Decomposing virulence: Host and pathogen perspectives on infection

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Introduction

Parasites and pathogens will affect all living organisms at some point in their lives. The negative effects that they have on their hosts might range from severe in the case of an ant infected with *Orphiocordyceps fungus* (Fig. 1), to mild effects such as when we are infected with a cold virus. In evolutionary terms we call the reduction in host fitness caused by an infection the virulence of an infection.

Virulence can be measured as the reduction in host survival after infection. For example, *Myxoma*, which causes the disease myxomatosis, is the deadliest vertebrate virus known, having killed hundreds of millions of rabbits. In a study examining rabbit survival after infection with one of two *Myxoma* viral strains, one strain was more virulent, i.e., resulted in more rabbit mortality, than the other strain (Kerr *et al.* 2022). This tells us that the pathogen strain influenced the virulence of the infection. However, it is a little more complex than this: one strain of rabbit survived infection with both viral strains better than another rabbit strain (Kerr *et al.* 2022). Therefore, there is something about the rabbit that is also determining the outcome of infection.

We can break the pathogen and host contributions to virulence down into two different components (Fig 2; Råberg & Stjernman 2012). From the pathogen side, variation in virulence could be due to exploitation (the ability to grow inside the host) or due to per parasite pathogenicity (PPP; the amount of damage each pathogen does) (Fig. 2a). From the host side,

Fig. 1. An *Acromyrmex octospinosus* ant queen infected by the fungal pathogen *Ophiocordyceps stilbelliformis.* The queen was wild-collected without obvious initial symptoms of the pathogen, and the fungus was identified in the article by Hughes *et al.* (2009).

Photo by S.A.O. Armitage.

one reason why there is variation in virulence is due to differences in how well the host immune system resists the infection, and another is due to host tolerance, i.e., the ability to deal with the damage that an infection causes (Fig. 2b). I will come back to these terms in more detail later.

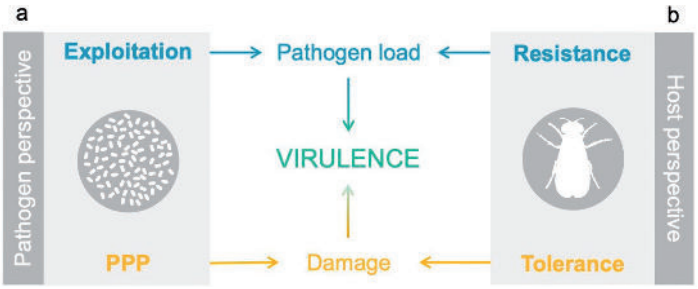

Fig. 2. Decomposing virulence into pathogen and host components. Variation in pathogen and host factors that can influence virulence. (a) From the pathogen perspective, pathogen load is affected by the pathogen's ability to exploit the host, e.g., how well it can grow inside the host. The amount of damage that a pathogen causes, is due to the per parasite pathogenicity (PPP). (b) From the host perspective, pathogen load is affected by the host's ability to resist the pathogen, and damage is affected by the host's ability to tolerate the damage caused by a given infection load. Concepts from Råberg & Stjernman (2012).

Therefore, understanding virulence is more complex than it might at first seem, and it is not trivial to determine the effects that are due to the host and to the pathogen. How can we unpick this problem, and decompose virulence into the host and pathogen contributions? This is a central question that we are working on in my group, and one which I focus on here.

Our model host and pathogens

In my group we are working with the fly *Drosophila melanogaster* as our host, which has long been used as a model for understanding about innate immunity. There are several known pathogens and parasites of this host, and we are mostly working with bacterial pathogens that have been isolated from wild-collected flies.

But how do infections get into the fly in the first place? Infections can occur orally, or they could occur through the cuticle, for example through wounds. Wounding is a widespread phenomenon, found across animals, plants and fungi, and it can have ecological and evolutionary consequences. In insects wounding activates the immune system, but the frequency of wounding in nature is infrequently systematically assessed. Recent work on wild-collected flies by a doctoral candidate in my group, Bengisu Subasi, has shown that wounds or damage can be frequent in nature; for example in the populations that she studied around 31 % of flies were wounded or damaged (Fig. 3; Subasi *et al.* 2024) . Wounds can be due to mites (Fig. 4; Subasi *et al.* 2024), or interactions with predators and conspecifics, and they can have important evolutionary and ecological consequences. Of particular interest to us is the potential for pathogens to enter through these wounds.

In our experiments we usually inject bacteria into the fly to mimic pathogen entry through a cuticular wound in the thorax or abdomen. Pathogens injected in this way can be virulent, i.e., result in mortality for some flies, but other flies can survive for many weeks with a persistent

infection, and others still can clear the infection (Acuña Hidalgo et al. 2022).

Decomposing the pathogen side of virulence: exploitation and per parasite pathogenicity

Now I will come back to our question of how the pathogen and the host affect virulence. I will start by presenting a study that focused on the pathogen side of variation in virulence (Fig. 2a). There are two pathogen components that will affect virulence: first, the so-called infection intensity, which is the pathogen's ability to grow inside the host, or to exploit the host. We measure this as the number of pathogens inside the host. Second, the virulence of the infection will be affected by

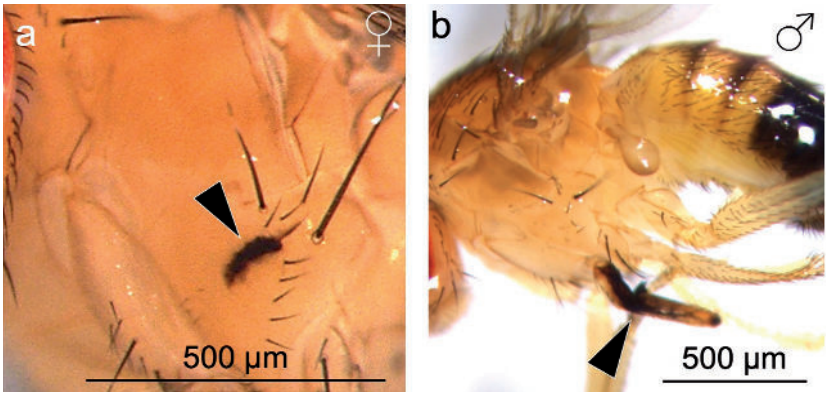

Fig. 3. Examples of wounding in wild-collected *D. melanogaster*. The arrows indicate melanised areas on (a) the thorax of a female and (b) the leg of a male fly. Images from Subasi *et al.* (2024).

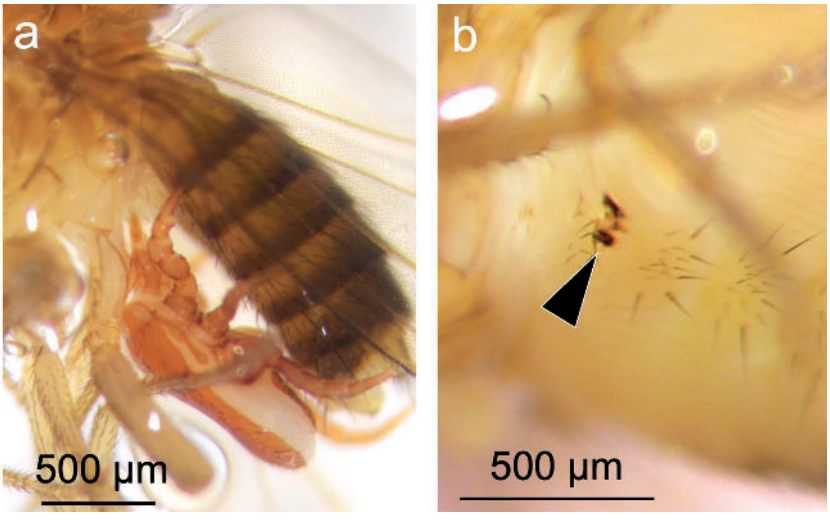

Fig. 4. Mite and wound on wild-collected *D. melanogaster*. (a) *Macrocheles sp.* attached to a female D. melanogaster. (b) Melanised areas (black arrow) that are indicative of wound-healing are visible on the abdomen of a female *D. melanogaster* after removing a mite. Images from Subasi *et al.* (2024).

the amount of harm done by parasites, i.e., PPP. There has been much more focus on exploitation (pathogen numbers) than on PPP, and I here show that PPP can also be used to understand variation in virulence.

In this project we used the framework by Råberg & Stjernman (2012; Fig. 2) in a novel context, i.e., to ask whether we could decompose the factors that affect variation in virulence across pathogen species. Beatriz Acuña Hidalgo and Luís Silva, former doctoral candidates in my group, injected flies with one of three species of bacteria at a range of doses, and survival and bacterial load were then assayed to quantify exploitation and PPP. We found that virulence, depended strongly on the infecting bacteria (Acuña Hidalgo et al. 2022), ranging from low virulence *Enterobacter cloacae* to *P. burhodogranariea*, and to the most virulent bacterium, *Lactococcus lactis* (Fig. 5a). We then asked whether variation in exploitation or PPP explain this gradient in virulence. We found an increase in bacterial load with an increase in virulence (Fig. 5b). Exploitation therefore explains some of the variation in virulence across these species (Acuña Hidalgo et al. 2022). We then tested whether PPP also explains some of the variation in virulence. To do this, we plotted our measure of virulence against the bacterial load. *E. cloacae* caused less harm per pathogen to the host compared to the other two species, because the reaction norm is relatively flat for this species (Fig. 5c). On the other hand, *L. lactis and P. burhodogranariea* caused more harm per pathogen than *E. cloacae* as they have negative slopes, meaning that for an increase in bacterial load there is an increase in mortality.

Altogether these results suggest that *E. cloacae* is less virulent compared to *L. lactis* and *P. burhodogranariea* because

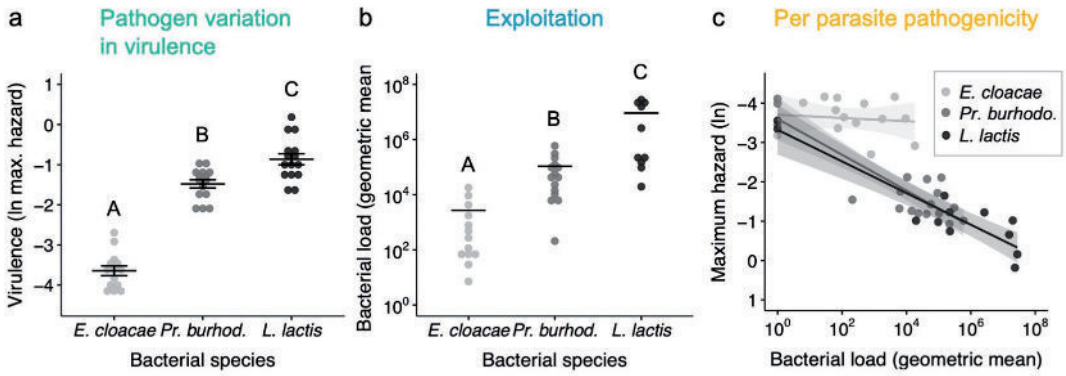

Fig. 5. Decomposing virulence across different bacterial species. (a) Virulence, measured as the inverse of maximum hazard (a value extracted from survival curves) ranges left-to-right from low (*E. cloacae*), to medium (*P. burhodogranariea*) and high *(L. lactis*). (b) Exploitation, measured as the mean bacterial load, increases with increasing virulence. (c) Per parasite pathogenicity, given as the slope of the relationship between maximum hazard and bacterial load, is higher for the two more virulent species, compared to the least virulent species (*E. cloacae*). Letters indicate significantly different treatments. Figure modified from Acuña Hidalgo et al. (2022).

it has lower exploitation and lower PPP. Furthermore, *L. lactis* is more virulent than *P. burhodogranariea* because of higher exploitation of the host (Acuña Hidalgo *et al.* 2022). If we had only considered exploitation as a source of variation, we might have concluded that load alone explains the differences that we found in virulence, which is not the case. We can therefore see that such a decomposition of virulence can provide insight into the causes of variation in virulence. We are missing a potentially huge impact of the pathogen if we only count pathogen numbers and ignore the damage that each of those pathogens can do. Like tolerance, PPP can also give insight into infection (Bertels et al. 2018), and a better understanding of the contributions of PPP and exploitation towards virulence, may help to predict the evolutionary implications of medical treatments.

Decomposing the host side of virulence: resistance and tolerance

I would now like to look at the host perspective on virulence (Fig. 2b). Infection intensity will not only be determined by the pathogen, but also by the host. In particular, the host's immune system, i.e., resistance mechanisms. Resistance reduces the risk of infection and/or the replication rate of the parasite, and such mechanisms might include antimicrobial peptides. We can test for variation in resistance by injecting the same pathogen into different host genetic backgrounds (e.g., Kutzer, Kurtz & Armitage 2018). But there is another aspect to infections, and that is that they can negatively affect host fitness and health, for example, there can be costs of using the immune system, or immunopathology due to infection. Host tolerance describes the ability of the host to limit the negative effects (damage) of a given infection load (Kutzer & Armitage 2016b). More specifically, in the context of the following results, fecundity tolerance describes how many offspring hosts can produce despite a given infection load. Our previous work has shown that variation in fecundity tolerance can be context- and genotype-dependent (Kutzer & Armitage 2016a; Kutzer, Kurtz & Armitage 2019). Resistance and tolerance lead to different ecological and evolutionary interactions between hosts and parasites. Tolerance is important and increasingly now seen as a defence strategy in its own right, for example, it has helped us to understand about HIV infection progression (Regoes et al. 2014). Furthermore, there is selection on tolerance under natural conditions (Hayward et al. 2014), and the evolution of pathogen tolerance might be important in relation to reservoir hosts and emerging infections: tolerance could increase disease circulation and the infectious period (Seal, Dharmarajan & Khan 2021). However, to date, there are no empirical data on the evolution of tolerance.

To address questions related to the evolution of tolerance and resistance, Luís Silva selected flies for tolerance or resistance against a bacterial pathogen. After several generations of selecting the flies, he tested whether there was indeed increased resistance or tolerance in the evolved flies. The selection lines then allowed us to answer the question of how pathogens respond to resistant and tolerant hosts. Resistance has a negative effect on

pathogen fitness and selects for bacteria that can survive the immune response, which might result in them being more virulent bacteria when introduced into a novel host. Tolerance, on the other hand, does not have a negative effect on pathogen fitness and the effect that it has on virulence is not known. It has been predicted theoretically that tolerant hosts could allow pathogens to evolve greater virulence towards non-tolerant or migrant hosts who encounter the pathogen (Miller, White & Boots 2006; Little *et al.* 2010), but we do not know empirically what effect tolerance has on pathogen virulence. Therefore, Luís injected resistant-, tolerant, or control-selected flies with the pathogen. He left the bacteria inside the host for several days, retrieved them, and injected them into the host stock population, which had never seen the pathogen. The results are unpublished, but they allowed us to evaluate whether host tolerance has consequences for the virulence or exploitation of the pathogen, which in turn might have implications for the evolutionary consequences of tolerance. In the future it would be interesting to test the generalisability of our results for other systems, particularly given the interest in designing drugs for improved tolerance to infection.

In summary, virulence is more complex than it might seem at first glance. From the pathogen perspective, the ability to grow/replicate and damage the host are important, and from the host perspective, the ability to reduce the pathogen growth and the damage from the infection are important. Therefore, to fully understand virulence, we need to explore both the host and pathogen perspectives. Challenges for the future include understanding how these four components individually and together affect virulence, and the generalisability across different model systems of the contributions of the components of virulence.

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