Tolerance of infection: a role for animal behavior, potential immune mechanisms, and
consequences for parasite transmission

James S. Adelman^{a,*} and Dana M. Hawley^b

Abstract

Infected organisms can resist or tolerate infection, with tolerance defined as minimizing per-parasite reductions in fitness. Although tolerance is well studied in plants, researchers have only begun to probe the mechanisms and transmission consequences of tolerance in animals. Here we suggest that research on tolerance in animals would benefit from explicitly incorporating behavior as a component of tolerance, given the importance of behavior for host fitness and parasite transmission. We propose two distinct manifestations of tolerance in animals: tissue-specific tolerance, which minimizes fitness losses due to tissue damage during infection, and behavioral tolerance, which minimizes fitness losses by maintaining normal, fitness-enhancing behaviors during infection. Here we briefly review one set of potential immune mechanisms underlying both responses in vertebrate animals: inflammation and its associated signaling molecules. Inflammatory responses, including broadly effective resistance mechanisms like the production of reactive oxygen species, can incur severe costs in terms of damage to a host's own tissues, thereby reducing tissue-specific tolerance. In addition, the signaling molecules involved in these responses also facilitate stereotypical behavioral changes during infection, or sickness behaviors, which include lethargy and anorexia, reducing normal behaviors and behavioral tolerance. We consider how tissue-specific and behavioral tolerance may vary independently or in conjunction and outline potential consequences of such covariation for the transmission of infectious diseases. We put forward the distinction between tissue-specific and behavioral tolerance not as a definitive framework, but to help stimulate and broaden future research by

considering animal behavior in the mechanisms and consequences of tolerance in animals.

Keywords

Pathogen, parasite, infectious disease, disease ecology, ecological immunology, cytokines, Th1/Th2, transmission, sickness behavior, animal behavior

Introduction

When an animal becomes infected, the ensuing suite of immune and neuroendocrine changes can serve to kill off the invading parasite (resistance) or to reduce the negative impacts of infection, without necessarily reducing parasite numbers (tolerance). More specifically, tolerance of infection refers to a host's ability to minimize per-parasite reductions in fitness (survival and reproductive success) (Caldwell et al., 1958; Medzhitov et al., 2012; Råberg, 2014; Råberg et al., 2009; Simms, 2000; Simms and Triplett, 1994). This stands in contrast to immunological self-tolerance, the immune system remaining unresponsive to a host's own healthy tissues (Goldsby et al., 2000). Tolerance of infection is most often measured using a reaction norm approach, specifically the slope of the relationship between fitness and parasite burden (Simms, 2000; Fig. 1). Because measuring fitness directly is not practical in many animal disease systems, studies of tolerance in animals often use health-based proxies in place of fitness, incorporating metrics such as the severity of pathology, mass loss, or anemia (Råberg, 2014). Under this system, regardless of the exact variable placed on the y-axis, more tolerant hosts show a shallower slope than

less tolerant hosts (Fig. 1A,C). Unlike resistance, which maintains fitness by killing parasites, tolerance allows hosts to carry higher burdens of parasites without experiencing large reductions in fitness. This also means that tolerant animals can harbor infection for longer periods of time, potentially moving across larger distances, presenting very different opportunities for the transmission and evolution of parasites (Boots et al., 2009).

Although plant biologists have long studied both resistance and tolerance (e.g., Caldwell et al., 1958; Simms and Triplett, 1994), research into tolerance among animals is a nascent and rapidly developing area (Graham et al., 2011; Jackson et al., 2014; Louie et al., 2016; Medzhitov et al., 2012; Råberg, 2014; Råberg et al., 2009; Råberg et al., 2007). As such, critical questions about animal tolerance remain largely unanswered, notably: what immune mechanisms underlie tolerance and how does tolerance impact the transmission of parasites? Here we argue that to answer these questions most effectively, tolerance in animals should be viewed as manifesting in two principal and distinct ways: 1) tissue-specific tolerance (t_{ts}), which limits fitness losses by reducing tissue damage during infection (Medzhitov et al., 2012), and 2) behavioral tolerance (t_{beh}), which limits fitness losses by maintaining normal, fitness-enhancing behaviors during infection. We define tissue-specific tolerance (t_{ts}) as the slope of the relationship between the severity of tissue pathology (e.g., anemia or infection-induced lesions) and infection intensity (Fig. 2A). This is distinct from t_{beh} , which we define as the slope of the relationship between the expression of normal behaviors and infection intensity (Fig. 2B). This pattern can also be represented as the slope of the relationship between sickness behaviors, the

stereotypical behavioral changes that occur during infection like lethargy and anorexia (see below), and infection intensity (Fig. 2B). Prior work on tolerance in animals has focused almost exclusively on what we would define as tissue-specific tolerance (e.g., Adelman et al., 2013b; Hayward et al., 2014; Jackson et al., 2014; Råberg et al., 2007; Sears et al., 2013), leaving the question of behavioral tolerance largely unexamined.

The core reasoning for this proposed distinction is two-fold: first, the reductions in normal reproductive, social, and defense behaviors during infection can have significant and direct fitness consequences for hosts, including lost opportunities for reproduction (e.g., Avitsur and Yirmiya, 1999; Bonneaud et al., 2003; Ilmonen et al., 2002; Lee et al., 2005; Råberg et al., 2000) and an increased probability of predation (e.g., Adelman et al., *In Review*; Lindström et al., 2003; Männiste et al., 2013; Otti et al., 2012). Thus, there should be strong selection on hosts in many cases to “behaviorally” tolerate infections by minimizing fitness losses associated with changes in normal behavior. Second, decomposing tolerance into tissue-specific and behavioral components can help clarify the impacts of tolerance on the transmission of infectious diseases. There are two principal routes by which tolerance should alter transmission: 1) increasing the amount and/or duration of parasite shedding and 2) increasing the potential for contact among infected and susceptible hosts. The first would be directly affected by t_{ts} , while the second would be directly affected by t_{beh} . Given these potentially distinct manifestations of tolerance, to understand the impacts of tolerance on the dynamics and evolution of animal

diseases, we will need to uncover how t_{ts} and t_{beh} are likely to covary and what mechanisms facilitate the expression of each.

Here we discuss potential mechanisms and transmission consequences underlying t_{ts} and t_{beh} in a particular subset of animals, vertebrates. We focus on vertebrates because several recent studies have pointed to a specific suite of immune responses as particularly likely to modulate tolerance during infection: inflammatory immune responses (Adelman et al., 2013b; Long et al., 2008; Råberg et al., 2009; Sears et al., 2011). These include the acute phase response, which encompasses the recruitment of immune cells to sites of infection, upregulation of potentially damaging defenses like the production of free-radicals, and the whole-organism responses of fever and sickness behaviors (notably lethargy and anorexia) (Adelman and Martin, 2009; Ashley et al., 2012; Hart, 1988; Shattuck and Muehlenbein, 2015; Tizard, 2008). Sickness behaviors are of particular importance, as they carry significant opportunity costs (Adelman and Martin, 2009; Owen-Ashley and Wingfield, 2007), can increase the likelihood of predation (Adelman et al., *In Review*), and have the direct potential to modulate contact rates among infected and susceptible individuals. Specifically, we discuss how signaling molecules associated with sickness behaviors and inflammatory responses, particularly cytokines and their receptors, could play pivotal roles in mediating t_{ts} , t_{beh} , and the links between them. We also present scenarios of how covariation between t_{ts} and t_{beh} should impact the dynamics of different types of infectious diseases, specifically vector-borne versus directly transmitted infections. Incorporating the distinction between t_{ts} and t_{beh} into studies

of animal infectious disease will help inform how tolerance can alter parasite transmission and host-parasite co-evolution in animals.

Animal Behavior as a Component of Tolerance

Because tolerance of infection in animals is a relatively recently studied phenomenon, research in this area has mainly focused on the patterns of fitness losses (or proxies thereof) during infection, without exploring the particulars of how fitness is altered. Notable exceptions, however, do exist. For example, Boots and colleagues' (2009) reviewed how achieving tolerance through longevity versus fecundity should have very different consequences for the parasite fitness (transmission). In addition, Sears et al. (2013) explicitly brought animal behavior into the study of tolerance: tadpoles that were able to behaviorally avoid trematode parasites (through evasive swimming) also altered the principal site of infection, showing less-severe mass loss for a given infection intensity (i.e., higher tolerance).

Although Sears and colleagues' work (2013; 2015) illustrates that animal behavior can impact tolerance, the role of behavior in their experimental system occurs pre-infection. In contrast, Prendergast (2008) introduced the term "behavioral tolerance" to describe changes that occur specifically post-infection. That work, however, focused on a more traditional immunological definition of tolerance—diminished inflammatory immune responses with repeated challenges of the same antigen (akin to self-tolerance)—rather than per-parasite changes in host behavior.

Because the present paper is concerned with tolerance of infection specifically, we define behavioral tolerance (t_{beh}) differently, using the reaction norm approach (Fig. 2). However, several aspects of Prendergast's (2008) behavioral tolerance remain salient for t_{beh} and t_{ts} , particularly the importance of inflammatory immune responses and sickness behaviors.

Several potential concerns arise when using sickness behaviors, or other proxies for fitness, in defining tolerance. First, substituting sickness behaviors, such as lethargy, anorexia, or diminished social behaviors, in place of “normal behaviors” in Fig. 2B, requires only a slight modification: because sickness behaviors represent the opposite of normal, healthy behaviors, we orient the y-axis so that more severe sickness behaviors fall closer to the origin. A steeper slope between sickness behaviors and infection intensity still constitutes lower t_{beh} ; a shallower slope still constitutes higher t_{beh} . A more important complication of replacing fitness with sickness behaviors on the y-axis of Fig. 2B is that some level of these behaviors may serve to promote resistance, for example by conserving energy to be used in costly immune responses like fever (Hart, 1988), or potentially facilitate tissue repair (t_{ts} , Medzhitov et al., 2012). However, because sickness behaviors carry significant opportunity costs, in terms of time not spent performing other fitness enhancing activities (Adelman and Martin, 2009; Hart, 1988; Lopes, 2014; Owen-Ashley and Wingfield, 2007; Shattuck and Muehlenbein, 2015; Tizard, 2008), high levels of sickness behaviors likely reduce fitness. This balance between the costs and benefits of sickness behaviors likely means that the fitness consequences of these behaviors are non-linear (e.g., Lopes, 2014), though for heuristic purposes here, we treat them

as linear. This complication is not unique to sickness behaviors, but should be true for many proxies of fitness, such as mass loss, or any inflammatory mediators that are critical for resistance, and thus increase fitness at low levels, but reduce fitness at high levels (Graham et al., 2005; Råberg, 2014; Råberg et al., 2009; Sears et al., 2011).

It should also be noted that although some parasites can manipulate host behaviors, facilitating transmission to susceptible individuals (reviewed in Moore, 2002), such patterns are compatible with the inclusion of animal behavior in the study of tolerance. Indeed, in many cases, increases in t_{beh} could aid a parasite's transmission, with physiological mechanisms that underlie t_{beh} being coopted by parasites. This would not be the case for all modes of host manipulation, however. For instance, manipulation that reduces or eliminates anti-predator behaviors would effectively reduce t_{beh} , but has been shown to increase trophically-driven transmission (Dobson, 1988; Moore, 2002; Poulin, 1994). Nevertheless, the relationships among t_{beh} and transmission that we explore later (Fig. 3) could be applied in such cases, though with slightly different assumptions.

Although sickness behaviors occur in both vertebrates and invertebrates (Sullivan et al., 2016), the molecular mechanisms underlying their expression have been particularly well studied in vertebrates (Besedovsky and del Rey, 2001; Dantzer and Kelley, 2007; Shattuck and Muehlenbein, 2015). Specifically, many of the same signaling molecules that promote sickness behaviors in vertebrates also promote other inflammatory responses (see below), which can prove damaging to a host's own

tissues, and serve as a potential link to tissue specific tolerance (t_{ts}) (Råberg et al., 2009; Sears et al., 2011). As such, further discussions below will be focused on vertebrate sickness behaviors and immune responses, though uncovering the relationships between t_{ts} and t_{beh} in invertebrates should also be a highly fruitful avenue for future research.

Potential Immune Mechanisms Underlying t_{ts} and t_{beh}

Revealing the mechanisms that underlie t_{ts} and t_{beh} will be important in understanding how these traits might function independently or in conjunction, which in turn will have important consequences for the spread of infectious disease (see “Transmission Consequences of t_{ts} and t_{beh} ”, below). As discussed above, we focus on the potential role of inflammatory signaling in vertebrates for several reasons, including the potential for cross-talk between cellular and molecular effectors of inflammation and behavioral responses to infection. Because several recent papers have reviewed these topics in some depth (e.g., Sears et al., 2011; Shattuck and Muehlenbein, 2015), we do not attempt an exhaustive review here. Rather, we provide a brief overview of immune mechanisms involved in sickness behaviors and inflammation generally, highlighting potential pathways that may be more or less likely to facilitate covariation or independence between t_{ts} and t_{beh} .

For several decades, the field of psychoneuroimmunology has been probing mechanisms associated with behavioral changes during infection, particularly among

vertebrate hosts (Besedovsky and del Rey, 2001; Dantzer and Kelley, 2007; Shattuck and Muehlenbein, 2015). Cytokines, signaling molecules associated with the vertebrate immune system, are chief players in such mechanisms. These peptides are secreted by a variety of cell types, with receptors on an even wider array of tissues (Vilček, 2003), though cells of the immune and neuroendocrine systems are of particular concern in the study of sickness behaviors. Such widespread signaling means that similar sets of molecules can coordinate both local and whole-organism (e.g., behavioral) responses to infection. As such, they provide a critical window into the coordination of t_{ts} and t_{beh} .

Although cytokines as a whole can induce myriad effects, for the purposes of t_{ts} and t_{beh} , we consider two broad classes: pro-inflammatory or anti-inflammatory. Pro-inflammatory cytokines promote responses such as vasodilation, production of reactive oxygen species, fever, and sickness behaviors; anti-inflammatory cytokines rein-in such powerful immune responses before they become overly dangerous to the host's own tissues (Ashley et al., 2012). These two classes of cytokines are often associated with different classes of helper T-cells (Th cells) in the vertebrate immune system. Notably, responses facilitated by Th1 and Th17 cells typically help clear microparasites (e.g., viruses, bacteria) and promote inflammation through the release of cytokines such as interleukin-2 (IL-2), IL-12, tumor necrosis factor- α (TNF- α), IL-17A, IL-22 and interferon- γ (IFN- γ) (Ashley et al., 2012; Glimcher and Murphy, 2000; Mosmann and Coffman, 1989; Ouyang et al., 2008; Romagnani, 2000). Many of these cytokines help promote the activity of phagocytic cells, like macrophages (Romagnani, 2000), which play a critical role in sickness behaviors, producing

cytokines such as IL-1 β , TNF- α , and IL-6, which can act centrally and peripherally to induce sickness behaviors (Shattuck and Muehlenbein, 2015). In contrast to Th1 and Th17 cells, differentiation of Th2 cells typically occurs during macroparasite (e.g., helminth) infection and these cells produce cytokines such as IL-4, IL-10, and transforming growth factor- β (TGF- β), which can have powerful anti-inflammatory effects, including inhibiting the transformation of undifferentiated Th cells into Th1 cells (Ashley et al., 2012; Romagnani, 2000). In addition, Th2 cytokines also facilitate the activity of regulatory T-cells (T_{reg}), which also produce anti-inflammatory cytokines, including IL-10 and TGF- β (Mills and McGuirk, 2004).

The cytokines most strongly associated with sickness behaviors are pro-inflammatory in nature, including interleukin-1 β (IL-1 β), interleukin-6, and tumor necrosis factor- α (TNF- α) (Shattuck and Muehlenbein, 2015), although other powerful pro-inflammatory signals, like interferon- γ (IFN- γ), have been associated with sickness behaviors in some circumstances (Vollmer-Conna et al., 2008). Importantly, receptors for these cytokines exist in the brain (Maier and Watkins, 1999), indicating potential for direct actions in the central nervous system that would promote sickness behaviors. However, there is much debate over how cytokines would enter the brain, given their size and their difficulty crossing the blood-brain barrier. While active transport mechanisms and diffusion across weak points in the blood-brain barrier remain possibilities (Laflamme et al., 1999; Saper and Breder, 1994), interaction with the peripheral nervous system also occurs, with subsequent expression of cytokines in microglia and astrocytes within the brain (Wynne et al., 2009). In particular, the vagus nerve appears to play an important role in communicating the actions of

peripheral cytokines to the central nervous system, as receptors for IL-1 β have been found in the vagal paraganglia (Goehler et al., 1997) and severing this nerve can block sickness behaviors (Bluthé et al., 1994; Hansen et al., 1998).

Sears and colleagues (2011) suggested that animals biased toward Th2/T_{reg}/anti-inflammatory responses would likely manifest more-tolerant phenotypes, given the potential for inflammation to damage a host's own tissues ("immunopathology", Graham et al., 2005). We place such mechanisms under the category of t_{ts} , with reduced inflammatory signaling likely reducing immunopathology and associated fitness losses due to tissue malfunction. Given the extent to which pro-inflammatory signals also facilitate sickness behaviors, the same is likely true for t_{beh} . However, both physiological and behavioral responses to infection can vary greatly across populations, species, season, and environmental or social contexts (Adelman and Martin, 2009; Lochmiller and Deerenberg, 2000; Lopes, 2014; Martin et al., 2008; Owen-Ashley and Wingfield, 2007). Moreover, physiological and behavioral responses do not necessarily change in tandem with such factors (e.g., Adelman et al., 2013b; Aubert et al., 1997; Lopes et al., 2012), though the precise mechanisms by which such differences arise remain unclear. Overall, the common signaling mechanisms that underlie tissue-specific and behavioral inflammatory processes would point to a high likelihood of positive covariation between these two components of tolerance in many cases (Fig. 3A). However, in some systems ecological pressures can impact sickness behaviors without altering other inflammatory responses (e.g., Aubert et al., 1997; Lopes et al., 2012), suggesting that tissue-specific and behavioral tolerance may in some cases act, and therefore evolve, independently of one another (Fig. 3B). This

potential “divorcing” of covariation between tissue-specific and behavioral tolerance may be a universal response to infection in some systems, or may be context-specific, occurring only during times when the maintenance of fitness-enhancing behaviors are particularly critical (e.g., high social stress, breeding season).

Uncovering the potential mechanisms and circumstances in which t_{ts} and t_{beh} vary in tandem or independently would provide critical insight into the transmission consequences of tolerance (Fig 3). A physiological regulatory network (PRN) approach (Cohen et al., 2012) could be highly valuable in uncovering likely molecular targets for covariation of t_{ts} and t_{beh} . Briefly, such approaches consider connections among signaling molecules and their targets in a network topology, allowing identification of highly connected molecules, termed “date-hubs” or “integrators”, which impact a disproportionate number of other molecules and systems (Cohen et al., 2012; Martin et al., *In Press*; Martin and Cohen, 2015; Martin et al., 2011). For example, glucocorticoids, steroids released in response to myriad stressors, both physiological and social, have wide effects on physiology, including reducing pro-inflammatory cytokine production (Borghetti et al., 2009; Sapolsky et al., 2000). As such, these hormones have been put forward as potential integrators, particularly with regard to defense against transmission of infectious agents (Martin et al., 2011). Given their impacts on cytokines involved in both inflammation and sickness behaviors (Borghetti et al., 2009), differences in glucocorticoid production would be likely to lead to concurrent changes in both t_{ts} and t_{beh} .

A PRN approach could also be useful for identifying mechanisms that facilitate independence between t_{ts} and t_{beh} (Fig. 3B). For example, molecules that are less connected in a regulatory network, including those that control the expression of cytokine receptors in different tissues, particularly in the central and peripheral nervous systems (Konsman et al., 2000; Olofsson et al., 2012), might be especially likely to generate independence of t_{ts} and t_{beh} . For example, with three distinct promoter regions controlling tissue-specific expression of the type I IL-1 receptor (in mice and humans, Chen et al., 2009; Ye et al., 1996), myriad possibilities exist for fine-tuning cell-type specific impacts of the pro-inflammatory IL-1 on the nervous system (Ingiosi and Opp, 2016).

Another important way in which t_{ts} and t_{beh} could function independently involves tissue-repair mechanisms that would guard against the physiological damage of inflammatory immune effectors, without impacting sickness behaviors. In such cases, high levels of pro-inflammatory signaling could lead to extreme sickness behaviors (low t_{beh}), though immunopathology could be minimized in tissues via repair mechanisms (high t_{ts}). Although we do not delve into these mechanisms here, Medzhitov et al. (2012) discuss such possibilities in some detail.

Finally, in introducing potential mechanisms impacting t_{ts} and t_{beh} , we have chosen not to separate those that vary within an individual over time versus those that vary across pedigrees, populations, or species. Although differences in tolerance are often depicted as population- or species-level phenomena, immune responses that likely contribute to t_{ts} and t_{beh} have proven plastic with regards to season, social

settings, and ontogeny (Goenka and Kollmann, 2015; Lopes, 2014; Martin et al., 2008). As such, it is highly likely that individual animals express more- or less-tolerant phenotypes at different times (Gervasi et al., 2015; Martin et al., *In Press*) and the mechanisms underlying such differences need not be distinct from those underlying differences at the population or species levels (Sears et al., 2011). Indeed, as studies of animal tolerance move forward, those that manipulate individual phenotypes should be of particular value in identifying mechanisms that facilitate the evolution of t_{ts} and t_{beh} across populations or species (Sinervo and Svensson, 1998).

Transmission Consequences of t_{ts} and t_{beh}

Surprisingly few studies have explicitly explored the transmission consequences of host tolerance. Enhanced transmission from tolerant hosts is implicit in ESS models of host tolerance and resistance (reviewed in Boots et al., 2009), but the way in which transmission dynamics might be influenced by host tolerance across pathogen transmission modes and spatial scales has received almost no attention. It is generally assumed that directly-transmitted pathogens will have higher transmission when hosts exhibit tolerance, because tolerant hosts often harbor high loads of infectious pathogen yet still engage in behaviors relevant for direct transmission (Adelman, 2015; Martin et al., *In Press*). However, the extent of correlation between t_{ts} and t_{beh} within hosts, and the way in which each component of tolerance influences pathogen shedding or contact rates, will be critical for determining resulting transmission consequences.

To conceptualize potential transmission consequences of tissue-specific versus behavioral tolerance, we consider the transmission term β from a standard Susceptible-Infectious-Recovered (SIR) model as having distinct contributions from behavioral factors that influence the contact rate between susceptible and infectious hosts (β_c) and physiological factors that influence the likelihood of transmission given a successful contact (β_p) (Hawley et al., 2011). Prior work has predicted that tolerance (specifically t_{ts} , using our definition) will positively influence components of transmission related to host physiology (β_p), because tolerant hosts can shed high quantities of pathogen (e.g., *Salmonella*; Gopinath et al., 2012) or harbor high loads of pathogen in the blood-stream for vectors to ingest, all while minimizing host mortality (e.g., Råberg et al., 2007). Thus, regardless of transmission mode or spatial scale, t_{ts} would often be predicted to increase β_p (Fig. 3C,K). However, in some cases, increased pathology (i.e., lower t_{ts}) might enhance β_p (Fig. 3G,O). Inflamed, non-tolerant tissues may shed pathogen at higher rates (Doenhoff, 1998; Graham et al., 2005) or may trigger behaviors such as coughing and sneezing which increase the likelihood of transmission given a contact between hosts. For example, house finches with more severe conjunctival lesions from experimental infection with *Mycoplasma gallisepticum* deposit higher relative amounts of this pathogen onto bird feeders (Adelman et al., 2013a), the main route of transmission in the wild (Adelman et al., 2015). Thus, in some systems, pathology itself may enhance transmission, leading to a non-linear or even negative relationship between t_{ts} and the physiological component of transmission, β_p (Fig. 3G,O).

The consequences of behavioral tolerance (t_{beh}) for contact rates, β_c , are likely to depend on the mode and spatial scale of pathogen spread (Fig 3, left). For directly transmitted pathogens, hosts with high behavioral tolerance are able to maintain rates of contact with susceptible conspecifics during infection, resulting in higher β_c , the behavioral component of transmission (Fig. 3D,H). In contrast, hosts with low behavioral tolerance by definition exhibit infection-induced changes in behavior, such as sickness behaviors, that can dampen pathogen spread through a population by reducing rates of contact (Ezenwa et al., 2016). For vector-transmitted pathogens, the effects of behavioral tolerance on transmission are likely to be more complex and dependent on vector behavior. If individuals exhibiting higher behavioral tolerance (t_{beh}) are more likely to exhibit vector avoidance behaviors, which have been shown to prevent successful feeding in many systems (e.g., Cully et al., 1991; Day and Edman, 1983; Waite et al., 2014), then behavioral tolerance would be predicted to result in decreased rates of contact between hosts and vectors (β_c) at local spatial scales (Fig. 3L,P). On the other hand, individuals with high behavioral tolerance would also be more likely to travel longer distances in spite of high loads of pathogen, which could result in higher rates of contact with vectors at regional scales (Fig. 3D,H).

The impact of behavioral tolerance on overall transmission, β , will depend on the extent of correlation between t_{ts} and t_{beh} (Fig. 3, top row) and the way in which each form of tolerance influences the key components of the transmission rate, β_p and β_c (Fig. 3, left two columns). Importantly, if t_{ts} and t_{beh} positively covary (Fig. 3A), and t_{ts} increases β_p (Fig. 3C), then behavioral tolerance will strongly increase transmission for directly transmitted pathogens and for vector-transmitted pathogens

at regional scales (Fig. 3E). In contrast, for vector-borne pathogens at local scales, under the same assumptions, the highest parasite transmission will occur at intermediate levels of behavioral tolerance (Fig. 3M). We predict that malaria infections would follow such patterns: increased t_{ts} should enhance parasite numbers available to feeding mosquitoes (B_p , Fig. 3C,K), whereas increased t_{beh} should enhance mosquito avoidance behaviors and overall locomotion in the host, dampening local transmission (Fig. 3L), but increasing the probability of long-distance movements by the host (Fig. 3H). Patterns would shift for systems in which pathology facilitates transmission (*i.e.*, t_{ts} decreases B_p , Fig. 3G,O), as is likely for *Mycoplasma* conjunctivitis in house finches. For instance, if t_{ts} and t_{beh} are positively correlated in that system (Fig. 3A), we would predict maximal transmission to result from intermediate levels of t_{beh} (Fig. 3I).

The above transmission consequences only apply when t_{ts} and t_{beh} positively covary, but in some cases, sickness behavior can likely be divorced from other physiological responses to infection, leading to no covariation between t_{ts} and t_{beh} (Fig 3B). When both t_{ts} and t_{beh} have similar effects on their respective transmission components (*e.g.*, Fig. 3C,D), a lack of covariation between t_{ts} and t_{beh} only subtly alters the predictions for overall transmission (Fig. 3E vs. 3F; Fig. 3Q vs. 3R). However, when the two types of tolerance have opposing effects on components of transmission (*e.g.*, Fig. 3G,H), the effects of t_{beh} on overall transmission are quite distinct (Fig. 3I vs. 3J; Fig. 3M vs. 3N). Importantly, when t_{beh} can act (and evolve) independently of t_{ts} , higher levels of t_{beh} should always result in increasing transmission probability for pathogens where host movement is critical for

transmission (e.g., directly-transmitted pathogens and vector-borne pathogens at regional scales). Finally, in rare cases, there may even be negative covariation between t_{ts} and t_{beh} , although we do not graphically explore that possibility here. This pattern may occur under conditions that favor terminal investment, for example in semelparous species toward the end of their lives (e.g., salmonids, *Antechinus sp.*), in which any longevity conferred by increased t_{ts} would essentially be wasted, whereas increased t_{beh} could ensure additional matings.

For simplicity, we have limited our consideration of tolerance and transmission to scenarios in which tissue-specific tolerance only influences the physiological component of transmission, β_p (Fig 3, left column). However, there are also interesting possibilities whereby tissue-specific tolerance may impact contact rates relevant for transmission, β_c . For example, if vectors prefer biting asymptomatic individuals, as would be predicted if disease severity acts as a cue of host quality for vectors (Witsenburg et al., 2015), then t_{ts} may result in an increase in β_c even at local spatial scales (Zeilinger and Daugherty, 2014). Here, the specific mechanisms generating t_{ts} and the way in which these mechanisms alter vector preference cues such as host body temperature and production of volatiles will be critical for understanding potential transmission consequences.

Conclusions

Tolerance of infection has only recently been explored as an important response to parasitism among animals and promises to be an exciting avenue of future research. In particular, although all organisms should be capable of mitigating the fitness costs of infection through tissue-specific tolerance (t_{ts}), mitigating these costs through behavior, which we term behavioral tolerance (t_{beh}), is an under explored aspect of tolerance unique to animals. Although many of the mechanisms underlying t_{beh} are likely shared with t_{ts} , including networks that control the release of and sensitivity to pro-inflammatory cytokines, these components of tolerance can have very different consequences for the transmission of parasites and the dynamics of infectious diseases.

Revealing how and when t_{ts} and t_{beh} vary in tandem or independently will be critical in understanding disease spread and the evolutionary dynamics of hosts and their parasites. It is likely that some hosts will favor one strategy over the other, either at ecological or evolutionary time-scales, placing distinct selection pressures on infecting parasites by either facilitating or impeding transmission. Because the consequences of tolerance for host-parasite coevolution can be complex (Best et al., 2014), exploring the unique contributions of t_{ts} and t_{beh} , both empirically and theoretically, may aid in understanding phenomena like the maintenance of diverse host defense strategies and the evolution of parasite virulence.

Acknowledgements

Thanks to Cassandra Nuñez for comments on an earlier version of this manuscript.

This work was supported by the National Science Foundation [grant number IOS-1054675 to DMH]. JSA was also supported by Iowa State University.

References

- Adelman, J.S., 2015. Immune systems: Linking organisms, populations, and evolution through disease, in: Martin, L.B., Ghalambor, C.K., Woods, H.A. (Eds.), Integrative Organismal Biology. Wiley, Hoboken, pp. 169-185.
- Adelman, J.S., Carter, A.W., Hopkins, W.A., Hawley, D.M., 2013a. Deposition of pathogenic *Mycoplasma gallisepticum* onto bird feeders: host pathology is more important than temperature-driven increases in food intake. Biol. Lett. 9, 20130594.
- Adelman, J.S., Kirkpatrick, L., Grodio, J.L., Hawley, D.M., 2013b. House finch populations differ in early inflammatory signaling and pathogen tolerance at the peak of *Mycoplasma gallisepticum* infection. Am. Nat. 181, 674-689.
- Adelman, J.S., Martin, L.B., 2009. Vertebrate sickness behaviors: Adaptive and integrated neuroendocrine immune responses. Integr. Comp. Biol. 49, 202-214.
- Adelman, J.S., Mayer, C., Hawley, D.M., *In Review*. Infection reduces anti-predator behaviors in house finches.
- Adelman, J.S., Moyers, S.C., Farine, D.R., Hawley, D.M., 2015. Feeder use predicts both acquisition and transmission of a contagious pathogen in a North American songbird. Proc. R. Soc. Lond. B. 282, 51429-51429.
- Ashley, N.T., Weil, Z.M., Nelson, R.J., 2012. Inflammation: mechanisms, costs, and natural variation. Ann. Rev. Ecol. Evol. Syst. 43, 385-406.
- Aubert, A., Goodall, G., Dantzer, R., Gheusi, G., 1997. Differential effects of lipopolysaccharide on pup retrieving and nest building in lactating mice. Brain. Behav. Immun. 11, 107-118.

- Avitsur, R., Yirmiya, R., 1999. The immunobiology of sexual behavior: Gender differences in the suppression of sexual activity during illness. *Pharmacology Biochemistry and Behavior* 64, 787-796.
- Besedovsky, H., del Rey, A., 2001. Cytokines as mediators of central and peripheral immune-neuroendocrine interactions, in: Ader, R., Felten, D.L., Cohen, N. (Eds.), *Psychoneuroimmunology*, 3rd ed. Academic Press, San Diego, pp. 1-17.
- Best, A., White, A., Boots, M., 2014. The coevolutionary implications of host tolerance. *Evolution* 68, 1426-1435.
- Bluthé, R.M., Walter, V., Parnet, P., Laye, S., Lestage, J., Verrier, D., Poole, S., Stenning, B.E., Kelley, K.W., Dantzer, R., 1994. Lipopolysaccharide induces sickness behavior in rats by a vagal mediated mechanism. *C R Acad Sci Ser 3 Sci Vie* 317, 499-503.
- Bonneaud, C., Mazuc, J., Gonzalez, G., Haussy, C., Chastel, O., Faivre, B., Sorci, G., 2003. Assessing the cost of mounting an immune response. *Am. Nat.* 161, 367-379.
- Boots, M., Best, A., Miller, M.R., White, A., 2009. The role of ecological feedbacks in the evolution of host defence: What does theory tell us? *Phil. Trans. R. Soc. Lond. B.* 364, 27-36.
- Borghetti, P., Saleri, R., Mocchegiani, E., Corradi, A., Martelli, P., 2009. Infection, immunity and the neuroendocrine response. *Veterinary Immunology and Immunopathology* 130, 141-162.
- Caldwell, R.M., Schafer, J.F., Compton, L.E., Patterson, F.L., 1958. Tolerance to cereal leaf rusts. *Science* 128, 714-715.

- Chen, Q., Zhang, H., Li, Q., An, Y., Herkenham, M., Lai, W., Popovich, P., Agarwal, S., Quan, N., 2009. Three promoters regulate tissue- and cell type-specific expression of murine interleukin-1 receptor type I. *J. Biol. Chem.* 284, 8703-8713.
- Cohen, A.A., Martin, L.B., Wingfield, J.C., McWilliams, S.R., Dunne, J.A., 2012. Physiological regulatory networks: ecological roles and evolutionary constraints. *Trends Ecol. Evol.* 27, 428-435.
- Cully, J.F., Jr., Grieco, J.P., Kissel, D., 1991. Defensive behavior of eastern chipmunks against *Aedes triseriatus* (Diptera: Culicidae). *J. Med. Entomol.* 28, 410-416.
- Dantzer, R., Kelley, K.W., 2007. Twenty years of research on cytokine-induced sickness behavior. *Brain. Behav. Immun.* 21, 153-160.
- Day, J.F., Edman, J.D., 1983. Malaria renders mice susceptible to mosquito feeding when gametocytes are most infective. *J Parasitol* 69, 163-170.
- Dobson, A.P., 1988. The population biology of parasite-induced changes in host behavior. *Q. Rev. Biol.* 63, 139-165.
- Doenhoff, M.J., 1998. Granulomatous inflammation and the transmission of infection: schistosomiasis - and TB too? *Immunology Today* 19, 462-467.
- Ezenwa, V.O., Archie, E.A., Craft, M.E., Hawley, D.M., Martin, L.B., Moore, J., White, L., 2016. Host behaviour-parasite feedback: an essential link between animal behaviour and disease ecology. *Proc. R. Soc. B* 283, 20153078.
- Gervasi, S.S., Civitello, D.J., Kilvitis, H.J., Martin, L.B., 2015. The context of host competence: a role for plasticity in host-parasite dynamics. *Trends Parasitol.* 31, 419-425.

- Glimcher, L.H., Murphy, K.M., 2000. Lineage commitment in the immune system: the T helper lymphocyte grows up. *Genes Dev.* 14, 1693-1711.
- Goehler, L.E., Relton, J.K., Dripps, D., Kiechle, R., Tartaglia, N., Maier, S.F., Watkins, L.R., 1997. Vagal paraganglia bind biotinylated interleukin-1 receptor antagonist: A possible mechanism for immune-to-brain communication. *Brain Res. Bull.* 43, 357-364.
- Goenka, A., Kollmann, T.R., 2015. Development of immunity in early life. *J. Infect.* 71, S112-S120.
- Goldsby, R.A., Kindt, T.J., Osborne, B.A., 2000. *Kuby Immunology*, 4 ed. W.H. Freeman and Company, New York.
- Gopinath, S., Carden, S., Monack, D., 2012. Shedding light on *Salmonella* carriers. *Trends Microbiol.* 20, 320-327.
- Graham, A.L., Allen, J.E., Read, A.F., 2005. Evolutionary causes and consequences of immunopathology. *Ann. Rev. Ecol. Evol. Syst.* 36, 373-397.
- Graham, A.L., Shuker, D.M., Pollitt, L.C., Auld, S.K.J.R., Wilson, A.J., Little, T.J., 2011. Fitness consequences of immune responses: strengthening the empirical framework for ecoimmunology. *Funct. Ecol.* 25, 5-17.
- Hansen, M.K., Taishi, P., Chen, Z.T., Krueger, J.M., 1998. Vagotomy blocks the induction of interleukin-1 beta (IL-1 beta) mRNA in the brain of rats in response to systemic IL-1 beta. *J Neurosci* 18, 2247-2253.
- Hart, B.L., 1988. Biological basis of the behavior of sick animals. *Neurosci. Biobehav. Rev.* 12, 123-137.

- Hawley, D.M., Etienne, R.S., Ezenwa, V.O., Jolles, A.E., 2011. Does animal behavior underlie covariation between hosts' exposure to infectious agents and susceptibility to infection? Implications for disease dynamics. *Integr. Comp. Biol.* 51, 528-539.
- Hayward, A.D., Nussey, D.H., Wilson, A.J., Berenos, C., Pilkington, J.G., Watt, K.A., Pemberton, J.M., Graham, A.L., 2014. Natural selection on individual variation in tolerance of gastrointestinal nematode infection. *Plos Biology* 12.
- Ilmonen, P., Taarna, T., Hasselquist, D., 2002. Are incubation costs in female pied flycatchers expressed in humoral immune responsiveness or breeding success? *Oecologia* 130, 199-204.
- Ingiosi, A.M., Opp, M.R., 2016. Sleep and immunomodulatory responses to systemic lipopolysaccharide in mice selectively expressing interleukin-1 receptor 1 on neurons or astrocytes. *Glia* 64, 780-791.
- Jackson, J.A., Hall, A.J., Friberg, I.M., Ralli, C., Lowe, A., Zawadzka, M., Turner, A.K., Stewart, A., Birtles, R.J., Paterson, S., Bradley, J.E., Begon, M., 2014. An immunological marker of tolerance to infection in wild rodents. *PLoS Biology* 12, e1001901.
- Konsman, J.P., Luheshi, G.N., Bluthé, R.M., Dantzer, R., 2000. The vagus nerve mediates behavioural depression, but not fever, in response to peripheral immune signals; a functional anatomical analysis. *Eur. J. Neurosci.* 12, 4434-4446.
- Laflamme, N., Lacroix, S., Rivest, S., 1999. An essential role of interleukin-1 beta in mediating NF-kappa B activity and COX-2 transcription in cells of the blood-brain

- barrier in response to a systemic and localized inflammation but not during endotoxemia. *J Neurosci* 19, 10923-10930.
- Lee, K.A., Martin, L.B., Wikelski, M.C., 2005. Responding to inflammatory challenges is less costly for a successful avian invader, the house sparrow (*Passer domesticus*), than its less-invasive congener. *Oecologia* 145, 244-251.
- Lindström, K.M., Van der Veen, I.T., Legault, B.A., Lundström, J.O., 2003. Activity and predator escape performance of Common Greenfinches *Carduelis chloris* infected with Sindbis virus. *Ardea* 91, 103-111.
- Lochmiller, R.L., Deerenberg, C., 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* 88, 87-98.
- Long, G.H., Chan, B.H.K., Allen, J.E., Read, A.F., Graham, A.L., 2008. Blockade of TNF receptor 1 reduces disease severity but increases parasite transmission during *Plasmodium chabaudi chabaudi* infection. *Int. J. Parasitol.* 38, 1073-1081.
- Lopes, P.C., 2014. When is it socially acceptable to feel sick? *Proc. R. Soc. Lond. B.* 281.
- Lopes, P.C., Adelman, J., Wingfield, J.C., Bentley, G.E., 2012. Social context modulates sickness behavior. *Behav. Ecol. Sociobiol.* 66, 1421-1428.
- Louie, A., Song, K.H., Hotson, A., Tate, A.T., Schneider, D.S., 2016. How many parameters does it take to describe disease tolerance? *Plos Biology* 14.
- Maier, S.F., Watkins, L.R., 1999. Bidirectional communication between the brain and the immune system: implications for behaviour. *Anim. Behav.* 57, 741-751.
- Männiste, M., Sepp, T., Hõrak, P., 2013. Locomotor activity of captive greenfinches involves two different behavioural traits. *Ethology* 119, 581-591.

- Martin, L.B., Burgan, S.A., Adelman, J.S., Gervasi, S.S., *In Press*. Host competence: an organismal trait to integrate immunology and epidemiology. *Integr. Comp. Biol.*
- Martin, L.B., Cohen, A.A., 2015. Physiological regulatory networks: the orchestra of life?, in: Martin, L.B., Ghalambor, C.K., Woods, H.A. (Eds.), *Integrative Organismal Biology*, pp. 137-152.
- Martin, L.B., Liebl, A.L., Trotter, J.H., Richards, C.L., McCoy, K., McCoy, M.W., 2011. Integrator networks: illuminating the black box linking genotype and phenotype. *Integr. Comp. Biol.* 51, 514-527.
- Martin, L.B., Weil, Z.M., Nelson, R.J., 2008. Seasonal changes in vertebrate immune activity: mediation by physiological trade-offs. *Phil. Trans. R. Soc. Lond. B.* 363, 321-339.
- Medzhitov, R., Schneider, D.S., Soares, M.P., 2012. Disease tolerance as a defense strategy. *Science* 335, 936-941.
- Mills, K.H.G., McGuirk, P., 2004. Antigen-specific regulatory T cells - their induction and role in infection. *Semin. Immunol.* 16, 107-117.
- Moore, J., 2002. *Parasites and the Behavior of Animals*. Oxford University Press, Oxford, UK.
- Mosmann, T.R., Coffman, R.L., 1989. Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. *Ann. Rev. Immun.* 7, 145-173.

- Olofsson, P.S., Rosas-Ballina, M., Levine, Y.A., Tracey, K.J., 2012. Rethinking inflammation: neural circuits in the regulation of immunity. *Immunological Reviews* 248, 188-204.
- Otti, O., Gantenbein-Ritter, I., Jacot, A., Brinkhof, M.W.G., 2012. Immune response increases predation risk. *Evolution* 66, 732-739.
- Ouyang, W., Kolls, J.K., Zheng, Y., 2008. The biological functions of T helper 17 cell effector cytokines in inflammation. *Immunity* 28, 454-467.
- Owen-Ashley, N.T., Wingfield, J.C., 2007. Acute phase responses of passerine birds: characterization and seasonal variation. *J. Ornithol.* 148, S583-S591.
- Poulin, R., 1994. Meta-analysis of parasite-induced behavioural changes. *Anim. Behav.* 48, 137-146.
- Prendergast, B.J., 2008. Behavioral tolerance to endotoxin is enhanced by adaptation to winter photoperiods. *Psychoneuroendocrinology* 33, 540-545.
- Råberg, L., 2014. How to live with the enemy: understanding tolerance to parasites. *PLoS Biology* 12.
- Råberg, L., Graham, A.L., Read, A.F., 2009. Decomposing health: tolerance and resistance to parasites in animals. *Phil. Trans. R. Soc. Lond. B.* 364, 37-49.
- Råberg, L., Nilsson, J.A., Ilmonen, P., Stjernman, M., Hasselquist, D., 2000. The cost of an immune response: vaccination reduces parental effort. *Ecol. Lett.* 3, 382-386.
- Råberg, L., Sim, D., Read, A.F., 2007. Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. *Science* 318, 812-814.

- Romagnani, S., 2000. T-cell subsets (Th1 versus Th2). *Annals of Allergy Asthma & Immunology* 85, 9-18.
- Saper, C.B., Breder, C.D., 1994. The neurologic basis of fever. *New Engl J Med* 330, 1880-1886.
- Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr. Rev.* 21, 55-89.
- Sears, B.F., Rohr, J.R., Allen, J.E., Martin, L.B., 2011. The economy of inflammation: when is less more? *Trends Parasitol.* 27, 382-387.
- Sears, B.F., Snyder, P.W., Rohr, J.R., 2013. Infection deflection: hosts control parasite location with behaviour to improve tolerance. *Proc. R. Soc. Lond. B.* 280.
- Sears, B.F., Snyder, P.W., Rohr, J.R., 2015. Host life history and host-parasite syntopy predict behavioural resistance and tolerance of parasites. *J. Anim. Ecol.* 84, 625-636.
- Shattuck, E.C., Muehlenbein, M.P., 2015. Human sickness behavior: ultimate and proximate explanations. *Am. J. Phys. Anthropol.* 157, 1-18.
- Simms, E.L., 2000. Defining tolerance as a norm of reaction. *Evolutionary Ecology* 14, 563-570.
- Simms, E.L., Triplett, J., 1994. Costs and benefits of plant responses to disease: resistance and tolerance. *Evolution* 48, 1973-1985.
- Sinervo, B., Svensson, E., 1998. Mechanistic and selective causes of life history trade-offs and plasticity. *Oikos* 83, 432-442.

- Sullivan, K., Fairn, E., Adamo, S.A., 2016. Sickness behaviour in the cricket *Gryllus texensis*: comparison with animals across phyla. *Behav. Processes* 128, 134-143.
- Tizard, I., 2008. Sickness behavior, its mechanisms and significance. *Anim. Health Res. Rev.* 9, 87-99.
- Vilček, J., 2003. The cytokines: an overview, in: Thomson, A.W., Lotze, M.T. (Eds.), *The Cytokine Handbook*, 4th ed. Academic Press, London, pp. 3-18.
- Vollmer-Conna, U., Piraino, B.F., Cameron, B., Davenport, T., Hickie, I., Wakefield, D., Lloyd, A.R., 2008. Cytokine polymorphisms have a synergistic effect on severity of the acute sickness response to infection. *Clin. Infect. Dis.* 47, 1418-1425.
- Waite, J.L., Henry, A.R., Owen, J.P., Clayton, D.H., 2014. An experimental test of the effects of behavioral and immunological defenses against vectors: do they interact to protect birds from blood parasites? *Parasites and Vectors* 7, (12 March 2014)-(2012 March 2014).
- Witsenburg, F., Schneider, F., Christe, P., 2015. Signs of a vector's adaptive choice: on the evasion of infectious hosts and parasite-induced mortality. *Oikos* 124, 668-676.
- Wynne, A.M., Henry, C.J., Godbout, J.P., 2009. Immune and behavioral consequences of microglial reactivity in the aged brain. *Integr. Comp. Biol.* 49, 254-266.
- Ye, K., Vannier, E., Clark, B.D., Sims, J.E., Dinarello, C.A., 1996. Three distinct promoters direct transcription of different 5' untranslated regions of the human interleukin 1 type I receptor: A possible mechanism for control of translation. *Cytokine* 8, 421-429.

Zeilinger, A.R., Daugherty, M.P., 2014. Vector preference and host defense against infection interact to determine disease dynamics. *Oikos* 123, 613-622.

Figure Captions

Figure 1. Tolerance and resistance can be depicted graphically, with tolerance represented as the slope of the relationship between infection intensity and fitness and resistance represented as the extent of infection intensity (Simms 2000, Raberg et al 2009). Individuals, populations, or species can differ in tolerance only (A), resistance only (B), or both (C).

Figure 2. Tolerance of infection, broken down into proposed components of behavioral tolerance (A, t_{beh}) and tissue-specific tolerance (B, t_{ts}). It should be noted that in the case of t_{beh} (A) high values of sickness behaviors will fall near the origin of the graph, indicating low t_{beh} , while low values fall at the extreme of the y-axis, indicating high t_{beh} . In both cases, a steeper slope constitutes lower tolerance; a shallower slope constitutes higher tolerance.

Figure 3. The impact of behavioral tolerance, t_{beh} , on the overall transmission of parasites, β , will depend upon its relationship with tissue-specific tolerance, t_{ts} , (top) and the ways in which t_{beh} and t_{ts} influence host-host or host-vector contact rates, β_c , and the probability of successful transmission given a contact, β_p (left two columns). Predicted transmission rates when t_{ts} and t_{beh} show positive correlation (A) are depicted with broad dashes (E,I,M,Q); transmission rates when t_{ts} and t_{beh} show no correlation (B) are depicted with short dashes (F,J,N,R). Grey rows indicate circumstances in which t_{ts} increases β_p (C,K), for example by allowing higher pathogen loads without host mortality. White rows indicate circumstances in which t_{ts}

decreases $\beta_p(G, O)$, as would be the case when pathology is required for successful pathogen shedding.

Fig.1

Fig. 2

Fig. 3