Supplementary Discussion for

Toward a mechanistic understanding of competence: a missing link in diversity-disease research

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How do we measure competence?

The general conceptual model

We model the infection pathway as a series of general steps encapsulating host-parasite interactions:

1) The host meets the parasite (or vector) in the environment,
2) the parasite enters the host (or settles on its surface, in the case of an ectoparasite),
3) the parasite develops or multiplies to achieve its transmittable form.

We consider these three steps to represent interaction states and associated transitions (which can be modeled as either rates or probabilities; Stewart Merrill et al. in review). The initial state of exposure is represented by **E** and following exposure, parasites enter their hosts with transition $\boldsymbol{\beta}$. The state of infection is represented by **I** and, once infection is achieved, parasites replicate or develop with transition $\boldsymbol{\lambda}$. Following development and/or replication, the parasite is in its transmittable form, **T**. This model is adapted from a Markov model for host susceptibility developed by Stewart Merrill et al. (in review).

The infection pathway

The model is meant to be generalizable across host-parasite systems, and each state and transition can be tailored to a specific interaction.

- **The exposed state E** may represent a host encountering: an infective stage in the environment; an infectious individual; or an infected vector.
- **The infection parameter $\boldsymbol{\beta}$** may represent: the infective stage crossing from the environment into the host; the active bite by a vector and subsequent injection of infective stages; or the settling of an ectoparasite on host tissue.
- **The infected state I** may represent a host that possesses a parasite in or on its body, that is not yet at a transmittable form (i.e., it has not yet developed or sufficiently replicated to be transmitted or produced transmittable offspring)
- **The development parameter $\boldsymbol{\lambda}$** may represent: morphological development to achieve a transmittable stage; morphological development to achieve an adult stage that can release infective stages; within-host replication to achieve intensity sufficient for transmission (i.e. viral replication to high viremia)
- **The transmittable state T** will vary greatly from system to system depending on whether the host releases all parasite propagules in one event (as with parasitoids and trophically transmitted parasites) or continually sheds parasites for the remainder of its lifespan or the lifespan of its infection (as with pathogens and some parasitic castrators).

How hosts kill parasites

We primarily consider two forms of parasite removal. First, parasites can be removed from the interaction at the point of exposure (**E**) if they prevent the invasion of the parasite (via μ). We call this prevention of infection **barrier resistance**. For environmentally-transmitted and direct contact-transmitted parasites, this can be accomplished with host physical or chemical barriers. In cases where the host must consume the parasite to be infected, these barriers often occur in the gastrointestinal tract. For vector-transmitted parasites, the **E→U** barrier is more likely to be behavioral, consisting of removal of the infected vector before it injects parasite infective stages. The second form of recovery is recovery from infection (**I**) using an internal immune defense (via γ). We call this recovery from infection **internal clearance**. While the specific form of immune action will vary from system to system, the phenomenon of killing parasites and thereby becoming uninfected (**I→U**) is general regardless of the type of parasite. Our conceptual model, provided in **Box 1**, does not demonstrate recovery after the parasite achieves its transmittable form (**T**), but this can occur for pathogen infections (e.g. viruses) and would importantly determine the period of time over which a host could transmit infection. We consider this for West Nile Virus in Supplementary Figure S1. Finally, the **U** class may or may not be susceptible to subsequent infection depending on the form of recovery and whether the host has immunological memory. That is, if an infected host clears an infection (moving from **I→U**) and possesses acquired immunity, they will not be vulnerable to reinfection.

Pre-transmission host mortality

If exposed or infected hosts die at rates that exceed the host's background mortality rate, there can be consequences for the parasite's transmission. Hosts can die at initial exposure (**E→D**, via m_1). For instance, high numbers of infective trematode cercariae can kill amphibian tadpoles as they penetrate tadpole tissue. High densities of feeding ticks have also resulted in exsanguination and death of moose, which precludes the transmission of parasites occurring inside the ticks. This form of mortality may not be common, and when it does not occur, the **E→D** link can be removed (i.e. $m_1 = 0$). Infected hosts can also die while their parasites develop or multiply (**I→D**, via m_2).

How the states and transitions inform competence

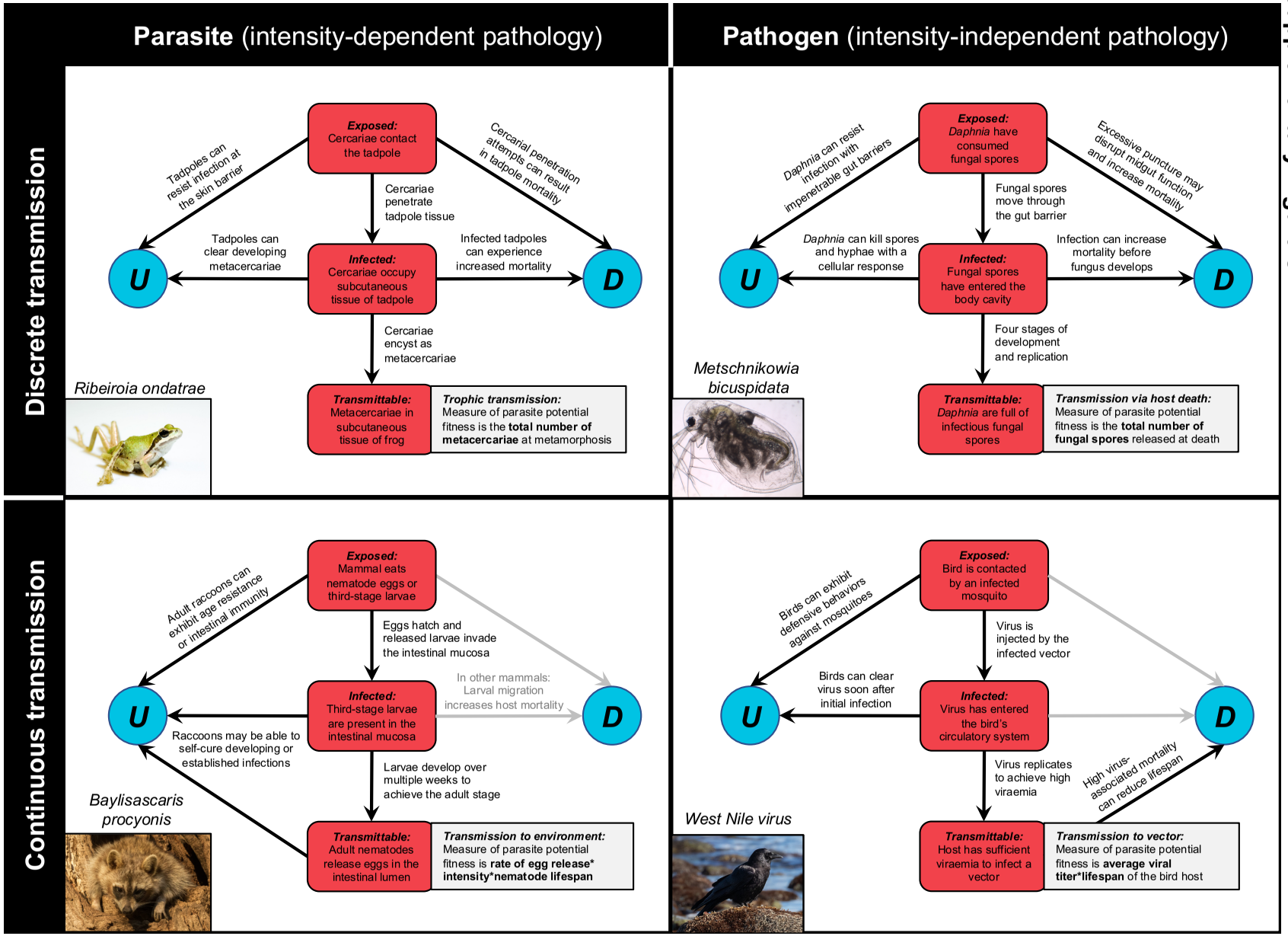
How parasites move through the infection pathway, and how they are removed by host defenses and host mortality, provides insight into the mechanistic basis of competence. Each of the states and transitions in our model can winnow down parasite numbers, from initial exposure to the transmittable state. Identifying which transitions represent the rate-limiting step for parasites provides a target for traits that encompass competence. For example, in Box 2 of the manuscript, we use experimental data to calculate the probabilities of each transition in the conceptual model, and then regress those probabilities against a standard value of competence. Ultimately, the approach showed us that barrier resistance (**E→U**) explains much of the variation in competence.

Applications of the model

We strongly encourage applying the conceptual model to hosts that are experimentally infected in the lab (as done in Stewart Merrill et al. 2020). In that way, one can ensure

that all host-parasite interactions begin in the exposed (**E**) class. By tracking the states of parasites (or hosts) as they progress through the infection process, one can empirically determine the proportion of **E** that become **I**, and the proportion of **I** that become **T** (as well as proportions of **E** and **I** that transition to **U** and **D**). These proportions provide an approximation of the associated transition probabilities. We demonstrate such application of the conceptual model in Box 2A of the manuscript. The mathematical model can also be applied to experimental data to quantify transition rates (and probabilities) using maximum likelihood. In this case, infection state data should be collected at evenly spaced intervals through time (as in Stewart Merrill et al. 2020). It is possible to apply the model to sentinel hosts in the field, or even natural field data. However, this will likely require modifications to the model and system-specific methods to determine which class hosts are in (in many systems, this will require sacrifice and dissection of the host).

Supplementary Figure S1



Supplementary Figure S1

We apply our model to four host-parasite interactions that vary in life history strategy and transmission. These interactions include **typical parasites** versus **pathogens** (columns), which are distinguished by whether the host experiences **intensity-dependent pathology** or **intensity-independent pathology** (Lafferty & Kuris 2002). Intensity-dependent pathology refers to parasites for which the host's pathology increases as a function of the number of infections acquired, whereas intensity-independent pathology refers to pathogens for which the host's pathology results from only one infection event alone. We also include parasites with **discrete** versus **continuous** transmission (rows); that is, whether transmission occurs in one discrete event (as with a parasitoid or trophically-transmitted parasite) or occurs continuously over the duration of the infection. Black arrows and accompanying text indicate transitions that are supported by the literature, where gray lines represent those transitions for which evidence has not been collected. Our application demonstrates that transitions can be removed or added to suit the specific interaction of a given host and parasite.

Ribeioria ondatrae in amphibians: *R. ondatrae* is a trematode typical parasite of amphibians. It causes **intensity-dependent pathology** in the form of limb malformations, with greater numbers of established metacercaria increasing the severity of malformations (Johnson et al. 1999). *R. ondatrae* is trophically-transmitted when the infected amphibian is consumed by an avian definitive host. Hence, metacercariae are transmitted in one **discrete transmission** event. In the infection pathway, amphibians are exposed to free-swimming cercariae which actively penetrate the host's skin. Following their movement from the environment into the host, they encyst as resting metacercariae within the host's subcutaneous tissue. *R. ondatrae* is transmittable once it achieves the metacercariae stage and the tadpole has undergone metamorphosis (because birds consume adult frogs). Both resistance and clearance have been observed in tadpole hosts of *R. ondatrae* (LaFonte & Johnson 2013). Tadpoles of some species have also been observed to die at high rates following exposure or before metamorphosis.

Metschnikowia bicuspidata in plankton: *M. bicuspidata* is an ascomycete fungus that infects freshwater zooplankton, such as *Daphnia*. It causes **intensity-independent pathology**, such that only one fungal spore is required to initiate infection. The spore then undergoes within-host development and replication to produce thousands of new infective spores. *M. bicuspidata* requires host death for its transmission, and spores are released to the environment in one **discrete transmission** event (Ebert 2005). In the infection pathway, *Daphnia* consume fungal spores that are free-floating in the water column. Fungal spores must then penetrate the *Daphnia* gut barrier and enter the body cavity to initiate infection. Then, the spores progress through multiple developmental stages (hypha, sporocyst, conidium, ascus; Stewart Merrill & Cáceres 2018) and replicate to fill the *Daphnia* body with the next generation of fungal spores. *Daphnia* can resist infection at the gut barrier and can clear infection using a cellular haemocyte response (Stewart Merrill et al. 2019). *Daphnia* have been observed to experience

elevated mortality following exposure (potentially due to spores damaging the gut epithelium) and during the course of infection, before the transmittable stage is achieved (Stewart Merrill personal observation). (*Daphnia* photo by Tara Stewart Merrill)

West Nile virus in birds: West Nile virus (a mosquito-transmitted *Flavivirus*) causes disease in humans and is known to infect a variety of bird species as reservoir hosts. The virus causes **intensity-independent pathology**, because the virus replicates inside the host, with increasing viremia causing symptoms and disease. The virus can be transmitted to individual mosquito vectors whenever mosquitoes take blood meals, so the parasite exhibits **continuous transmission** over the duration of the infection. The infection pathway begins when a bird is contacted by an infectious mosquito. During a bloodmeal, the mosquito then injects the virus into the bird host's tissue. The virus replicates internally to produce a sufficiently high viremia that virus will be ingested by a mosquito during a subsequent bloodmeal. At the point of contact with an infectious mosquito, birds can exhibit defensive behaviors (e.g., foot stomps, head movements, and wing shakes; Darbro et al. 2007) to resist infection. Following injection of the virus, some birds can also rapidly clear the virus before it is transmittable to vectors (Komar et al. 2003; Nemeth et al. 2006). The lifespan of the transmittable virus is ultimately determined by the host mortality rate (which can be amplified in species like crows; Komar et al. 2003; Nemeth et al. 2011) or further clearance by the host's immune response. (Photo by Loren Merrill)

Baylisascaris procyonis in raccoons: The nematode, *Baylisascaris procyonis*, causes substantial morbidity and mortality in mammals and birds. Raccoons serve as definitive hosts for the nematode, in which the nematode causes **intensity-dependent pathology**. *B. procyonis* exhibits **continuous transmission**: adult nematodes shed eggs daily in the intestinal tract which are then released to the environment with raccoon feces. The infection pathway begins when a raccoon consumes infective eggs in the environment or third-stage larvae in the tissue of an intermediate host. Following ingestion, the larvae invade the intestinal mucosa where they undergo morphological development to reach the adult stage. The infection becomes transmittable when adult nematodes move to the lumen of the intestine and release eggs. Nematode eggs are not infective to adult raccoons, potentially due to age resistance or intestinal immunity, and some evidence suggests that raccoons are able to self-cure and clear developing infections or adult worms (Kazacos 2001). Although there is limited evidence for parasite-induced mortality of raccoons (Weinstein et al. 2016), it should be noted that larval migrations in the tissue of other mammalian and bird hosts does ultimately result in increased mortality (Kazacos 2001). (Photo by Loren Merrill)

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