Pathogenicity across variation in microbial virulence and host resistance

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Data accessibility: Data and code is archived on GitHub (https://github.com/anjaligupta1210/Pathogen-Potential).

Abstract:

The pathogenicity of a microbe is difficult to define in a comparative context: across microbes with varying virulence or across host genotypes with varying susceptibilities. Several different statistical approaches have been employed to investigate pathogenicity and susceptibility. Simple measures like proportional mortality or morbidity at a given time are attractive due to their simplicity but represent a single snapshot. Survival curve approaches, such as the Cox proportional hazards model and risk scores provide a more nuanced picture of the course of infection. More recently, Casadevall introduced the concept of pathogenic potential, a composite measure encompassing both host susceptibility and pathogen virulence, which focuses on the pathogenicity of a single pathogenic microbe, and later expanded to include additional nuances. Using *Drosophila melanogaster*, we conducted infection experiments with diverse species of *Providencia* that naturally vary in virulence. We also used several infectious doses to tune infections. We employed different host genotypes that vary in susceptibility to *Providencia* infection. Our analysis incorporates factors such as host genotype, pathogen type, inoculum load, symptomatic fraction, and mortality to compare host- and pathogen-based measures of pathogenicity. We discuss the advantages and limitations of each method, providing insights into their applicability in deciphering the intricacies of host-pathogen interactions and guiding the choice of analytical approaches in infectious disease research.

Introduction:

Virulence is the relative capacity of a microbe to cause damage in a host (Casadevall & Pirofski, 1999). It is intricately linked to host susceptibility, and its measurement is context-dependent, varying across host systems or for interspecific microbial comparisons (Casadevall & Pirofski, 2001). But virulence lacks an absolute measure, emphasizing the need for a relative assessment of a microbe's impact on a host (Casadevall & Pirofski, 1999). Hosts may display symptoms when infected with a specific microbial threshold, exhibiting observable disruptions in normal physiological functions, including mortality in severe cases. This relationship is further complicated by the fact that virulence is not a static characteristic but can evolve in response to changes in host resistance, environmental conditions, and microbial interactions (Ewald, 1994; Read, 1994). For instance, highly virulent strains may dominate in environments where transmission opportunities are frequent and host mortality does not significantly impede pathogen spread (Frank, 1996). Conversely, in environments where hosts are scarce or transmission is limited, less virulent strains that prolong host survival may be favored (Ebert & Bull, 2003). Additionally, the expression of virulence factors can be modulated by the host immune response, with some pathogens evolving mechanisms to evade or suppress host defenses, thereby enhancing their pathogenic potential (Casadevall & Pirofski, 2003; Mims et al., 2001). Understanding these dynamics is crucial for developing effective disease management strategies and predicting the emergence of more virulent or drug-resistant strains (Anderson & May, 1982; Antia et al., 2003).

Moreover, the interplay between microbial virulence and host resistance underscores the importance of considering both pathogen and host genetics in the study of infectious diseases. Genetic variations in hosts can influence susceptibility to infections, leading to diverse clinical outcomes even within the same species (Hill, 2001; Schmid-Hempel & Ebert, 2003). Similarly, microbial genetic diversity can result in different virulence profiles, impacting the severity and spread of infections (Mackinnon & Read, 2004; Woolhouse et al., 2002). Therefore, a comprehensive understanding of virulence requires an integrated approach that examines both microbial and host factors, as well as their interactions within specific ecological and evolutionary contexts (Casadevall & Pirofski, 2001; Galvani, 2003).

In many studies, the quantification of host susceptibility and pathogen virulence relies on host mortality rates, often using the Cox proportional hazards or related models (Cox, 1972; Klein & Moeschberger, 2003). This model offers a robust framework for survival analysis, combining a non-parametric baseline hazard with a parametric model for covariates proportional to the baseline hazard (Therneau et al., 2000). The Cox model can be expressed as

$$\lambda(t; Z) = \lambda_0(t)\, exp(\beta^T Z) \qquad [1]$$

where β is the parameter vector for Z (Z represents a vector of covariates, which are the variables or factors that influence the hazard or risk of the event occurring (in this case, host mortality)), and λ□(t) is the baseline hazard. Risk scores, crucial in survival analysis, are calculated based on the Cox model, expressed as R = $\beta^T Z$ (Hosmer Jr et al., 2008). While risk scores have been employed to assess risk factors, they do not encompass a quantitative measure of pathogen virulence and solely rely on host mortality (Bradburn et al., 2003).

Simple statistics such as the proportion of hosts alive at different time points and the time to death (or survival time) are commonly used to summarize survival data. The proportion alive provides a straightforward measure of survival at specific times but lacks the nuance to account for varying follow-up times and censoring (Aalen et al., 2008; Cutler & Ederer, 1958). Time to death offers more detailed information but is limited by the need for complete follow-up data and may not accurately reflect the impact of covariates (Altman, 1990). The Kaplan-Meier estimator is another widely used method in survival analysis that provides an estimate of the survival function from lifetime data (Kaplan & Meier, 1958). This non-parametric statistic handles censored data effectively and allows for the estimation of survival probabilities at various time points. However, it does not account for the effects of multiple covariates simultaneously, limiting its utility in multifactorial analyses (Collett, 2023; Pocock et al., 2002). To address these limitations, the Cox proportional hazards model offers a more comprehensive approach by incorporating multiple covariates into the survival analysis, thus providing insights into the factors that influence host mortality rates (Cox, 1972). However, while this model is powerful for identifying risk factors, it does not directly quantify pathogen virulence, necessitating additional methods to capture the full complexity of host-pathogen interactions (Hosmer Jr et al., 2008).

Casadevall, 2017 introduced the concept of the pathogenic potential of a microbe, highlighting the variable nature of host damage during host-microbe interactions. Clinical symptoms manifest when host damage disrupts homeostasis, leading to death when damage surpasses the point of

repair and return to homeostasis becomes impossible. Pathogenic potential is conceptualized as a composite of host susceptibility and pathogen virulence, expressed as

$$PP = \frac{F_S}{I} 10^M \quad [2]$$

where PP represents pathogenic potential, F_S denotes the fraction symptomatic (host susceptibility factor), I is the infective dose (or pathogen burden), and M is the mortality fraction (Casadevall, 2017). To account for interactions resulting in death, this ratio is adjusted by multiplying it by the 10th power of the mortality fraction. To incorporate the time to disease, the pathogenic potential can be expressed as

$$PP_T = \frac{F_S}{I} 10^M \frac{1}{T} \quad [3]$$

where T represents time to disease manifestation (Casadevall, 2017).

In this model, the fraction symptomatic host (F_S) is a measure of host susceptibility, referring to the inherent or acquired traits of the host that affect its vulnerability to infection and disease progression. These traits can include genetic factors, immune status, and existing health conditions that influence how severely the host is affected by the pathogen (Casadevall, 2017). The infective dose (I) is a measure of pathogen virulence, pertaining to the ability of the pathogen to cause damage to the host, which is determined by its mechanisms of infection, replication rate, and production of virulence factors that harm the host (Casadevall, 2017). The mortality fraction (M) is a measure of the lethality of the infection, indicating the proportion of infected hosts that succumb to the disease (Casadevall, 2017). The time to disease (T) captures

the duration from infection to the onset of clinical symptoms, adding a temporal dimension to the pathogenic potential and reflecting the speed of disease progression (Casadevall, 2017).

To fully understand the dynamics of host-pathogen interactions, is it crucial to distinguish between host-based and pathogen-based measures of pathogenicity, or do these metrics ultimately capture similar aspects of disease dynamics? Host-based measures focus on the response of the host organism to the pathogen, including metrics such as survival rates, risk scores, and the proportion of symptomatic individuals (Schmid-Hempel, 2009). These measures provide insight into how the host's health and survival are affected by infection, often using tools like Kaplan-Meier survival curves and Cox proportional hazards models to analyze mortality data (Cox, 1972; Kaplan & Meier, 1958). But do they truly reflect the virulence of the pathogen itself, or are they more indicative of the host’s capacity to withstand infection (Kleinbaum & Klein, 1996)? In contrast, pathogen-based measures aim to quantify the inherent virulence of the pathogen, independent of the host's response. This includes assessing the pathogen's ability to cause disease at various doses and its potential to induce host mortality (Mideo et al., 2008; Read, 1994). Are these distinctions meaningful, particularly in cases of high-dose infections where it becomes difficult to attribute mortality to a specific pathogen due to overwhelming pathogen loads (Frank & Schmid□Hempel, 2008)? By exploring these measures, can we better understand the relationship between host resilience and pathogen virulence, or are these categories less distinct than they initially appear?

Here, we set out to investigate the informativeness of quantitative measures of pathogenicity, including simple metrics of survival, Cox survival analysis, and pathogen potential and its related measures. We conducted infections of *Drosophila melanogaster* using *Providencia* species as a pathogen due to their varied levels of virulence and interaction with the host immune system. *Providencia rettgeri* has been shown to cause differential survival outcomes in *D. melanogaster* depending on the host's *dpt* genotype, making it an excellent candidate for examining host genotype impact influences on infection dynamics (Hanson et al., 2019, 2023; Mullinax et al., 2023; Unckless et al., 2015, 2016). Additionally, the genetic diversity within the host population for the *dpt* genotypes, further enriches this model, allowing us to dissect the contributions of specific alleles to infection outcomes (Hanson et al., 2023; Mullinax et al., 2023). The wildtype serine allele (dpt^{S69}) is associated with higher resistance to *P. rettgeri*, while the segregating arginine allele (dpt^{S69R}) confers increased susceptibility (Hanson et al., 2023; Mullinax et al., 2023; Unckless et al., 2016). The null allele (Δ*dpt*) results in nearly all flies succumbing to infection within two days, highlighting the crucial role of the *dpt* gene in mediating immune responses (Hanson et al., 2023; Mullinax et al., 2023; Unckless et al., 2016). This variation allows for a robust analysis of how host genetics influence susceptibility and resistance to bacterial infections. *P. sneebia* and *P. alcalifaciens* are highly pathogenic to *D. melanogaster*, with even low doses leading to lethal infections (Galac & Lazzaro, 2011). In contrast, *P. burhodogranariea* Strain D exhibits lower virulence, making it a suitable model for studying infections that result in moderate survival rates (Galac & Lazzaro, 2011). This range of virulence across *Providencia* species enables comprehensive studies of pathogen-host interactions under varying conditions of pathogen aggression and host resistance. Our approach allowed us to vary infection outcomes along three axes: the intrinsic virulence of the pathogen,

the dose of the pathogen, and the susceptibility of the host. We then compared pathogenicity metrics across these three axes. We measured survival against bacterial infections for four different species of *Providencia* injected in variable doses (different ODs) in flies of three different *dpt* genotypes (homozygous serine *dpt* (wildtype, dpt^{S69}), homozygous arginine *dpt* (dpt^{S69R}), and *dpt* null (Δ*dpt*)) in a controlled genetic background. The *Providencia* strains we used represent a range from avirulent (*P. burhodogranariea* strain D) to very virulent (*P. alcalifaciens* and *P. sneebia*). The *dpt* genotypes are an allelic series at least in relation to *P. rettgeri* (Mullinax et al., 2023; Unckless et al., 2016). Finally, we considered advantages and limitations of our different pathogenicity measures, offering recommendations on when to use each method of analysis.

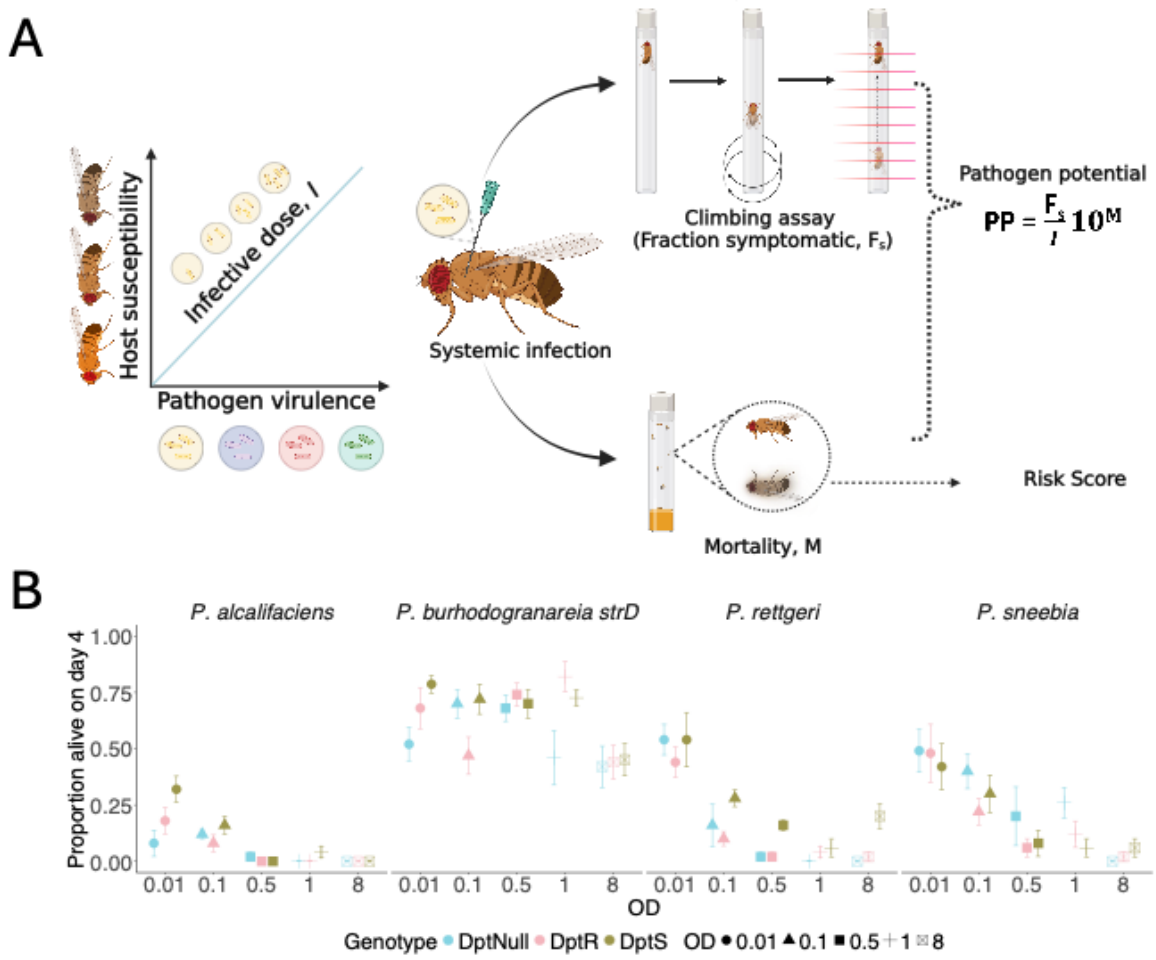

Figure 1: A. Experimental design (made using BioRender), **B.** Plot of the proportion of flies alive on day 4 post infection for various host genotypes (homozygous serine *dpt* (wildtype, dpt^{S69}), homozygous arginine *dpt* (dpt^{S69R}), and *dpt* null (*Δdpt*)), and variable pathogen strains (*P. burhodograneria* Strain D, *P. rettgeri*, *P. sneebia*, *P. alcalifaciens*) with different loads of infective inoculum (OD). The error bars represent the 95% confidence interval around the mean of the proportion of flies alive on day 4 post infection.

Materials & methods:

Fly stocks and maintenance: We used CRISPR/Cas9 edited flies with modified *diptericin A* gene (Mullinax et al., 2023). We used three lines of *D. melanogaster*, with the same genetic background, but polymorphic for *diptericin A* gene – homozygous serine *dpt* (wildtype, dpt^{S69}), homozygous arginine *dpt* (dpt^{S69R}), and *dpt* null (*Δdpt*, refers to lines with a 3 base pair deletion). Flies were maintained in a 23°C incubator with a 12h light:12h dark schedule on a cornmeal-molasses-yeast diet (64.3g/L cornmeal, 79.7mL/L molasses, 35.9g/L yeast, 8g/L agar, 15.4mL of food acid mix (50mL Phosphoric Acid + 418mL Propionic Acid + 532mL deionized water) and 1g/L Tegosept.

Bacterial strains: The following bacteria were used for systemic infections for this study: *Providencia rettgeri* (isolated from wild-caught *D. melanogaster* by B. Lazzaro in State College, PA, USA), *Providencia burhodograneria* Strain D (isolated from the hemolymph of wild *Drosophila*; P. Juneja and B. Lazzaro), *Providencia sneebia* (isolated from the hemolymph of wild *Drosophila*; P. Juneja and B. Lazzaro), and *Providencia alcalifaciens* (isolated from wild-caught *D. melanogaster* by P. Juneja and S. Short in Ithaca, NY, USA) (Galac & Lazzaro, 2011; Juneja & Lazzaro, 2009; Lazzaro, 2002). Bacteria were grown from glycerol stocks on LB plates at 37°C overnight. Individual colonies of bacteria were picked and grown in 2mL LB broth shaking overnight at 37°C for preparing infection cultures. The optical density (A_{600nm}) of the suspension was adjusted according to the desired infection dose.

Infection assays: We used bacterial suspensions for *P. rettgeri*, *P. burhodograneria* Strain D, *P. sneebia*, and *P. alcalifaciens* at an Optical Density (A_{600nm}) of approximately 0.01, 0.1, 0.5, 1, and 8 for our infections. We infected a total of 50 flies per genotype per bacteria per optical density. To induce systemic infection, 5-9 day old flies were pricked in the thorax with a needle dipped in a bacterial suspension. We also used LB media instead of bacterial suspension to poke flies as a control. Flies were incubated post infection at 23°C at a density of 10flies/vial with a 12h light:12h dark schedule and survival was tracked daily for 7 days post infection.

We used a climbing assay as a proxy for the fraction of symptomatic/sick flies post infection. We recorded the proportion of flies per vial that could climb up the walls of the vial within 5s after being tapped to the bottom daily for 7 days post infection. Figure 1A represents a schematic of our experimental design.

Bacterial load: To measure actual dosing in newly infected flies, single wildtype, dpt^{S69} flies were pricked using *P. rettgeri* and *P. sneebia* cultures at an optical density (A_{600nm}) of approximately 0.01, 0.1, 0.5, 1, and 8, followed by surface sterilization by washing in 70% ethanol and molecular grade water, and then, homogenized in 500µL LB and plated on LB plates immediately after the infection using a WASP touch spiral plater from Don Whitely Scientific. The LB agar plates were incubated overnight at 37°C. Gut commensal bacteria grow more slowly than *Providencia* under these conditions, so by limiting incubation to overnight we exclude any commensal bacteria from our assay. The number of colony forming units (CFU) on each plate was recorded using a Neutec Flash and Go colony counter. We used 5 replicates

(using single flies) per bacteria per A_{600nm} for quantifying the systemic bacterial load in infected flies.

Data and statistical analysis: We fitted our data to a mixed effects cox regression model to analyze the effect of the type and dose of pathogen and the host genotype. We performed a principal component analysis and a correlation matrix to look at the variation between the different measures of virulence and pathogenicity. Data analysis performed done in R version 4.3.1 (R Core Team, 2013). Data visualization was performed using R packages – ggplot2 (Wickham, 2011), survminer (Kassambara et al., 2017), wesanderson (Ram & Wickham, 2018), ggpubr (Kassambara & Kassambara, 2020), corrplot (Wei et al., 2017), RColorBrewer (Neuwirth & Neuwirth, 2014) and ggrepel (Slowikowski et al., 2018). Data was fitted to a cox-proportional hazards model to analyze the effect of host genotype, pathogen type and dose on survival [Table S2]. Statistical analysis was performed using R packages – lme4 (Bates, 2010) and coxme (Therneau & Therneau, 2015).

Results:

Host genotype, dose and pathogen strain interact to influence survival against infection

We measured survival against bacterial infections for four different species of *Providencia* injected in variable doses (different ODs) in flies of three different *dpt* genotypes (homozygous serine *dpt* (wildtype, dpt^{S69}), homozygous arginine *dpt* (dpt^{S69R}), and *dpt* null (*Δdpt*)) in a controlled genetic background. These *Providencia* strains represent a range from avirulent (*P. burhodogranereia*) to highly virulent (*P. alcalifaciens* and *P. sneebia*) bacteria (Galac & Lazzaro, 2011). The host *dpt* genotypes are an allelic series at least in relation to *P. rettgeri* where the wildtype serine allele is most resistant, the segregating arginine allele is more susceptible, and the null allele results in almost all flies dying within two days of infection (Mullinax et al., 2023). Our results from our infection experiments align with previously published findings - (i) flies homozygous for the serine allele survive systemic infection with *P. rettgeri* much better than those homozygous for the arginine allele (Unckless et al., 2016), (ii) *P. alcalifaciens* and *P. sneebia* are highly pathogenic to *D. melanogaster*, where small doses of infection with these bacteria can result in lethal infections (Galac & Lazzaro, 2011), (iii) most flies survive infections against *P. burhodograneria* Strain D at moderate doses (Galac & Lazzaro, 2011) [Figure 1B, Figure 2A].

We surveyed survival against infection using the Kaplan-Meier survival curves and proportion alive on day 4 post-infection to assess the effect of host genotype, dose, and pathogen strain [Figure 1B, Figure 2A]. We rationalized that 4 days post infection was a reasonable snapshot

because most flies destined to die had died by that point so the interaction between host immunity and pathogen was mostly over. Very small doses of *P. alcalifaciens* were sufficient to cause high mortality in flies regardless of their genotype. We saw a range of rates of mortality for survival against *P. sneebia* across genotypes and across different doses of infection. While for survival against *P. burhodograneria* Strain D, even high doses of injection did not prove to be lethal for most of the flies. We also quantified the bacterial load immediately after infection for the range of doses (optical densities) used in our assays. Dose was not linear with the optical density, instead the optical density was linearly related to log transformation of the dose (Figure S1, Table S1). We looked at the effect of host genotype, pathogen type and dose on survival using a cox-proportional hazards model [Table S2]. First, both the dose (OD) and pathogen species significantly affect survival, with *P. alcalifaciens* showing the highest pathogenicity (Table S2: coef = 1.273, $p < 0.001$). Second, host genotype markedly influences survival outcomes: *Δdpt* flies showed a higher mortality against all pathogens except *P. sneebia*, while the genotypic difference between dpt^{S69} and dpt^{S69R} did not significantly influence survival against the different pathogen strains except for *P. rettgeri* where flies with the serine allele (dpt^{S69}) have significantly better survival rates compared to those with the arginine allele (dpt^{S69R}) or null allele (Δ*dpt*) (Table S2: GenotypeDptS: coef = -1.116, p = 0.001). Third, interactions between OD, treatment, and genotype underscore the complexity of these relationships, highlighting specific combinations that significantly alter survival rates (Table S2: OD1 * Treatment*P. rettgeri* * GenotypeDptS (coef = -1.566, p = 0.005)).

Limitations of measures of pathogenicity

Host-based measures: We examined Kaplan-Meier survival curves, risk scores, and fraction symptomatic to assess host-based measures of pathogenicity [Figure 2]. Both risk scores and fraction symptomatic follow an approximately inverse relationship to host survival upon infection [Figure 2]. Fraction symptomatic is least sensitive to host genotype

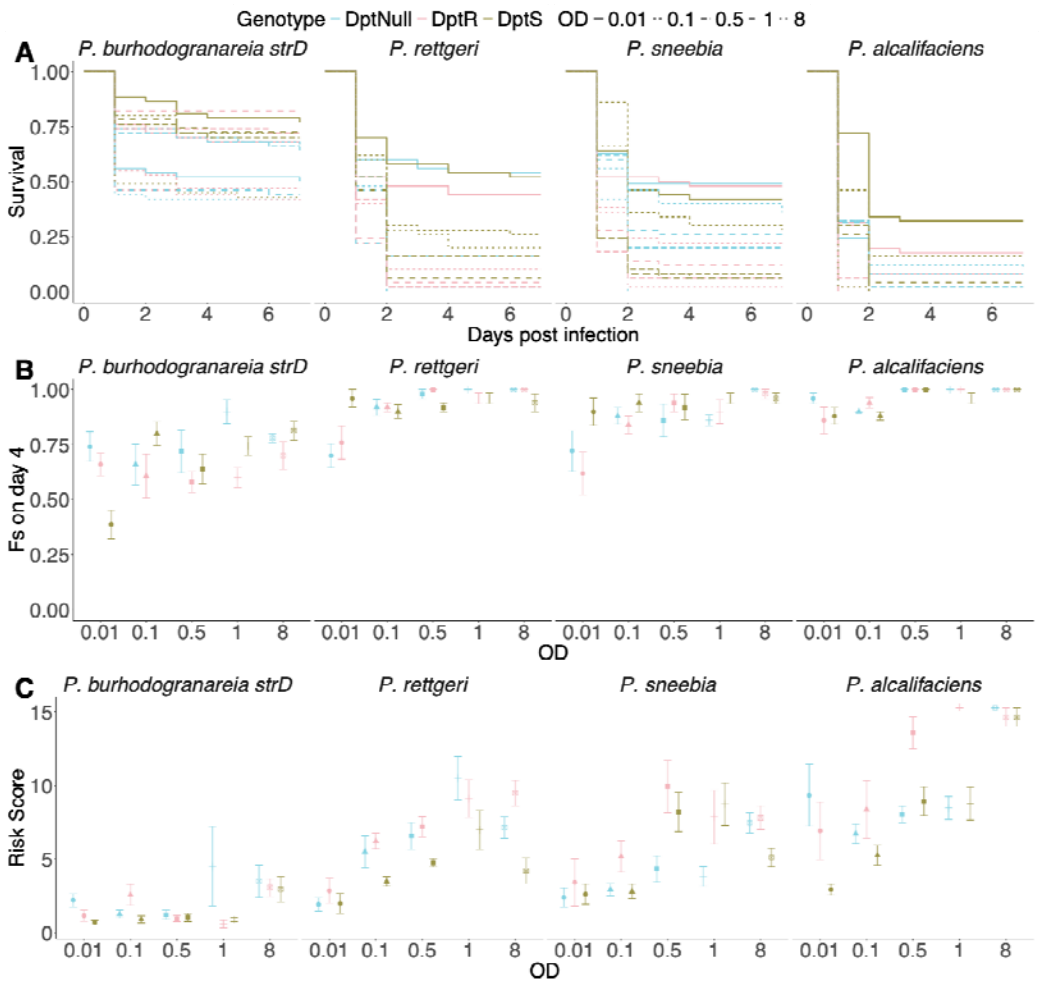

Figure 2: Host-based measures of pathogenicity: **A.** Survival curves, **B.** Fraction symptomatic on Day4, and **C.** Risk scores for infected flies of various host genotypes (homozygous serine *dpt* (wildtype, dpt^{S69}), homozygous arginine *dpt* (dpt^{S69R}), and *dpt* null (*Δdpt*)), and variable pathogen strains (*P. burhodograneria* Strain D, *P. rettgeri*, *P. sneebia*, *P. alcalifaciens*) with different loads of infective inoculum (OD).

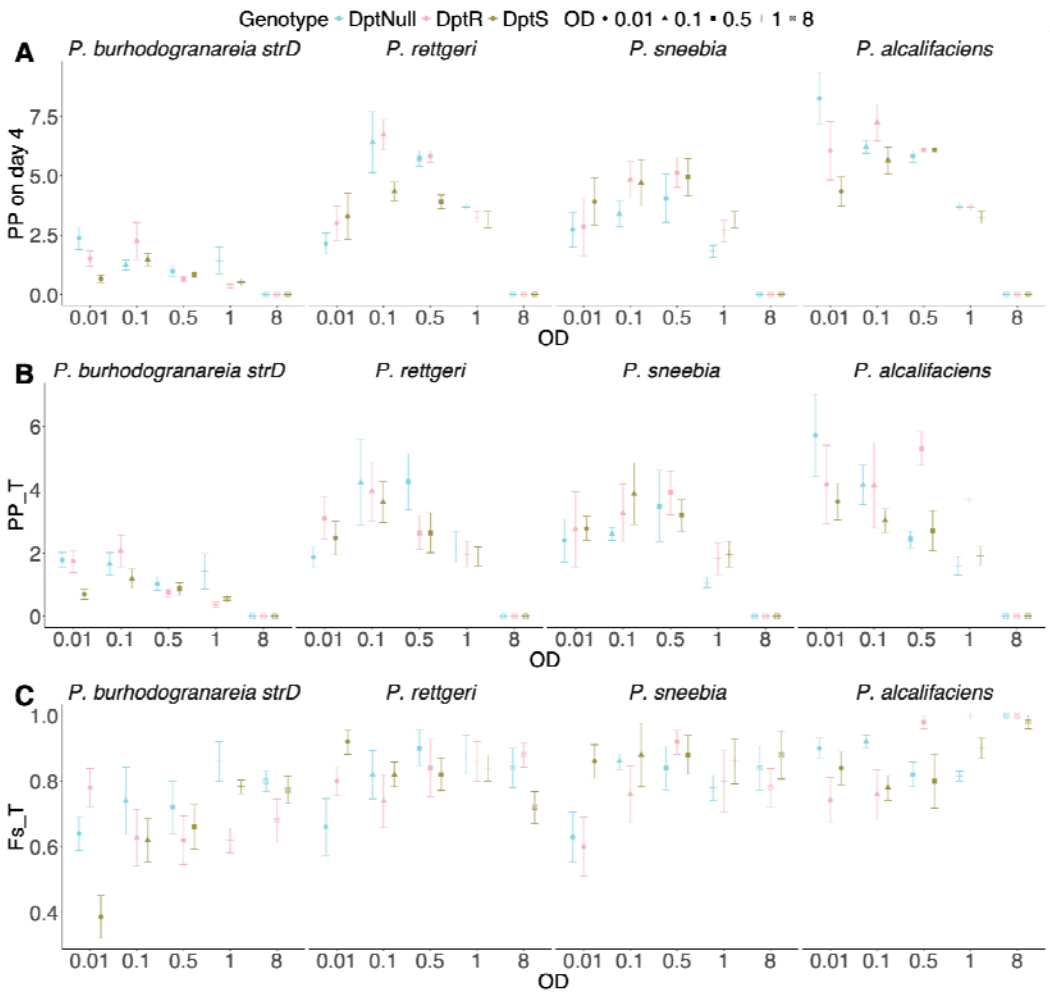

Figure 3: A. Pathogen potential on Day 4 post-infection, **B.** Pathogen potential on median survival day (T), and **C.** Fraction symptomatic on median survival day (T) for infected flies of various host genotypes (homozygous serine *dpt* (wildtype, dpt^{S69}), homozygous arginine *dpt* (dpt^{S69R}), and *dpt* null (*Δdpt*)), and variable pathogen strains (*P. burhodograneria* Strain D, *P. rettgeri*, *P. sneebia*, *P. alcalifaciens*) with different loads of infective inoculum (OD).

303 out of the three measures of host susceptibility [Figure 2]. Risk scores show variation with both

the dose and virulence of pathogens but fail to resolve the interaction between the dose of a pathogen and the host genotype [Figure 2]. These host-based measures of pathogenicity are easy to interpret, but they tell us little about pathogen virulence of individual microbes. It becomes

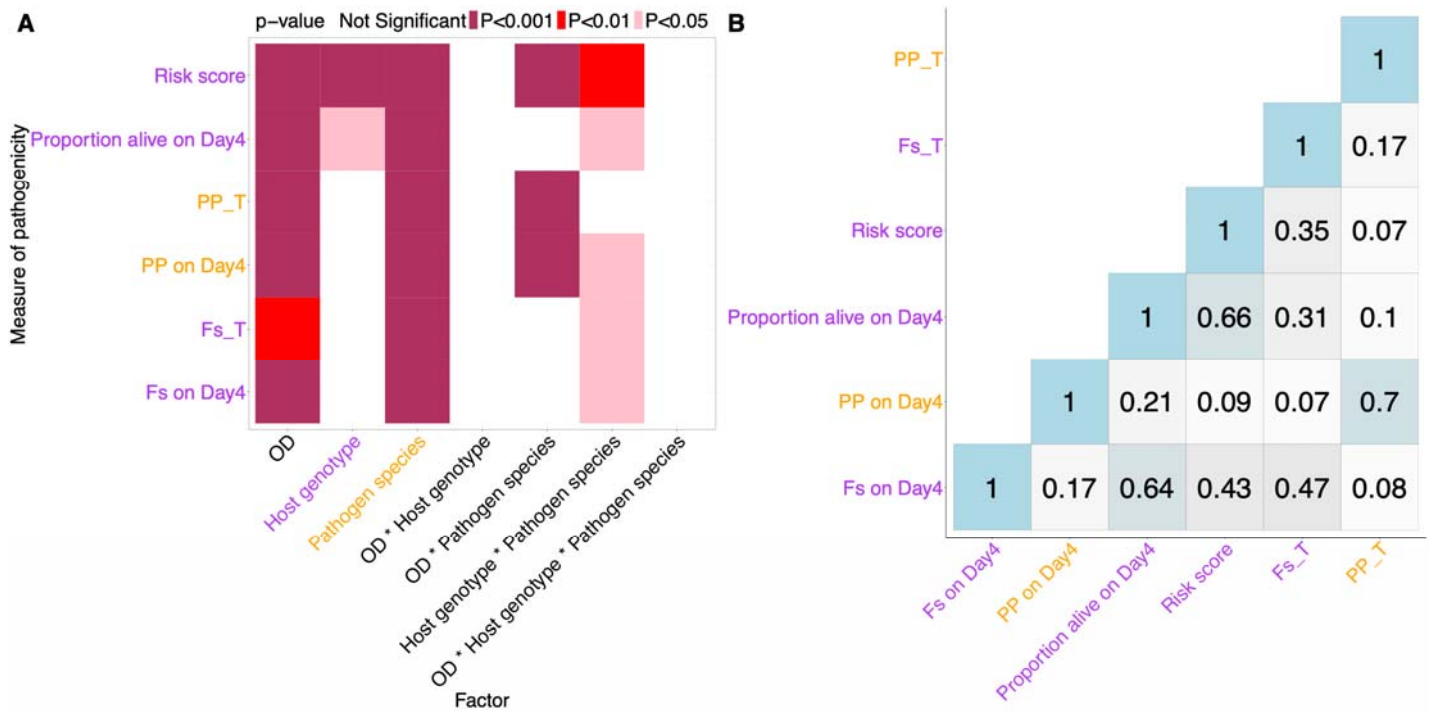

Figure 4: A. P-values from ANOVA to indicate the significance of each factor corresponding to each measure of pathogenicity (see Table S3 for more information), B. Heatmap showing R^2 (R=correlation coefficient) values to assess the differences between different measures of host susceptibility (survival, risk scores, and fraction symptomatic) and pathogen virulence (pathogen potential) (see Table S4 for more information). [Host-based measures are labelled purple while pathogen-based measures are labelled orange]

difficult to determine whether the differences in survival seen in the host is due to a highly virulent pathogen or infection with a high dose of a pathogen. Most pathogens become virulent enough to cause visible symptoms/mortality when administered to a single host at high doses.

Pathogen-based measures: We looked at the pathogen potential and related metrics to assess pathogen-based measures of pathogenicity [Figure 3]. We quantified the pathogen potential on day 4 post-infection, and on the day of median survival for each vial. Pathogen potential was proposed as a tool for adding quantitative rigor to comparisons in microbial pathogenesis (Casadevall, 2017, 2022; Casadevall et al., 2011; Casadevall & Pirofski, 1999, 2001, 2003; Smith & Casadevall, 2022). Pathogen potential is helpful in analyzing the dose-dependent effect of pathogen on host mortality, but there are limitations to the measure. Pathogen potential failed to capture the variation between host genotypes [Figure 4A]. The limitation in assessing pathogen potential arises when evaluating high doses or highly virulent microbes at moderate doses, wherein the likelihood of a specific pathogenic microbe being deemed the primary cause of mortality approaches zero, even though all individuals die. This counterintuitive phenomenon stems from the low probability that any specific pathogen cell within the high dose is the sole contributor to mortality. While this concept aligns with the understanding that the pathogen responsible for mortality may not be easily pinpointed, it presents a challenge in practical utility in our context, as the assessment becomes less informative with more virulent pathogens.

Comparative analysis: We took a comparative approach to determine how different measures of host susceptibility (survival, risk scores, and fraction symptomatic) and pathogen virulence (pathogen potential) were correlated with each other to assess how different they are in their analytical information. From the R^2 matrix, it was evident that all the different host-based measures of virulence and susceptibility were highly correlated to each other, but not to the pathogen-based measures (pathogen potential) [Figure 4B]. To further examine the relationship among metrics, we performed a principal component analysis (PCA). This revealed three main

groupings: risk score (alone), fraction alive and fraction symptomatic metrics, and pathogen-potential related metrics [Figure S2]. Pathogen potential metrics clustered away from the host-based measures as they incorporate quantitative information about the infecting pathogen as well as host response. Interestingly, risk scores also clustered away from all the different pathogenicity measures as risk scores were able to capture the information for the variation between host genotype, pathogen type and dose of the pathogen [Figure 4A, Figure S2].

Discussion:

Our study investigated the complex dynamics of host-pathogen interactions, focusing on the quantification of host susceptibility and pathogenicity. Traditionally, host mortality rates, analyzed through the Cox proportional hazards models, have been widely used to assess virulence. An alternative, pathogenic potential concept, introduced by Casadevall, 2017 offers a more nuanced perspective by considering the composite nature of host damage. In this study, we explore the advantages and limitations of traditional survival analysis and risk scores compared to the quantitative measures like pathogen potential. Our results suggest that, at least for our study system, the best measure depends on overall host mortality in relation to dose.

Our infection experiments revealed intricate interactions between host genotype, pathogen strain, and infection dose [Table S2]. The survival curves and fraction symptomatic provided clear patterns of host response. The observed higher mortality in Δ*dpt* flies against various pathogens, except *P. sneebia*, underscores the role of the diptericin A gene in systemic infection resilience

(Hanson et al., 2023; Mullinax et al., 2023; Unckless et al., 2016). *P. alcalifaciens* demonstrated high pathogenicity even at low doses, emphasizing the importance of considering both pathogen virulence and infection dose in assessments.

Traditional survival analysis and risk scores, rooted in Cox models, proved informative for overall trends and risk assessment. However, they fall short in providing a comprehensive understanding of pathogen virulence, particularly for avirulent or less virulent pathogens. The limitations of these host-based measures highlight the necessity for additional quantitative insights. The pathogen-based measure, pathogen potential, offers a quantitative lens but encounters limitations at high pathogen doses, where pinpointing the primary cause of mortality becomes less informative. It becomes evident that each measure contributes unique insights, emphasizing the importance of a nuanced and multifaceted approach in analyzing host-pathogen interactions. A combination of pathogen potential and risk scores seemed to be particularly informative in separating the variation between host susceptibility and pathogen virulence.

We propose recommendations for the optimal use of each measure, considering their strengths and limitations –

(i) <u>Survival Analysis (Kaplan-Meier Curves):</u> Survival analysis is a robust measure and should be used to assess the overall survival trends over time. It is particularly useful for understanding the impact of host genotype, dose, and pathogen strain on the course of infection. **Limitations:** While survival analysis provides valuable information about the overall outcome, it may not distinguish between the effects of pathogen virulence and the dose administered.

(ii) Risk Scores: Risk scores are informative for assessing variation in both dose and virulence of pathogens. They are especially useful when trying to understand how different combinations of host genotype, dose, and pathogen strain contribute to the risk of mortality. **Limitations:** Risk scores may not provide information about the virulence of avirulent or less virulent pathogens, and their utility diminishes for high doses of less virulent pathogens.

(iii) Fraction Symptomatic: Fraction symptomatic is useful for insights into the proportion of individuals exhibiting symptoms, giving a qualitative assessment of the infection. **Limitations:** Fraction symptomatic is less sensitive to pathogen virulence and may not provide detailed information about the severity of the infection.

(iv) Pathogen Potential: Pathogen potential is valuable for understanding the dose-dependent effects of pathogens on host mortality. **Limitations:** Pathogen potential may be less informative when mortality is high since it loses resolution, where the likelihood of pinpointing a specific pathogen as the primary cause of mortality decreases.

Survival analysis is robust for overall trends, risk scores offer detailed information on variation, fraction symptomatic provides qualitative insights, and pathogen potential contributes quantitative rigor. A comparative analysis aids in choosing the most relevant measure based on experimental goals.

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References:

Aalen, O., Borgan, O., & Gjessing, H. (2008). *Survival and event history analysis: a process point of view*. Springer Science & Business Media.

Altman, D. G. (1990). *Practical statistics for medical research*. Chapman and Hall/CRC.

Anderson, R. M., & May, R. M. (1982). Coevolution of hosts and parasites. *Parasitology*, *85*(2), 411–426.

Antia, R., Regoes, R. R., Koella, J. C., & Bergstrom, C. T. (2003). The role of evolution in the emergence of infectious diseases. *Nature*, *426*(6967), 658–661.

Bates, D. M. (2010). *lme4: Mixed-effects modeling with R*. Springer New York.

Bradburn, M. J., Clark, T. G., Love, S. B., & Altman, D. G. (2003). Survival analysis part II: multivariate data analysis–an introduction to concepts and methods. *British Journal of Cancer*, *89*(3), 431–436.

Casadevall, A. (2017). The Pathogenic Potential of a Microbe. *MSphere*, *2*(1). https://doi.org/10.1128/msphere.00015-17

Casadevall, A. (2022). *Expanding the Pathogenic Potential Concept To Incorporate Fulminancy, Time, and Virulence Factors*. https://journals.asm.org/journal/msphere

Casadevall, A., Fang, F. C., & Pirofski, L. (2011). Microbial virulence as an emergent property: consequences and opportunities. *PLoS Pathogens*, *7*(7), e1002136.

Casadevall, A., & Pirofski, L. (1999). Host-pathogen interactions: redefining the basic concepts of virulence and pathogenicity. *Infection and Immunity*, *67*(8), 3703–3713.

Casadevall, A., & Pirofski, L. (2001). Host□pathogen interactions: the attributes of virulence. *The Journal of Infectious Diseases*, *184*(3), 337–344.

Casadevall, A., & Pirofski, L. (2003). The damage-response framework of microbial pathogenesis. *Nature Reviews Microbiology*, *1*(1), 17–24.

Collett, D. (2023). *Modelling survival data in medical research*. Chapman and Hall/CRC.

Cox, D. R. (1972). Regression models and life□tables. *Journal of the Royal Statistical Society: Series B (Methodological)*, *34*(2), 187–202.

Cutler, S. J., & Ederer, F. (1958). Maximum utilization of the life table method in analyzing survival. *Journal of Chronic Diseases*, *8*(6), 699–712.

Ebert, D., & Bull, J. J. (2003). Challenging the trade-off model for the evolution of virulence: is virulence management feasible? *Trends in Microbiology*, *11*(1), 15–20.

Ewald, P. W. (1994). *Evolution of infectious disease*. Oxford University Press, USA.

Frank, S. A. (1996). Models of parasite virulence. *The Quarterly Review of Biology*, *71*(1), 37–78.

Frank, S. A., & Schmid□Hempel, P. (2008). Mechanisms of pathogenesis and the evolution of parasite virulence. *Journal of Evolutionary Biology*, *21*(2), 396–404.

Galac, M. R., & Lazzaro, B. P. (2011). Comparative pathology of bacteria in the genus Providencia to a natural host, Drosophila melanogaster. *Microbes and Infection*, *13*(7), 673–683.

Galvani, A. P. (2003). Epidemiology meets evolutionary ecology. *Trends in Ecology & Evolution*, *18*(3), 132–139.

Hanson, M. A., Grollmus, L., & Lemaitre, B. (2023). Ecology-relevant bacteria drive the evolution of host antimicrobial peptides in Drosophila. *Science*, *381*(6655), eadg5725.

Hanson, M. A., Lemaitre, B., & Unckless, R. L. (2019). Dynamic evolution of antimicrobial peptides underscores trade-offs between immunity and ecological fitness. *Frontiers in Immunology*, *10*, 2620.

Hill, A. V. S. (2001). The genomics and genetics of human infectious disease susceptibility. *Annual Review of Genomics and Human Genetics*, *2*(1), 373–400.

Hosmer Jr, D. W., Lemeshow, S., & May, S. (2008). *Applied survival analysis: regression modeling of time-to-event data* (Vol. 618). John Wiley & Sons.

Juneja, P., & Lazzaro, B. P. (2009). Providencia sneebia sp. nov. and Providencia burhodogranariea sp. nov., isolated from wild Drosophila melanogaster. *International Journal of Systematic and Evolutionary Microbiology*, *59*(5), 1108–1111.

Kaplan, E. L., & Meier, P. (1958). Nonparametric estimation from incomplete observations. *Journal of the American Statistical Association*, *53*(282), 457–481.

Kassambara, A., & Kassambara, M. A. (2020). Package ‘ggpubr.’ *R Package Version 0.1*, *6*(0).

Kassambara, A., Kosinski, M., Biecek, P., & Fabian, Sjdsc. (2017). Package ‘survminer.’ *Drawing Survival Curves Using ‘Ggplot2’(R Package Version 03 1)*, *3*.

Klein, J. P., & Moeschberger, M. L. (2003). *Survival analysis: techniques for censored and truncated data* (Vol. 1230). Springer.

Kleinbaum, D. G., & Klein, M. (1996). *Survival analysis a self-learning text*. Springer.

Lazzaro, B. P. (2002). *A population and quantitative genetic analysis of the Drosophila melanogaster antibacterial immune response*. The Pennsylvania State University.

Mackinnon, M. J., & Read, A. F. (2004). Immunity promotes virulence evolution in a malaria model. *PLoS Biology*, *2*(9), e230.

Mideo, N., Alizon, S., & Day, T. (2008). Linking within-and between-host dynamics in the evolutionary epidemiology of infectious diseases. *Trends in Ecology & Evolution*, *23*(9), 511–517.

Mims, C. A., Nash, A. A., & Stephen, J. (2001). *Mims' pathogenesis of infectious disease*. Gulf Professional Publishing.

Mullinax, S. R., Darby, A. M., Gupta, A., Chan, P., Smith, B., & Unckless, R. (2023). A suite of selective pressures supports the maintenance of alleles of a Drosophila immune peptide. *BioRxiv*, 2023–2028.

Neuwirth, E., & Neuwirth, M. E. (2014). Package 'RColorBrewer.' *ColorBrewer Palettes*.

Pocock, S. J., Clayton, T. C., & Altman, D. G. (2002). Survival plots of time-to-event outcomes in clinical trials: good practice and pitfalls. *The Lancet*, *359*(9318), 1686–1689.

R Core Team, R. (2013). *R: A language and environment for statistical computing*.

Ram, K., & Wickham, H. (2018). wesanderson: a Wes Anderson palette generator. *R Package Version 0.3*, *6*, 2018.

Read, A. F. (1994). The evolution of virulence. *Trends in Microbiology*, *2*(3), 73–76.

Schmid-Hempel, P. (2009). Immune defence, parasite evasion strategies and their relevance for ‘macroscopic phenomena’such as virulence. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1513), 85–98.

Schmid-Hempel, P., & Ebert, D. (2003). On the evolutionary ecology of specific immune defence. *Trends in Ecology & Evolution*, *18*(1), 27–32.

Slowikowski, K., Schep, A., Hughes, S., Lukauskas, S., Irisson, J.-O., Kamvar, Z. N., Ryan, T., Christophe, D., Hiroaki, Y., & Gramme, P. (2018). Package ggrepel. *Automatically Position Non-Overlapping Text Labels with ‘ggplot2*.

Smith, D. F. Q., & Casadevall, A. (2022). On the relationship between Pathogenic Potential and Infective Inoculum. *PLoS Pathogens*, *18*(6). https://doi.org/10.1371/journal.ppat.1010484

Therneau, T. M., Grambsch, P. M., Therneau, T. M., & Grambsch, P. M. (2000). *The cox model*. Springer.

Therneau, T. M., & Therneau, M. T. M. (2015). Package ‘coxme.’ *R Package Version*, *2*(5).

Unckless, R. L., Howick, V. M., & Lazzaro, B. P. (2016). Convergent Balancing Selection on an Antimicrobial Peptide in Drosophila. *Current Biology*, *26*(2), 257–262. https://doi.org/10.1016/j.cub.2015.11.063

Unckless, R. L., Rottschaefer, S. M., & Lazzaro, B. P. (2015). The Complex Contributions of Genetics and Nutrition to Immunity in Drosophila melanogaster. *PLoS Genetics*, *11*(3). https://doi.org/10.1371/journal.pgen.1005030

Wei, T., Simko, V., Levy, M., Xie, Y., Jin, Y., & Zemla, J. (2017). Package ‘corrplot.’ *Statistician*, *56*(316), e24.

Wickham, H. (2011). ggplot2. *Wiley Interdisciplinary Reviews: Computational Statistics*, *3*(2), 180–185.

Woolhouse, M. E. J., Webster, J. P., Domingo, E., Charlesworth, B., & Levin, B. R. (2002). Biological and biomedical implications of the co-evolution of pathogens and their hosts. *Nature Genetics*, *32*(4), 569–577.

Supplementary Information

Figure S1: Bacterial load (CFU/fly) vs OD (A_{600nm}) for different ODs and different pathogens. Single infected flies were homogenized in 500µL LB and plated on LB plates immediately after the infection. Statistical analysis can be seen in Table S1.

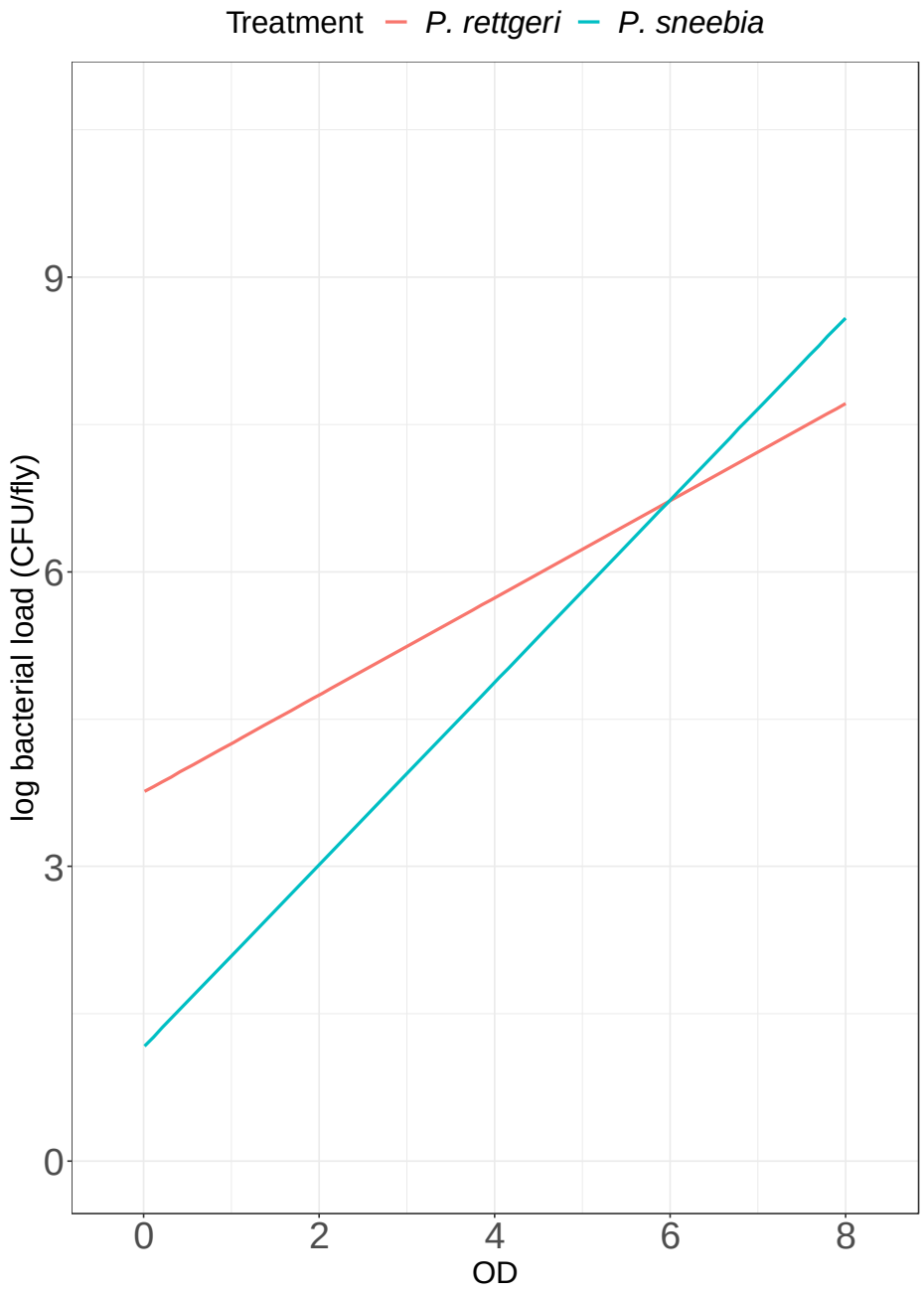


Figure S2: Principal component analysis (PCA) over different measures of host susceptibility (survival, risk scores, and fraction symptomatic) and pathogen virulence (pathogen potential). [Prop_DayX_Alive: Proportion survival on Day X post-infection, PP_DayX: Pathogen potential on Day X post-infection, PP_T: Pathogen potential on median survival day, F_s_T/T: Fraction symptomatic on median survival day/ Median survival day, Fs_DayX: Fraction symptomatic on Day X post-infection, Fs_T: Fraction symptomatic on median survival day].

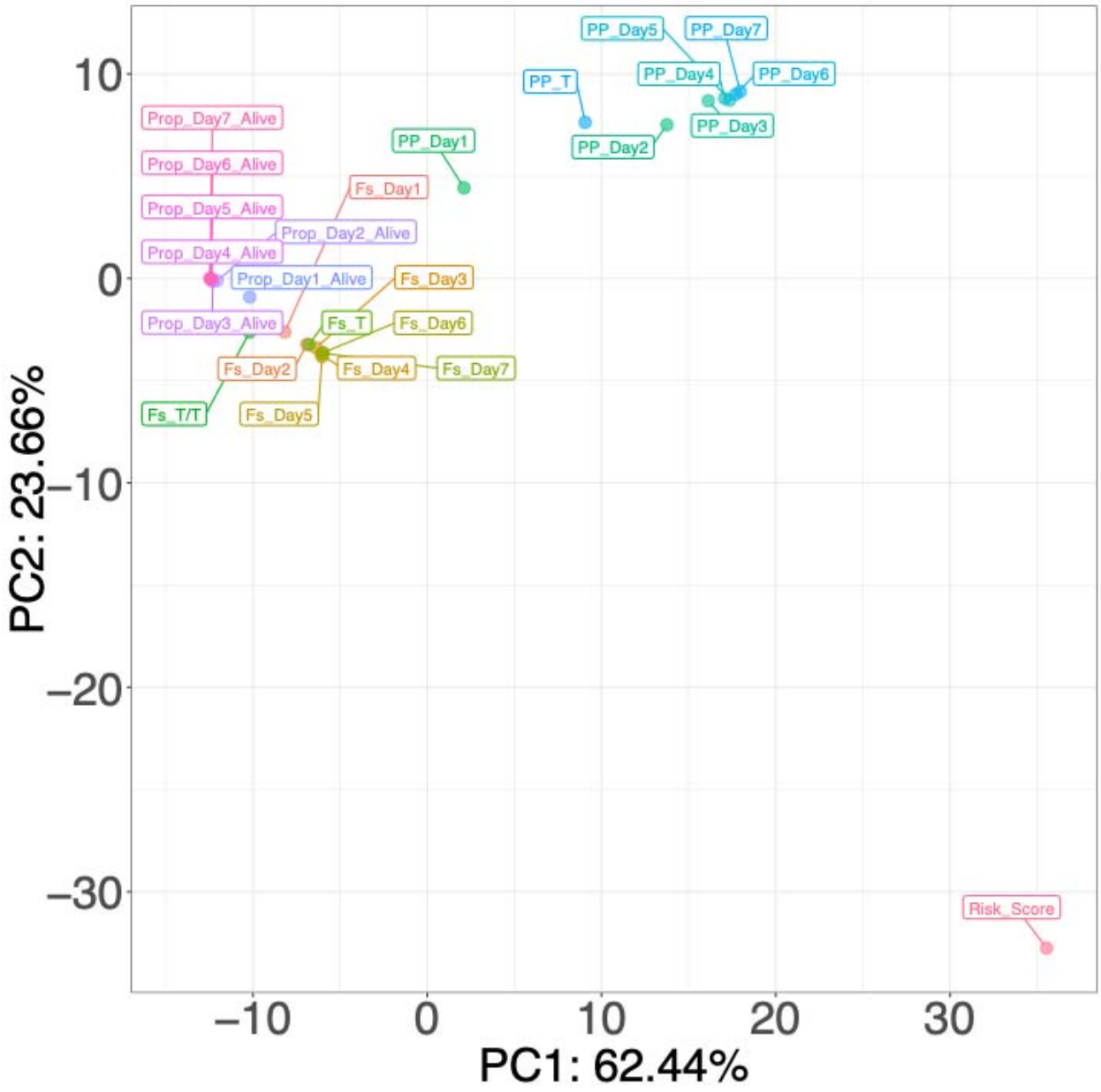


Table S1: Statistical analysis for analyzing the effect of OD and treatment (pathogen species) on log-transformed bacterial load. Data was fitted to a linear mixed-effects model.

[Model: lm(log(`Bacterial_Load_CFU/fly`+1) ~ OD + Treatment)]

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	3.265	0.523	6.239	<0.001 ***
OD	0.711	0.111	6.364	<0.001 ***
Treatment	-1.612	0.647	-2.489	0.015 *

Table S2: Statistical analysis for analyzing the effect of OD, treatment (pathogen species), and host genotype on survival. Data was fitted to a mixed-effects cox-proportional hazards model.

[Model: coxph(Survival ~ OD * Treatment * Genotype]

	coef	**exp(coef)**	**se(coef)**	**z**	**Pr(>|z|)**	
OD_0.1	-0.542	0.581	0.309	-1.753	0.079	
OD_0.5	-0.580	0.559	0.314	-1.848	0.064	
OD_1	0.235	1.265	0.275	0.854	0.392	
OD_8	0.312	1.366	0.272	1.143	0.252	
Treatment_P. rettgeri	-0.140	0.868	0.288	-0.486	0.626	
Treatment_P. sneebia	-0.003	0.996	0.280	-0.013	0.988	
Treatment_P. alcalifaciens	1.273	3.573	0.248	5.116	<0.001	***
Genotype_DptR	-0.664	0.514	0.320	-2.075	0.0379	*
Genotype_DptS	-1.116	0.327	0.351	-3.180	0.001	**
OD_0.1 * Treatment_P. rettgeri	1.512	4.536	0.403	3.745	<0.001	***
OD_0.5 * Treatment_P. rettgeri	1.774	5.897	0.403	4.394	<0.001	***
OD_1 * Treatment_P. rettgeri	1.384	3.990	0.373	3.704	<0.001	***
OD_8 * Treatment_P. rettgeri	0.984	2.677	0.371	2.648	0.008	**
OD_0.1 * Treatment_P. sneebia	0.843	2.325	0.406	2.075	0.037	*
OD_0.5 * Treatment_P. sneebia	1.209	3.353	0.402	3.002	0.002	**

OD_1 * Treatment_P. sneebia	0.283	1.328	0.375	0.755	0.449	
OD_8 * Treatment_P. sneebia	0.895	2.447	0.364	2.453	0.014	*
OD_0.1 * Treatment_P. alcalifaciens	0.375	1.456	0.373	1.006	0.314	
OD_0.5 * Treatment_P. alcalifaciens	0.597	1.818	0.375	1.592	0.111	
OD_1 * Treatment_P. alcalifaciens	-0.166	0.846	0.343	-0.484	0.627	
OD_8 * Treatment_P. alcalifaciens	0.321	1.379	0.341	0.943	0.345	
OD_0.1 * Genotype_DptR	1.282	3.605	0.441	2.903	0.003	**
OD_0.5 * Genotype_DptR	0.447	1.564	0.482	0.927	0.353	
OD_1 * Genotype_DptR	-0.889	0.410	0.499	-1.782	0.074	
OD_8 * Genotype_DptR	0.659	1.934	0.412	1.598	0.109	
OD_0.1 * Genotype_DptS	0.808	2.244	0.500	1.615	0.106	
OD_0.5 * Genotype_DptS	0.953	2.593	0.498	1.910	0.056	
OD_1 * Genotype_DptS	0.023	1.023	0.480	0.049	0.960	
OD_8 * Genotype_DptS	1.023	2.783	0.439	2.326	0.019	*
Treatment_P. rettgeri * Genotype_DptR	0.982	2.671	0.426	2.304	0.021	*
Treatment_P. sneebia * Genotype_DptR	0.749	2.116	0.423	1.769	0.076	
Treatment_P. alcalifaciens * Genotype_DptR	0.351	1.420	0.384	0.912	0.361	
Treatment_P. rettgeri * Genotype_DptS	1.100	3.005	0.456	2.410	0.015	*
Treatment_P. sneebia * Genotype_DptS	1.257	3.515	0.443	2.837	0.004	**
Treatment_P. alcalifaciens * Genotype_DptS	0.146	1.158	0.417	0.351	0.725	
OD_0.1 * Treatment_P. rettgeri * Genotype_DptR	-1.398	0.246	0.565	-2.470	0.013	*
OD_0.5 * Treatment_P. rettgeri * Genotype_DptR	-0.653	0.519	0.593	-1.101	0.270	
OD_1 * Treatment_P. rettgeri * Genotype_DptR	0.453	1.573	0.607	0.746	0.455	
OD_8 * Treatment_P. rettgeri * Genotype_DptR	-0.699	0.496	0.538	-1.299	0.193	
OD_0.1 * Treatment_P. sneebia * Genotype_DptR	-0.868	0.419	0.573	-1.513	0.130	
OD_0.5 * Treatment_P. sneebia * Genotype_DptR	0.249	1.282	0.596	0.417	0.676	
OD_1 * Treatment_P. sneebia * Genotype_DptR	1.408	4.090	0.613	2.296	0.021	*
OD_8 * Treatment_P. sneebia * Genotype_DptR	-0.709	0.491	0.536	-1.323	0.185	
OD_0.1 * Treatment_P. alcalifaciens * Genotype_DptR	-0.883	0.413	0.533	-1.656	0.097	
OD_0.5 * Treatment_P. alcalifaciens * Genotype_DptR	0.346	1.413	0.564	0.613	0.539	
OD_1 * Treatment_P. alcalifaciens *	1.768	5.863	0.579	3.050	0.002	**

Genotype_DptR						
OD_8 * Treatment_P. alcalifaciens * Genotype_DptR	-0.394	0.674	0.505	-0.778	0.435	
OD_0.1 * Treatment_P. rettgeri * Genotype_DptS	-1.134	0.321	0.621	-1.824	0.068	
OD_0.5 * Treatment_P. rettgeri * Genotype_DptS	-1.198	0.301	0.615	-1.949	0.051	
OD_1 * Treatment_P. rettgeri * Genotype_DptS	-0.443	0.641	0.597	-0.741	0.458	
OD_8 * Treatment_P. rettgeri * Genotype_DptS	-1.566	0.208	0.569	-2.752	0.005	**
OD_0.1 * Treatment_P. sneebia * Genotype_DptS	-0.989	0.371	0.618	-1.600	0.109	
OD_0.5 * Treatment_P. sneebia * Genotype_DptS	-0.461	0.630	0.607	-0.760	0.447	
OD_1 * Treatment_P. sneebia * Genotype_DptS	0.620	1.860	0.593	1.046	0.295	
OD_8 * Treatment_P. sneebia * Genotype_DptS	-1.534	0.215	0.554	-2.765	0.005	**
OD_0.1 * Treatment_P. alcalifaciens * Genotype_DptS	-0.095	0.908	0.589	-0.162	0.870	
OD_0.5 * Treatment_P. alcalifaciens * Genotype_DptS	0.086	1.089	0.583	0.147	0.882	
OD_1 * Treatment_P. alcalifaciens * Genotype_DptS	0.936	2.550	0.568	1.646	0.099	
OD_8 * Treatment_P. alcalifaciens * Genotype_DptS	-0.101	0.903	0.533	-0.189	0.849	

Table S3: ANOVA for analyzing the effect of OD, treatment (pathogen species), and host genotype on different measures of pathogenicity, corresponding to Figure 4A.

[Model: aov("Measure of pathogenicity" ~ OD * Treatment * Genotype]

A. Risk score

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
OD_group	1	457.7775	457.7775	57.810707	4.54036379e-13
Genotype	2	204.3041	102.1520	12.900337	4.40620663e-06
Treatment	3	2385.008	795.0028	100.39739	5.84776824e-44
OD_group:Genotype	2	6.813781	3.40689	0.4302412	0.65078763

OD_group:Treatment	3	212.4440	70.8146	8.9428743	1.129315565e-05
Genotype:Treatment	6	170.1871	28.3645	3.5820305	0.00195593
OD_group:Genotype:Treatment	6	85.26429	14.2107	1.7946085	0.10019399
Residuals	276	2185.522	7.91856		

B. Proportion alive on day 4

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
OD_group	1	1.6552	1.6552	53.1849	3.2203077932e-12
Genotype	2	0.1909	0.0954	3.0673	0.04813333
Treatment	3	13.3078	4.4359	142.5350	8.5207499643e-56
OD_group:Genotype	2	0.0115	0.0057	0.1855	0.83073304
OD_group:Treatment	3	0.1787	0.0595	1.9142	0.12747538
Genotype:Treatment	6	0.3985	0.0664	2.1342	0.0497153
OD_group:Genotype:Treatment	6	0.1214	0.0202	0.6503	0.68984172
Residuals	276	8.5896	0.0311		

C. Pathogen potential on median survival day (PP_T)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
OD_group	1	348.326304	348.326304	181.087145	4.42E-32
Genotype	2	9.26896418	4.63448209	2.40936478	0.09176047
Treatment	3	144.923737	48.3079122	25.1142156	2.14E-14
OD_group:Genotype	2	2.39122672	1.19561336	0.62157295	0.53784906
OD_group:Treatment	3	38.43589	12.8119633	6.66065651	0.00023403
Genotype:Treatment	6	17.3356943	2.88928239	1.50207404	0.17741778
OD_group:Genotype:Treatment	6	3.54631668	0.59105278	0.30727527	0.93289644
Residuals	276	530.893896	1.92352861		

D. Pathogen potential on day 4

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
OD_group	1	681.987327	681.987327	319.382172	5.48E-48
Genotype	2	8.37501878	4.18750939	1.96105674	0.14266536
Treatment	3	477.539564	159.179855	74.545678	2.41E-35
OD_group:Genotype	2	2.72747801	1.363739	0.63865399	0.52878111
OD_group:Treatment	3	125.482761	41.8275871	19.5883194	1.53E-11
Genotype:Treatment	6	28.389012	4.731502	2.21581447	0.04181231
OD_group:Genotype:Treatment	6	7.75694982	1.29282497	0.60544416	0.72592681

Residuals	276	589.351939	2.13533311		

E. Fraction symptomatic on median survival day (F_S_T)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
OD_group	1	0.21735177	0.21735177	10.2606548	0.00151824
Genotype	2	0.02829094	0.01414547	0.66777374	0.51367563
Treatment	3	1.50871066	0.50290355	23.7408687	1.06E-13
OD_group:Genotype	2	0.00398772	0.00199386	0.09412543	0.9101978
OD_group:Treatment	3	0.15593698	0.05197899	2.45380334	0.06354373
Genotype:Treatment	6	0.29539735	0.04923289	2.32416655	0.03314698
OD_group:Genotype:Treatment	6	0.17852128	0.02975355	1.40459345	0.2128092
Residuals	276	5.84651649	0.02118303		

F. Fraction symptomatic on day 4

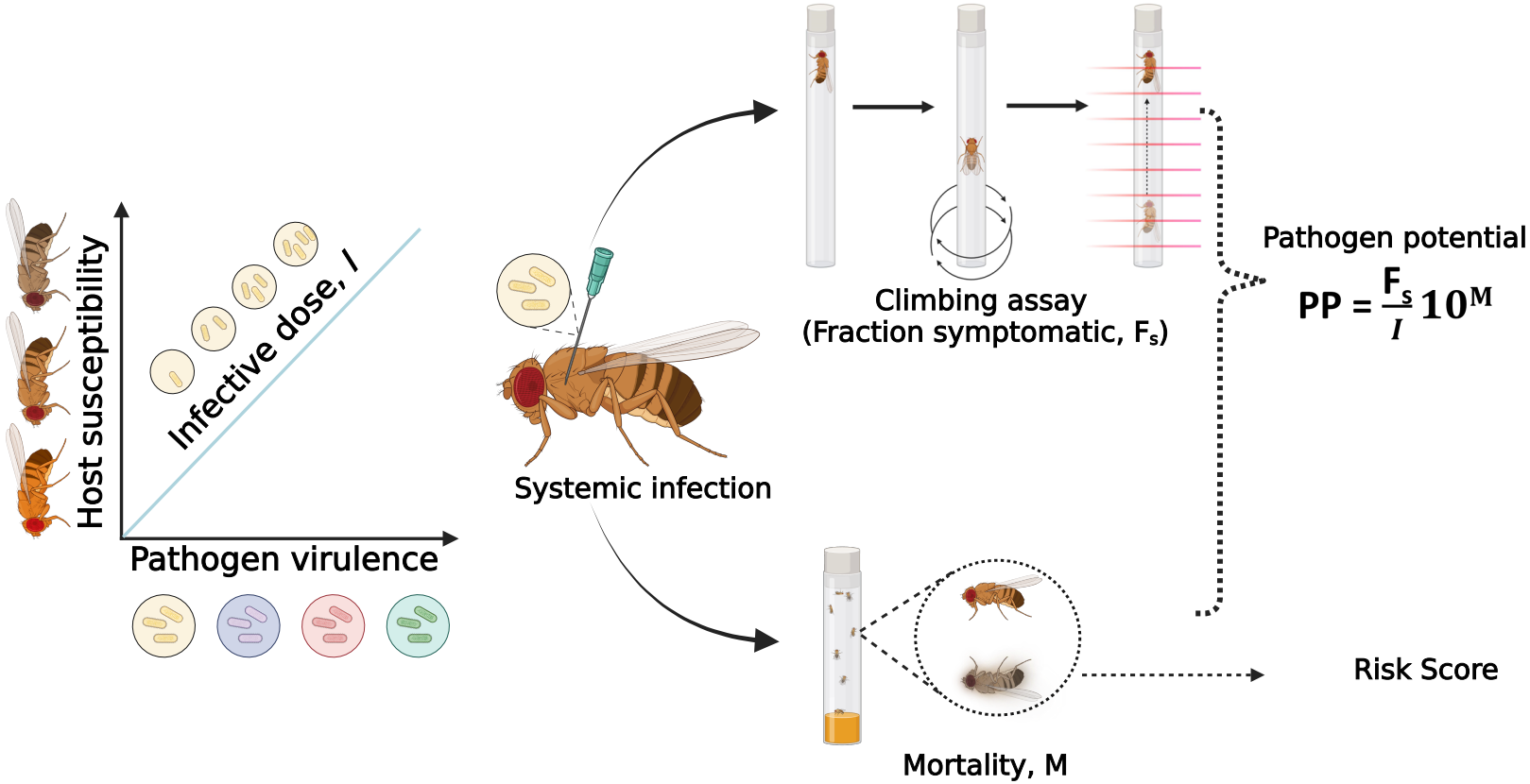
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
OD_group	1	0.4171	0.4171	27.7256	2.81539842813e-07
Genotype	2	0.0733	0.0366	2.4375	0.08925211
Treatment	3	3.3174	1.1058	73.4978	5.73644212150e-35
OD_group:Genotype	2	0.0069	0.0034	0.2324	0.79272734
OD_group:Treatment	3	0.0318	0.0106	0.7062	0.54898427
Genotype:Treatment	6	0.2548	0.0424	2.8228	0.01106243
OD_group:Genotype:Treatment	6	0.1245	0.02075	1.3796	0.22278305
Residuals	276	4.1525	0.0150		

Table S4: Correlation matrix for different measures of pathogenicity containing R values corresponding to Figure 4B.

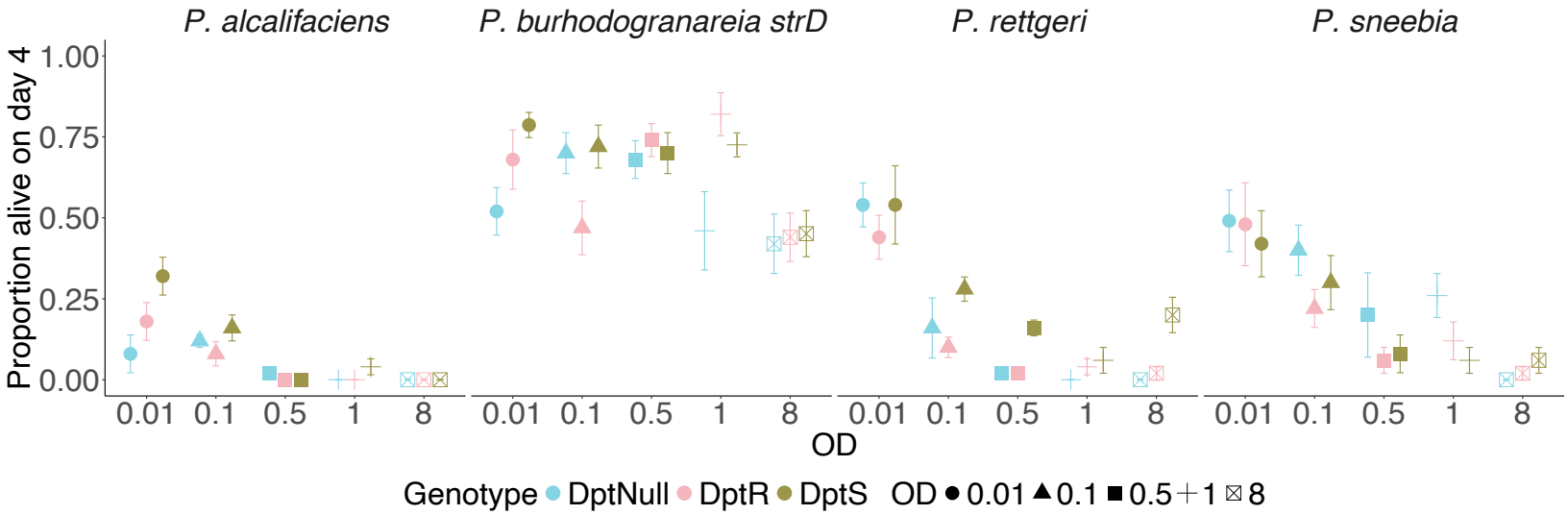
	Fs_Day4	PP_Day4	Prop_Day4_Alive	Risk_Score	Fs_T	PP_T
Fs_Day4	1	0.40802074	-0.8017966	0.65527477	0.68539485	0.27865825
PP_Day4		1	-0.4632633	0.30039245	0.2677697	0.8375262
Prop_Day4_Alive			1	-0.8110406	-0.5595822	-0.3118433
Risk_Score				1	0.5887811	0.2706994

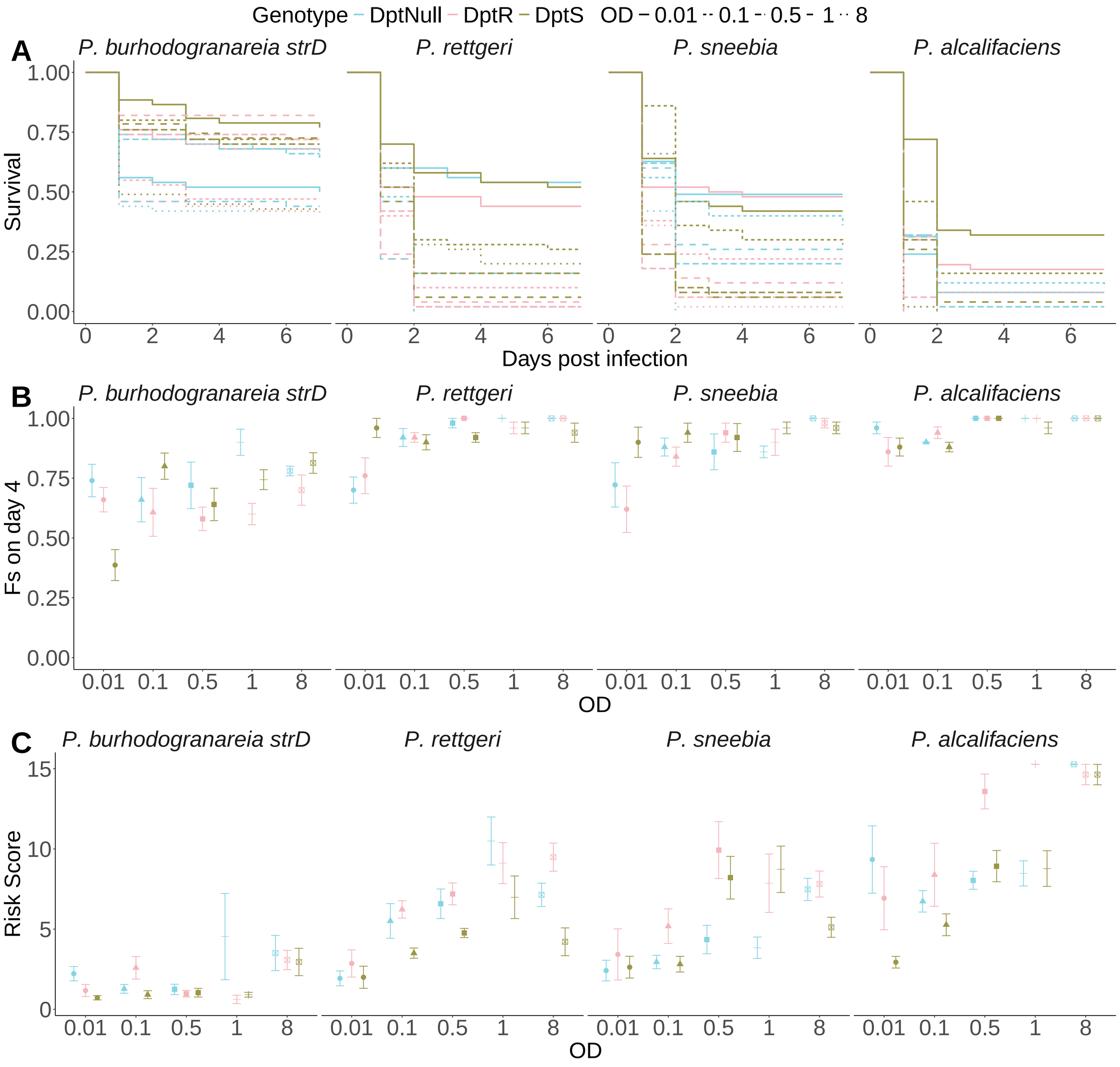
					1	3
Fs_T					1	0.4097006
PP_T						1

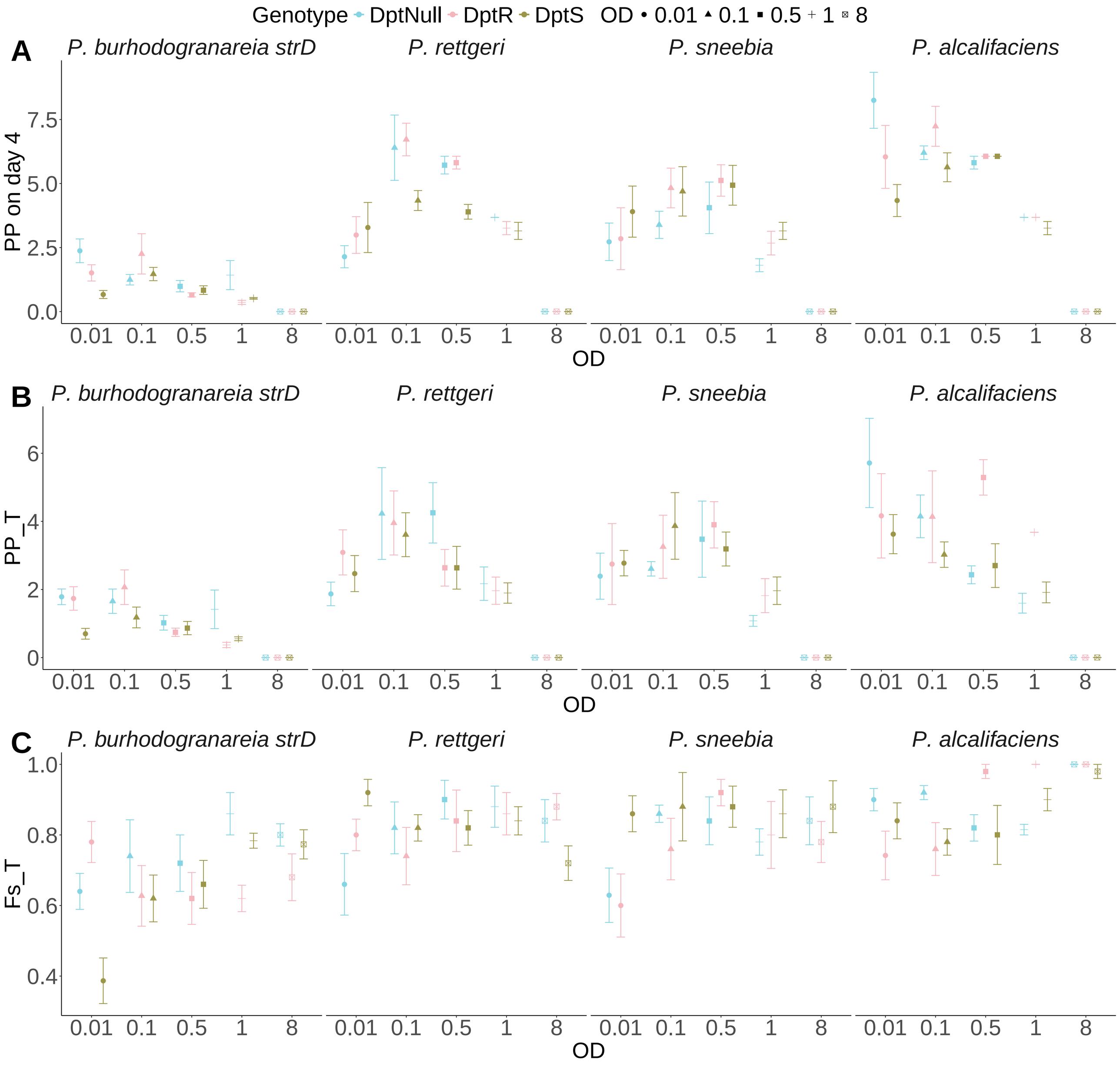
A

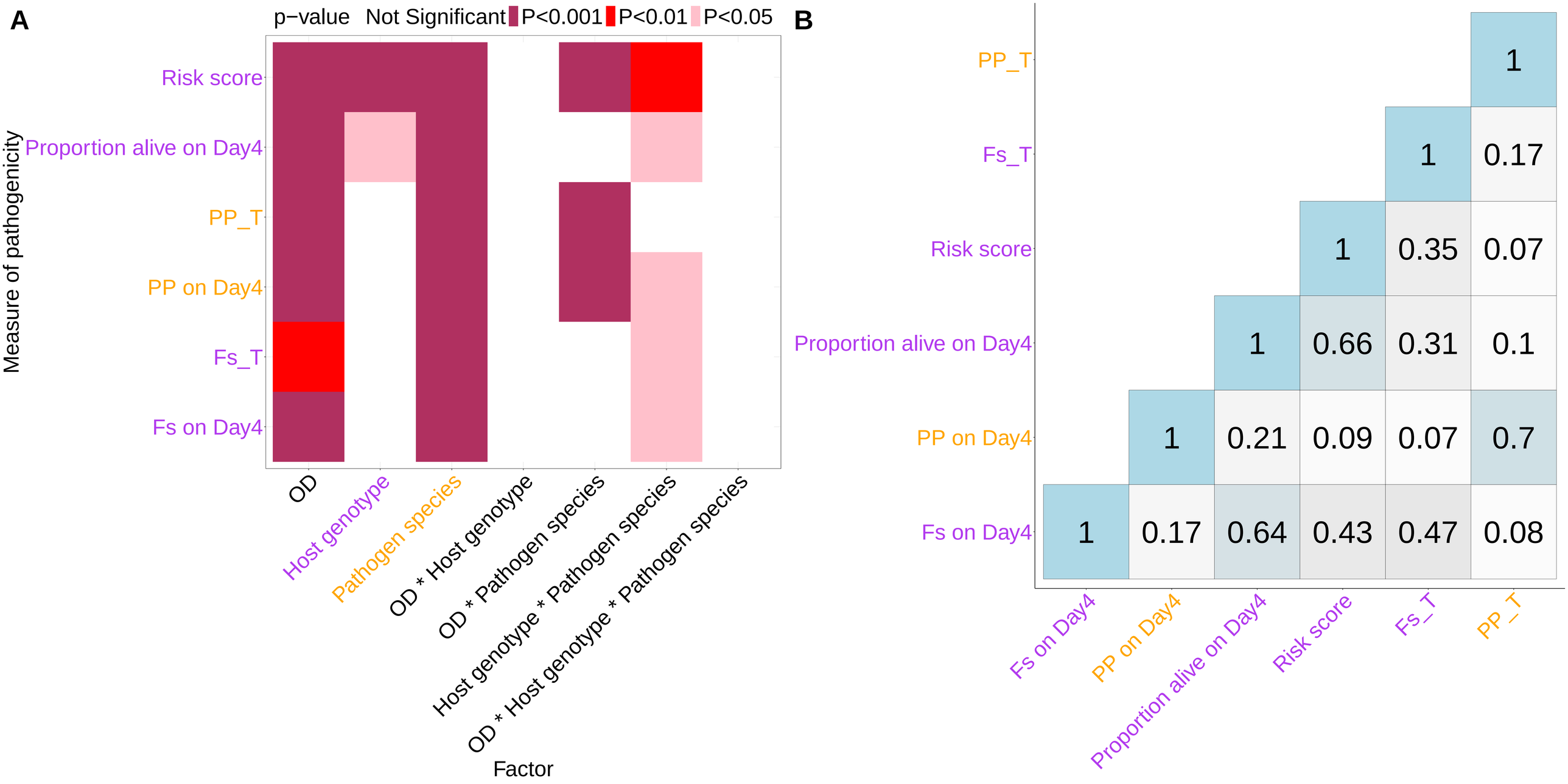

Host susceptibility
Infective dose, I
Pathogen virulence
Systemic infection
Climbing assay
(Fraction symptomatic, F_s)
Pathogen potential
$PP = \frac{F_s}{I} 10^M$
Risk Score
Mortality, M

B


P. alcalifaciens
P. burhodogranareia strD
P. rettgeri
P. sneebia
Proportion alive on day 4
1.00
0.75
0.50
0.25
0.00
0.01 0.1 0.5 1 8
OD
Genotype DptNull DptR DptS
OD 0.01 0.1 0.5 1 8


Genotype DptNull DptR DptS OD 0.01 0.1 0.5 1 8
A
P. burhodogranareia strD
P. rettgeri
P. sneebia
P. alcalifaciens
Survival
1.00
0.75
0.50
0.25
0.00
0 2 4 6
Days post infection
B
Fs on day 4
0.01 0.1 0.5 1 8
OD
C
Risk Score
15
10
5
0


Genotype DptNull DptR DptS OD 0.01 0.1 0.5 1 8
A
P. burhodogranareia strD
P. rettgeri
P. sneebia
P. alcalifaciens
PP on day 4
7.5
5.0
2.5
0.0
0.01 0.1 0.5 1 8
OD
B
PP_T
6
4
2
0
C
Fs_T
1.0
0.8
0.6
0.4


A
p−value
Not Significant
P<0.001
P<0.01
P<0.05
Measure of pathogenicity
Risk score
Proportion alive on Day4
PP_T
PP on Day4
Fs_T
Fs on Day4
OD
Host genotype
Pathogen species
OD * Host genotype
OD * Pathogen species
Host genotype * Pathogen species
OD * Host genotype * Pathogen species
Factor
B
PP_T
Fs_T
Risk score
Proportion alive on Day4
PP on Day4
Fs on Day4
1
1 0.17
1 0.35 0.07
1 0.66 0.31 0.1
1 0.21 0.09 0.07 0.7
1 0.17 0.64 0.43 0.47 0.08
Fs on Day4
PP on Day4
Proportion alive on Day4
Risk score
Fs_T
PP_T