**SIGNIFICANCE.**

***Duration of infection is a determinant of both individual health and public health*.** The number of days, weeks, months, or even years that an infection persists in a host has important health implications on multiple biological scales. For an infected individual, for example, the time it takes to clear an infectious agent can affect the likelihood that the infection will become lethal (e.g., for pneumonia {Aston, 2019 #7832}), the cumulative severity of symptoms such as anemia (e.g., during chronic malaria {Chen, 2016 #7833}), and the amount of tissue damage that must be repaired if the individual is to recover (e.g., from viral hepatitis {Wursthorn, 2008 #7834} or gastrointestinal helminthiasis {Pearson, 2012 #7835}). The work of physicians therefore tends to be easier when duration of infection is short.

Equally striking is the impact of infection duration on the population scale: the longer individuals are infected, the longer they tend to be infectious to others, whether by persistent coughing (e.g., for tuberculosis; {Jones-Lopez, 2016 #7836}), spreading of fecal matter (e.g., by Typhoid Mary {Marineli, 2013 #7837} and modern analogues {Prasad, 2018 #7838}), or availability of transmissible propagules to biting vectors (e.g., for malaria {Vallejo, 2016 #7839}). Accordingly, infection duration and its inverse, clearance rate, are canonical parameters in models of dynamic epidemiology {Anderson, 1979 #21;Anderson, 1991 #28;May, 1979 #373}. For example, the mean and variance of infection duration in a population predict epidemic outbreak risk (e.g., for norovirus {Milbrath, 2013 #7840}) and ease of control by public health interventions (e.g., for sexually-transmitted {Robinson, 2012 #7841} and vector-borne {Childs, 2015 #7842} infections). The work of public health officials – in blocking spread of infections from one individual to the next – therefore tends to be easier when the duration of infection is short.

***Despite these profound individual- and population-scale impacts upon human health, we are unable to predict infection duration,* a priori*, for any host-parasite system.*** This lack of predictive ability means that we still do not understand important aspects of the immune-parasite interaction that are yet to be uncovered. Infection duration will be shaped by the dynamic interactions between host defenses and parasite attacks, which are in turn influenced by both host and parasite genetics. For example, “resistant” host genotypes clear parasites more rapidly than “susceptible” genotypes do, often via powerful immune responses that mobilize appropriate effector mechanisms (e.g., secretion of mucus by goblet cells, accelerated epithelial turnover, and peristalsis to expel gastrointestinal nematodes {Grencis, 2015 #7699}). Furthermore, parasite genotypes that immunosuppress (e.g., {D'Elia, 2009 #7671}) or metabolically co-opt (e.g., {Sondberg, 2016 #7844}) the host most vigorously often generate infections of longest duration.

Indeed, infection duration is known to vary among host genotypes (e.g., {Wahid, 1989 #7846;Venkatesan, 1993 #7848;Pullinger, 2007 #7849;Brown, 2016 #7850}), parasite genotypes (e.g., {Tong, 2007 #7851;Trottier, 2008 #7852;Pichette-Jolette, 2019 #7853}), and to depend, in some cases, on the combination of host and parasite genotypes (e.g., GHxGP interactions for duration {Andrade, 1990 #7854;Prentice, 2014 #7855}). Considerable effort has been expended to understand the molecular and cellular mechanisms behind the effects of host and parasite genetic backgrounds, but these efforts have not yet produced a framework that allows prediction of infection duration, in large part because **genetics (even GHxGP interactions) are not the only driver of variation in infection duration**.

**Infection duration often varies with dose** (e.g., {Ebert, 2000 #2262;Paterson, 2008 #2048}). Even more perplexing, the change in duration with dose often varies among host genotypes. For example, mouse strains described as “resistant” due to their ability to clear a high dose of nematodes become chronically infected when exposed to a low dose, whereas other mouse strains are chronically infected regardless of dose {Bancroft, 1994 #7858;Bancroft, 2001 #7857;Else, 1994 #7859}. More vexing still, infection duration often varies among individuals of a given genotype exposed to the same parasite dose, even in controlled experiments {Robinson, 1989 #7847;Wahid, 1993 #7845;Wahid, 1989 #7846}, and even with clonal isolates of parasites (such as rodent malaria {Fenton, 2008 #1574} or streptococcus {Gingles, 2001 #1585} infections). The variation in infection duration among individual hosts of the same genotype can be as large as the variation among host genotypes (e.g., {Saccareau, 2017 #7860}). This variation among individuals is typically ignored, treated as unwanted noise that potentially obscures differences among treatment means, rather than as an object of study in its own right. **Yet if our clinical goal is to be able to predict infection duration, we must grapple with this within-genotype variation and determine its causes.**

**Infection duration often varies with host environment** (e.g., Scott 1991, 1996, Leung 2018).For example, our experiments with inbred mice infected with the nematode *Trichuris muris* show that infection durations observed under natural conditions do not match those found in the lab: when inbred mouse strains are “rewilded” by moving them into outdoor enclosures before infection, clearance is drastically reduced (Fig. 1). Moreover, the variation in infection duration among individuals also varies with host environment: mice exposed to the natural environment only prior to infection (“short-term wild”) had more variable parasitemia than those that spent the entire infection in the lab or in the wild (Fig 1).

By definition, infection duration is determined by the dynamics of parasite growth and mortality: the infection lasts until the immune response kills or expels the parasite, or, failing that, until the parasite dies. Parasite growth and mortality are determined by the interactions between the immune system and the parasite. These essentially ecological interactions are complex, as evidenced by the contingent responses of infection duration to changes in host or parasite genetics, parasite dose, and host environment (as reviewed above). **Here, we propose to use infectious dose to “probe” the dynamics of the immune-parasite interaction across environments, thereby revealing the fundamental biological processes that drive variation in infection duration (Fig. 2).** To accomplish this, we will develop and test novel mathematical theory to quantify the positive and negative feedbacks that regulate host-parasite interactions, with the goal of improving prediction of infection duration, in both controlled lab settings and under greater environmental realism. We will focus on the biology of gastrointestinal nematode parasites. Our emphasis on nematodes is pragmatic: nematode infection duration varies widely even in controlled conditions (e.g., some hosts take days, others take years to purge worms) and infection duration is strongly correlated with morbidity in human GI nematode infections {Chan, 1994 #6982}. Our aims will address within-host processes that drive varied infection duration across host genotypes, among individuals within a genotype, and as hosts move into a more natural environment, revealing general principles that likely apply to many infectious disease systems.

**INNOVATION.**

Our primary innovations are: A) to build a novel mathematical model of “Allee effects” that uniquely enables prediction of a broad continuum of infection durations; B) to test the model using inoculating dose as an experimental probe; and C) to field-test the model in an unparalleled natural system; we will thus generate one of the most complete time-series of within-host ecological dynamics ever assembled. Our combined expertise in mathematics, immunoparasitology and fieldwork enables a powerful approach to explaining variation in duration not attributable to genetics but instead arising from within-host ecological dynamics.

In particular, we introduce the ecological concept of **Allee effects** to explain how within-host dynamics lead to variation in infection duration. Allee effects arise when positive feedback loops generate a positive relationship between *per-capita* growth rate and population density. **The key dynamical signature of Allee effects are persistence thresholds** (e.g., {Nelson, 2008 #7830;Tobin, 2011 #7829}): when density is below the threshold, the population declines to extinction; above it, the population persists. Near the threshold, subtle differences in system state can produce strikingly different persistence times. Positive feedback loops are likely to be ubiquitous in immune-parasite systems: e.g., the positive feedback between cytokine production and T cell activation means that the per-cell growth rate of a T cell population increases as more T cells are activated; positive feedback between parasite biomass and immunomodulation means that the per-gram growth rate of the parasite may increase with biomass (e.g., due to escalating manipulation). Our preliminary results suggest that including such positive feedback mechanisms into mathematical models of the immune-parasite interaction generates Allee effects for both the parasite and the immune response (Fig. 3). **Allee effects** **might be the key to predicting when parasites will “go extinct” within the host.**

To address this novel idea, we will work in parallel, developing mathematical theory for Allee effects in host-parasite interactions and testing the predictions of theory using innovative experimental approaches. We will pair conventional immunoparasitological dose-response experiments in the lab with **experiments in** **a unique outdoor system that enables us to control host genetics and inoculating dose while titrating in natural variation** (e.g., in gut microbes) likely to modulate within-host dynamics. Recent studies have highlighted the divergence between immune phenotypes of wild and laboratory mice {Abolins, 2017 #7747;Abolins, 2018 #7746} and advocated the virtues of naturalizing mice in various ways {Beura, 2016 #7712;Leung, 2018 #7710;Reese, 2016 #7721;Rosshart, 2019 #7801;Rosshart, 2017 #7728}. This includes our own “rewilding” approach. As outlined below, our preliminary data show that **rewilding mice for even a couple of weeks prolongs nematode infections**. We hypothesize that this increase in duration arises because rewilding pushes the system across a mathematical persistence threshold: a parasite Allee effect in action.

**APPROACH.**

Our approach combines the power of mathematical ecology with the tractability of mouse genetic models in immunoparasitology, to illuminate causes of varied infection duration for gastrointestinal helminths. We first argue that our mathematical approach will fill a knowledge gap in within-host dynamics, unify previous insights and resolve previously unexplained variation in infection duration. We then address unique suitability of our team for this undertaking before explaining how we will use mathematics and empirical data on mouse-nematode interactions to address each of our Aims in turn.

***Do feedbacks in within-host ecology predictably determine infection duration?*** This is the core question motivating this proposal. And we have good reason to suspect that the answer is yes! Recent empirical work has suggested that the within-host interaction between parasites, immunity, and host physiology can generate a dynamical threshold between acute and chronic infections. For example, during experimental infections of fruit flies {Duneau, 2017 #7861} and flour beetles {Tate, 2017 #7703}, the duration of infection was acute in some insects, and chronic in others, despite stringent controls.Analyses of these datasets indicate that **variation in infection duration was caused by** **subtle differences in the initial rates of immune response induction and parasite replication**; such subtle dependence on initial conditions is a hallmark of Allee effects and indicative of strong feedback mechanisms.

Working with **collaborators Professor Anieke van Leeuwen and Professor Sarah Budischak**, we recently developed and parameterized a mathematical model of within-host ecology of gastrointestinal helminths that can reproduce this threshold in infection duration {van Leeuwen, 2019 #7862}. Unlike all previous mathematical modeling approaches, **whether an infection is acute or chronic is an *emergent property* of the within-host ecological dynamics in our model** (instead of being pre-programmed into the math, as in the past (e.g., {Alizon, 2008 #7823;Fenton, 2006 #2337})**.** Our result is due to an Allee effect in parasite growth, driven by parasite manipulation of resources: when parasites wrest control of resources, they tip the system towards chronic infection; when they don’t, infection is acute.

***We propose that within-host Allee effects are a general property of host-parasite interactions and that by quantifying the feedbacks that give rise to them, we can explain variation in infection duration among individuals and environments***.To test this proposal, we will build on the conceptual framework of van Leeuwen et al. (2019) by developing and validating a model that encompasses key immunological feedbacks and then testing its predictions experimentally, as follows, on mice (*Mus musculus*) infected with whipworms (*Trichuris muris*), drawing upon the expertise of our **collaborators, Professor Kathryn Else and Professor Richard Grencis**.

Within-host ecology is characterized by both positive and negative feedback loops (**Fig. 2A**). Most obviously, there is a negative feedback loop between parasite growth and the immune response: parasite biomass is reduced by an effective immune response; reduction of parasite biomass then reduces immune stimulation. For gastrointestinal nematodes, a Type 2 immune response promoted by T-helper (Th) 2 cells is effective, whereas neither a Th1 response nor a regulatory T cell (T-reg) response leads to clearance of worms {Grencis, 2015 #7699}. Positive feedback loops are also ubiquitous within the immune system itself, and can act to drive clearance or chronicity. For example, cytokine production drives activation of T-helper cell populations that then secrete those same cytokines and suppress secretion of opposing cytokines (e.g., Th2 cells promoting Th2 while inhibiting a Th1 response, and vice versa {Yates, 2000 #1305;Yates, 2004 #1315}). If a Th2 response is launched and propogated, these feedbacks are clearance-promoting; on the other hand, if the worm “gets the upper hand” via immunomodulation (e.g., by promoting either a T-reg response {Harnett, 2014 #7803} or a Th1response, as *T. muris* does {Bancroft, 2001 #7857;Bancroft, 2019 #7863;Else, 1994 #7859}), these same feedbacks become chronicity-promoting, allowing the parasite to grow more rapidly and gain further control, delaying clearance.

It is easy to imagine that changing the host environment can shift infection duration by altering the strength of feedback processes.

However, identifying which processes are most affected would remain an important challenge. Fortunately, mathematical theory provides some essential insight. If negative feedbacks dominate the dynamics of the system, then infection duration would be expected to vary smoothly with changes in the environment, and changes in the initial state of the system should have little effect on duration (Fig. 2B; Strogatz 2000, Angeli 2004). Mathematically, the system would have a single stable attractor and Allee effects would not occur. However, if positive feedbacks dominate, then infection duration will exhibit threshold (“tipping point”) behavior, and the system will exhibit multistability. In such a system, duration can change suddenly if a tipping point is crossed, and changing the initial conditions (e.g., dose) can lead the system towards different attractors, the hallmark of Allee effects (Fig. 2C). **Currently, it is unknown whether Fig. 2B or C is a better characterization of the dynamics of infection duration, and thus we do not know which processes are the most important drivers of infection outcome.**

We have found that models that include both negative- and positive-feedback mechanisms will always have the potential to produce Allee effects. For example, **Fig. 3** shows outcomes for a simple model, inspired by *T. muris*. The model includes all of the negative and positive feedbacks indicated in Fig. 2a, including self-promotion of Th1 and Th2 response, Th1-Th2 cross-inhibition, and parasite promotion of Th1 and Th2 responses. **Analysis of this model reveals several important truths.** First, multistability is rampant. Examination of the Th2-parasite subsystem reveals bistability between acute and chronic infections (Fig. 3A), and examination of the Th1-Th2 subsystem reveals multistability between a variety of empirically observed immune states, including (critically), Th1 and Th2 polarization (Fig. 3B). Second, observed infection outcomes depend on the strength of the clearance-promoting and chronicity-promoting processes driving immune dynamics. Third, variation in the initial dose of parasites or the initial state of the immune system can lead to variation in infection duration and reveal which feedback processes are most important to system dynamics. In particular, If clearance-promoting feedback loops are stronger than chronicity-promoting loops and the immune system is initially Th1-biased, then low doses will lead to a chronic infection but high doses will be cleared (C1, moving from gray to black); if the immune system is initially Th2-biased, then infections will be cleared rapidly, regardless of dose (D1). If chronicity-promoting feedback loops are stronger than clearance-promoting loops and the immune system is initially Th1-biased, then chronic infections occur regardless of dose (C2); if the immune system is initially Th2-biased, then low doses are cleared, but high doses will lead to a chronic infection (D2).

***Dose-dependence of trichurid nematodes is a powerful model system for duration studies***. To determine whether the theoretical results depicted in **Fig. 2-3** can help shed light on the results observed in **Fig. 1**, we will use mouse strains that differ in resistance against *Trichuris muris*, a natural gastrointestinal nematode parasite of mice {Else, 1988 #7856;Hurst, 2013 #7679;Klementowicz, 2012 #7672}. *Trichuris spp*. (whipworms) are transmitted via the fecal-oral route and inhabit the caeca of many mammals {Hansen, 2013 #7665}. They burrow into the epithelium and, at high burdens, cause host wasting (e.g., *T. trichiura* in people {Tshikuka, 1997 #6339}). The *T. muris* system thus balances experimental tractability with global health relevance.

As in many helminth infections, rapid clearance of *T. muris* requires the development of a Th2-polarized immune response, and chronicity is associated with dominance of other T-helper subsets, especially Th1 {Bancroft, 2001 #7857;Bancroft, 2019 #7863;Else, 1994 #7859}. Th2 cells coordinate the activation of effector mechanisms such as mucins and antibodies that purge nematodes from the gut, whereas Th1 cells promote ineffective mechanisms such as phagocytosis {Grencis, 2015 #7699}. The polarization of T helper cell phenotype is directed by cytokines such as interferon (IFN)- (for Th1) and interleukin (IL)-4 (for Th2) which induce master regulator transcription factors T-bet (for Th1 polarization) and GATA-3 (for Th2 polarization) {van den Ham, 2008 #7806}. Induction of the master regulators begets further production of IFN- or IL-4, setting off feedback loops that ultimately polarize T-helper populations into Th1 or Th2, respectively {Schrom, 2017 #7827;Yates, 2004 #1315}. The nematodes, unsurprisingly (given that Th1 promotes worm survival), secrete and excrete products that immunomodulate the host {Eichenberger, 2018 #7864} into deploying Th1- rather than Th2-associated effectors (e.g., {Cliffe, 2005 #1937}), including a recently described, highly abundant protein (p43) that ablates a key Th2 effector cytokine, interleukin(IL)-13 *in vitro* and *in vivo* {Bancroft, 2019 #7863}. **Host-parasite battles over Th2ness, especially downstream effectors that actually clear the worms, thus appear a likely determinant of infection duration in this system.**  Our preliminary experimental work (depicted in **Fig. 1**) suggests that moving mice from the lab to the field tilts the battle towards the parasite, making it easier to skew the system towards Th1ness. Indeed, mice with the highest worm burdens (and thus the longest infection durations) also had the highest levels of CD4+ T-cells expressing Th1 cytokines. The outdoor farmlike environment of the mouse enclosures at Princeton’s research station alters a number of immunologically important factors for mice {Budischak, 2018 #7744} that make the impact upon nematode susceptibility unsurprising. For *T. muris* infections, for example, microbial diversity leads the nematodes to exhibit higher hatching rates than in sterile conditions {Hayes, 2010 #2382}, and the nematodes appear to select microbial taxa within the colon that promote chronicity of infection {White, 2018 #7875}. These microbes are likely to promote Th1 and Th17 (among other immunological changes observed by {Beura, 2016 #7712;Reese, 2016 #7721;Rosshart, 2017 #7728;Rosshart, 2019 #7801}); **we thus expect that natural environments will always benefit the worms and promote long duration of infection.**

**Our theoretical results suggest two possible explanations for the observed shifts in infection duration and immune phenotype when mice are rewilded.** Rewilding could shift the system towards chronicity by altering the strength of clearance-promoting versus chronicity-promoting feedbacks or by altering the initial state of the immune system towards a Th1 bias. Furthermore, our theoretical results suggest that variation in dose is a powerful probe of the system dynamics that can help to reveal the immunoparasitological mechanisms underlying the observed change in duration. **?**

Moreover, our results can potentially help to explain the long-standing, albeit vexing, observation that **mouse strains have strikingly different dose-dependence in susceptibility to *T. muris*.** Given a high dose of eggs, “susceptible” mouse strains produce a Th1-polarized response and become chronically infected, whereas “resistant” strains produce a Th2-polarized response and clear the infection quickly ({Bancroft, 1994 #7858;Bancroft, 2001 #7857;Else, 1994 #7859}, reviewed in {Hurst, 2013 #7679;Klementowicz, 2012 #7672}). **This pattern changes if the inoculating dose is reduced: now “resistant” strains become chronically infected, too.** This pattern cannot be explained by changes in the strength of processes that generate negative feedback. For example, it is possible that worm establishment, growth, and fecundity is density-dependent (a negative feedback mechanism), such that all three are increased in low-dose infections, leading to higher infection durations. However, this has been ruled out by experimental work {Michael, 1989 #7876}. We will test whether this puzzle is solved via the logic of **Fig. 3C1**, which shows that reducing dose leads to a chronic infection because the clearance-promoting Th2 positive feedback loop is never engaged, allowing the parasite to ‘fly beneath the radar’ of the immune system.

**The close integration of experiments and mathematics that our team is poised to deliver is essential to reveal causes of varied duration of infection**. Our team is uniquely suited to meet these challenges. **PI Cressler** is a mathematical ecologist with an excellent track record in infectious disease research (e.g., {Cressler, 2016 #7797;Cressler, 2014 #7664;Cressler, 2014 #7680;Budischak, 2018 #7866;Hite, 2019 #7865}) who has worked extensively to pair theory with experiment. Of particular relevance to this proposal is Cressler’s work using mathematics to disentangle complex within-host dynamics and discover resource-dependence of immune defense across different host-parasite systems {Cressler, 2014 #7664;Cressler, 2014 #7680}. **PI Graham** is an ecological immunoparasitologist who uses experimental (e.g., {Leung, 2018 #7710}), observational (e.g., {Hayward, 2014 #7546}) and clinical trial (e.g., {Budischak, 2018 #7743}) study designs to elucidate genetic and environmental drivers of parasite (often nematode) dynamics within mammalian hosts. The 2 PIs also have a track record of working together on theory to predict how optimal immune strategy varies according to the costs of immune defense and varied parasite virulence {Cressler, 2015 #7663} and how infection duration emerges from details of within-host dynamics {van Leeuwen, 2019 #7862}. We also have a track record of collaborating to ground the latter theory in the tractable experimental system proposed here (M. musculus infected by T. muris; {Budischak, 2018 #7744;van Leeuwen, 2019 #7862}). Our collaborative team includes an eco-physiologist (**Budischak**), two immunoparasitologists with unrivalled expertise on the experimental system (**Else** and **Grencis**, on the host genetics of susceptibility (e.g., {Sahputra, 2019 #7867}) and immunomodulation by the parasite (e.g., {Bancroft, 2019 #7863}), respectively) and a mathematical ecologist (**van Leeuwen, of** {van Leeuwen, 2019 #7862}), which will ensure we have the knowledge and support required to complete the project.

We propose to fully test our theoretical predictions in that empirical system, and we envision it as an iterative process: we will begin with experiments inspired by predictions of the initial mathematics; as we learn from our empirical findings, we will return to modify the mathematics to improve accuracy of the predictions. Ultimately, **we will test whether acute-to-chronic thresholds are general across mouse genotypes and increasingly realistic environments**, achieving three Aims.

**Aim 1. Leverage host genetic variation in dose-response to quantify the relative strengths of feedbacks that drive variation in infection duration among genotypes.**

Drawing on our previous theoretical work {van Leeuwen, 2019 #7862} and pilot results (**Fig. 2-3**), we propose that variation in the relative magnitude of Th2- versus Th1-mediated feedbacks (**Fig. 1**) can explain the previously puzzling variation in infection duration in general, and in the dose-dependence of *T. muris* in particular. We specifically hypothesize that “resistant” host strains exhibit stronger Th2-escalation with increasing parasite doses but that low doses remain Th1 prone, whereas “susceptible” strains fall prey to Th1 manipulation at low doses, and higher doses amplify the Th1 feedbacks. **The key challenge we address in Aim 1 is thus to identify and quantify the feedback mechanisms driving the opposing responses to dose observed in susceptible and resistant mouse strains. In so doing, we will gain novel insight into the *processes* that determine infection dynamics in this system.** We will do this via dose-response experiments to generate high-resolution data on immune and parasite dynamics. We will then use these data to develop, parameterize and test a more mechanistic mathematical model of the *Mus*-*Trichuris* interaction than represented in our preliminary mathematical results (**Fig. 2a,b**).

***Dose variation as an experimental tool across different host strains in naturalized environments.*** The goal of these experiments is to leverage host genetic variation across environmental manipulations, to quantify feedbacks between immune responses and parasite growth, and to test whether Allee effects govern duration as hypothesized. We will begin with several inbred mouse strains that are the focus of foundational immunological research on *T. muris* infection (C57BL/6 and BALB/c as “resistant” and B10.BR and AKR as “susceptible,” independent of their Major Histocompatibility Complex (MHC) genotype {Hurst, 2013 #7679;Klementowicz, 2012 #7672}) – including divergent dose-dependencies. However, our experiments will be novel in several crucial ways.

An especially important refinement is that we will semi-naturalize the mice for all experiments. Our previous work {Bar, 2020 #7949;Leung, 2018 #7710;Lin, 2020 #7888;Yeung, 2020 #7887} and that of others {Beura, 2016 #7712;Reese, 2016 #7721;Rosshart, 2019 #7801;Rosshart, 2017 #7728} reviewed in {Hamilton, 2020 #7919} suggests that **the single most important bridge between lab mice and real adult mammals goes via microbial and thus antigenic exposure**. For example, providing antigenic experience by co-housing lab mice with “dirty roommates” {Beura, 2016 #7712} or giving lab mice fecal transplants from wild mice {Rosshart, 2017 #7728} makes their immune cell distributions better resemble that of adult mammals and dramatically alters their susceptibility to challenge infections. **One week prior to nematode inoculations, we will therefore orally gavage each mouse with a standardized slurry of cecal microbes** (pooled from 100 helminth-negative mice that had been kept outdoors for up to 3 months but that tested negative for over 30 mouse pathogens). We have previously found that such microbial transplants confer a stable, naturalized immune phenotype {Yeung, 2020 #7887}. We have also found that microbial exposure outdoors extends the duration of *T. muris* infection, even in the host strains and at doses associated with the most acute dynamics in conventional lab housing {Leung, 2018 #7710}. FIG? Thus, although dose-dependent susceptibility of C57BL/6, BALB/c, B10.BR and AKR mice to *T. muris* has been described in the lab {Hurst, 2013 #7679;Klementowicz, 2012 #7672} we expect our ***GH x dose*** data to reflect microbe-dependent shifts in immune responses and extended durations of infection.

A further refinement will concern the array of doses of *T. muris*. Because we are interested in the feedbacks that drive switchlike system-level behavior (toggling between Th1 and Th2 dominance) and in **identifying any host-strain dependent tipping points**, we must ultimately expose each host genotype to finer-scale variation in dose than has been previously undertaken. For our initial round of experiments, the doses of *T. muris* that we will deploy, all via oral gavage (as in our previous work {Budischak, 2018 #7744;Leung, 2018 #7710}), are: 20, 40, and 200 embryonated eggs per mouse. This relatively limited dose range will allow us to study all 4 host strains and both sexes, to establish immune and parasite dynamics of the ***GH x dose*** comparisons in the presence of diverse gut microbes. This variation can immediately help to identify whether the system is better characterized by Fig. 2B (suggesting strong negative feedbacks), or Fig. 3B (suggesting strong positive feedbacks). While we expect strong positive feedbacks based on the foregoing discussion, more critical is to identify the *processes that are driving system dynamics.* If we find evidence for tipping point behavior as in Fig. 3C, we will identify the most interesting subset of host strains and sexes for follow-up experiments, where we will use a broader dose ranges such as 10, 20, 40, 100, 200, and 400 embryonated eggs per mouse. This broadens and more finely divides the range used in past experiments (which often compared 40 vs either 200 or 400; {Hurst, 2013 #7679;Klementowicz, 2012 #7672}). We expect that together, these experiments may reveal a refined dose range relevant to the tipping points of each strain. For example, one strain may tip to chronicity below a dose of 100 eggs, while another may only tip to chronicity below a dose of 20 eggs.

We will use 20 adult mice per sex per ***GH x dose*** combination per experiment (with 2 host strains, 1 sex and 3 dose levels per experiment, culled at 4 different time points). We will conduct at least 2 independent experiments per strain-by-dose combination. This accords with sample sizes identified in power calculations, given the magnitude of differences among strains and within-genotype variance in immune response induction in preliminary (**Fig. 3**) and prior results {Fairlie-Clarke, 2010 #7591;Graham, 2005 #907;Leung, 2018 #7710}. Each experiment will also include uninfected controls to capture baseline immunophenotypic variation among strains and cohorts of mice.

\*\*\*FOR REFERENCE, HERE’S THE ORIGINAL CORE EXPERIMENTAL DESCRIPTION OF THIS AIM\*\*\*

We will begin with several inbred mouse strains that are the focus of foundational immunological research on *T. muris* infection (C57BL/6 and BALB/c as “resistant” and B10.BR and AKR as “susceptible,” independent of their Major Histocompatibility Complex (MHC) genotype {Hurst, 2013 #7679;Klementowicz, 2012 #7672}) – including divergent dose-dependencies. Because we are interested in the feedbacks that drive switchlike system-level behavior (toggling between Th1 and Th2 dominance) and in **identifying any host-strain dependent tipping points**, we must expose each host genotype to finer-scale variation in dose than has been previously undertaken. The doses of *T. muris* that we will deploy, all via oral gavage (as in our previous work {Budischak, 2018 #7744;Leung, 2018 #7710}), are: 10, 20, 40, 100, 200, and 400 embryonated eggs. This broadens and more finely divides the range used in past experiments (which often compared 40 vs either 200 or 400; {Hurst, 2013 #7679;Klementowicz, 2012 #7672}). For logistical reasons, 10-40-400 doses of eggs will be run in separate experiments from the 20-100-200-egg experiments. We expect that these experiments may reveal a refined dose range relevant to the tipping points of each strain. For example, one strain may tip to chronicity below a dose of 100 eggs, while another may only tip to chronicity below a dose of 20 eggs. We will therefore conduct follow-up experiments that more finely divide the large dose gaps around the strain-specific tipping point. \*\*\*END\*\*\*

***Collection of rich immunoparasitological data.*** We will quantify duration and dynamics of whipworm burden in terms of the number, developmental stage (e.g., larval stage L3 vs L4 vs adult), and biomass of nematodes (as we’ve measured previously {Budischak, 2018 #7744;Leung, 2018 #7710} as well as ATP content {Hasnain, 2012 #7670} of the nematodes collected from the caecum at four serial cull timepoints per experiment (2, 4, 6 and 8 weeks post-infection). These different ways of capturing worm survival, growth, development, and energy richness may reveal differential associations with immune responses or other aspects of host physiology. **We will culture isolated nematodes (as in {Bancroft, 2019 #7863}), to collect E/S products and purify/quantify production of the immunomodulatory (IL-13-blocking) molecule p43; we will then test whether, as expected, larger and later-stage worms are capable of greater immunomodulation (an as-yet untested assumption of Fig. 1).** We will also collect fecal egg counts from all mice, starting when infection is expected to become patent (around 4 weeks post-infection {Hurst, 2013 #7679;Klementowicz, 2012 #7672}) and working both forwards and backwards (in our collection of fecal pellets over time) to ensure we capture all shedding of eggs. **In one experiment for each *GH-by-dose* treatment combination, we will also include a separate group of mice that will be followed until fecal egg counts drop to zero** (in case it takes until worms die of old age at ~14 weeks, for example; {Hurst, 2013 #7679;Klementowicz, 2012 #7672}). We will quantify health and nutritional plane via weekly changes in host body weight, serum albumin, and total protein {Rothschild, 1969 #7676}, as well as endpoint measures of epithelial damage by histopathology {D'Elia, 2009 #7671} and body composition via both leptin and carcass weight (as we did in {Budischak, 2018 #7744}).

We will quantify immune dynamics in terms of weekly fecal concentrations of resistance-associated mucins and REsistin-Like Molecule (RELM)-ß {Hasnain, 2010 #7668} and susceptibility-associated calprotectin and lipocalin 2 {Konikoff, 2006 #7675}, and weekly serum antibody profiles, in which IgG2a:IgG1 ratio approximates the Th1:Th2 bias {Hayes, 2014 #7669;Le Goff, 2002 #668}. We will also carry out weekly flow cytometric analysis of Peripheral Blood Mononuclear Cell (PBMC) fractions expressing Tbet vs GATA-3 (to quantify Th1 & Th2 master regulator expression, respectively) and IFN- vs IL-4/IL-13 (to quantify Th1 & Th2 cytokine expression, respectively), alongside standard markers of T cell phenotype (e.g., CD3, CD4, CD8). Key endpoint measurements will entail phenotyping of mesenteric lymph node (MLN) and lamina propria cells (all of the above as we measured in {Leung, 2018 #7710}), including T cell fractions expressing Tbet, GATA-3, IFN-, IL-4 and IL-13, restimulation of MLN cells with *T. muris* antigen and subsequent production of a full panel of cytokines. We will also analyze PBMC via flow cytometry and antibodies via ELISA to verify patterns observed longitudinally in smaller sample volumes from the same animals. Given the larger number of cells available for flow cytometry at experimental endpoints, we will also include markers of proliferative and gut-homing potential (e.g., Ki67 & CCR6, respectively) in our flow cytometry panel.

***Hypothesis tests and tipping points for within-host Allee effects.*** We hypothesize that when we measure the dynamics of parasite expulsion in relation to Th1 and Th2 transcription factors and cytokines across these genotypes and doses, **we will discover the immunological tipping points (likely differing by host strain) that determine duration**. However, because we expect greatest parasite growth once hosts have tipped to Th1, we also expect a considerable contribution of biomass-dependent parasite feedbacks to sustaining the Th1 milieu.

As an essential test of our Allee effect hypothesis, we will use the empirical data to estimate the parameters of our mechanistic mathematical description of the system dynamics. Building on existing theory for Th1-Th2 interactions (e.g., Yates 2000, 2004; van den Ham & de Boer 2008; Schrom 2020), we consider the following model of immune-parasite interaction:

|  |  |
| --- | --- |
|  | (1) |

The parameter captures the non-parasite induction of Th*-i* immunity; we might expect that is larger in the mesocosm than in the lab, as the hosts are exposed to a wider variety of microbes. The term captures the induction of Th-*i*  immunity by *T. muris*. The term captures self-promotion of Th-*i* immunity by, for example, production of cytokines (e.g., IL-13, IFN-). The term captures cross-inhibition of Th-*i* immunity by Th-*j* cells. Existing theory-data syntheses give reasonable starting estimates for many of the parameters of these self-promotion and cross-inhibition terms (Schrom 2020), as well as identifying straightforward extensions of these models that more explicitly incorporate the interactions of, e.g., master regulator transcription factors (GATA3 and T-bet) with both cytokines and T-cell populations (Yates 2004, van den Ham & de Boer 2008, Schrom 2020). Parasite biomass growth rate is presumed to be density dependent , and parasites are killed by effector cells activated by Th2 cells at a rate proportional to . Note that negative feedback processes in this model come primarily through the parasite growth terms, whereas positive feedback processes dominate the immune terms.

While we can use existing estimates of the parameters of this model to gain some preliminary insights, evidence for changes in the strength of positive and negative feedback mechanisms across genotypes will be revealed by fitting the model to observed data. In this case, the most relevant data are the various measures of Th2ness and parasite biomass, which map onto predictions of **Fig. 2** and are measured directly from the serial culls and indirectly from fecal egg counts.

We will use iterated filtering {Ionides, 2006 #6880;Breto, 2018 #7870} to fit the dynamical system specified by equation (1) to the experimental data. Fitting is a well-studied problem {He, 2010 #6876;Ionides, 2006 #6880;Breto, 2018 #7870}, and existing software packages (e.g., **pomp** {King, 2016 #7871} provide considerable flexibility for estimating the parameters of dynamical systems from noisy, incomplete data. **The fitting process will thus test our hypothesis that variation in infection duration is driven by Allee effects by estimating the magnitude of the parameters that underlie positive and negative feedbacks in the model.**

***Expected outcomes and potential pitfalls.*** The work proposed under this Aim is relatively low risk and high reward. We anticipate that the primary challenges would arise from the complexity of our *in vivo* experiments. However, we have experience with all of the protocols required {Budischak, 2018 #7744;Leung, 2018 #7710;van Leeuwen, 2019 #7862}, including prior immunological dose-response work {Fairlie-Clarke, 2015 #7872;Metcalf, 2011 #6604}. We therefore do not anticipate difficulty in completing the experiments. Furthermore, we expect the experimental data *per se* to represent an important advance: a nuanced understanding of how rates of immune response induction as well as outcome of infection depend upon host genotype and dose will be of broad interest in immunology.

LOGISTICS OF INOCULATIONS & BACKUP PLANS FOR ENVIRONMENTAL NATURALIZATINO: MYCOBIOTA🡪GAVAGE For logistical reasons, 10-40-400 doses of eggs will be run in separate experiments from the 20-100-200-egg experiments. … Due to the evidence that microbes are the largest contributors to immune naturalization, for the experiments under this Aim, we will focus on that rather than other immunologicaly relevant axes of environmental realism (opportunity to exercise, natural rhythms in temperature as well as light).

The theory development is an important advance as well. Current mathematical approaches to studying within-host interactions have not advanced our understanding of the determinants of infection chronicity, despite offering other insights {Fenton, 2006 #2337;Fenton, 2010 #2336;Alizon, 2008 #7823;Childs, 2015 #7842}. The theory-data integration we propose here will provide novel information about the magnitudes of key immunological and parasitological processes. This will open up novel theoretical research addressing the cross-scale interaction between within-host and between-host processes in disease systems {Day, 2011 #6803;Mideo, 2011 #6576;Handel, 2015 #7825} while providing empirical tests of predicted switchlike behavior in mammalian T cell populations {Yates, 2004 #1315;Schrom, 2017 #7827}.

A particularly compelling conceptual motivation for studying the role of Allee effects in driving parasite persistence is the key role of Allee effects for understanding and deriving management strategies for invasive species {Tobin, 2011 #7829}. There are strong conceptual similarities between the question of whether a parasite can establish in a host and whether an invasive species can establish in an ecosystem; feedback processes are critical in both cases. **Furthermore, leveraging Allee effects has been an important strategy for managing invasive species.** For example, mathematical models that incorporated positive feedback between pine beetle exploitation and pine defense were able to accurately predict invasion thresholds across environments {Nelson, 2008 #7830}, and inform management strategies focused on shifting establishment thresholds {Borden, 1989 #7831;Tobin, 2011 #7829}. Understanding Allee effects in host-parasite systems may similarly inform treatment strategies.

One potential pitfall would be if the above model proves too simple and is unable to reproduce observed infection dynamics. Decades of experimental work indicates that we will certainly observe variation in infection duration. The most likely reason for the model’s inability to fit the data well would be a lack of immunological detail, for example on the dynamics of cytokines or transcription factors. Fortunately, as noted above, other authors have considered more detailed models that can be brought to bear on the data. If this were the case, we can extend the model to include additional variables, such as the dynamics of immune cell activation and proliferation in response to cytokines {Yates, 2000 #1305;Yates, 2004 #1315;van den Ham, 2008 #7806;Bergmann, 2001 #7807}}, the dynamics of cytokine expression by Antigen-Presenting Cells (APCs) and T helper cells {De Boer, 1995 #7809;Fishman, 1993 #7808}, and the dynamics of immune cell polarization and master regulator expression {Höfer, 2002 #7810;van den Ham, 2008 #7806}}. We can extend the model above to include some of these other variables to gain deeper insights into the mechanisms underlying the observed infection durations. **Importantly, even if we do not find strong evidence for tipping point behavior, either empirically or theoretically, our theory-data integration will still provide valuable insights into the processes that *do* drive variation in infection duration.**

**Aim 2: Validate a modeling framework for predicting infection duration of individuals by experimentally manipulating the relative strengths of immunological feedbacks.**

Mathematical models indicate that subtle variation in baseline immune state interacts with feedback processes to drive quantitative variation in infection duration among individuals across or within genotypes (and corresponding qualitative variation; i.e., acute vs chronic infections) (**Fig. 2**). However, the theoretical work above does not identify which immune pathways cause varied infection duration, though statistical analysis of the experimental data under Aim 1 will provide clues. To enable robust prediction of duration, we must identify key immune variables and manipulate them experimentally.

***Manipulation of immune feedbacks to alter dose-dependence of duration.*** Here, we will manipulate feedbacks directly. Dose manipulation (under Aim 1) alters both the induction and manipulation of immune signaling by parasites, while manipulation of immune feedbacks independent of parasite dose allows us to experimentally decouple host and parasite agency in response induction. We note that manipulation of the master regulators of Th1 and Th2 is best achieved by manipulation of the cytokine signals that induce them, IFN- vs IL-4. We further note that manipulation of IL-13 in vivo is expected to alter effector mechanisms downstream of the Th2 induction. We will therefore use additions of recombinant cytokines as well as monoclonal antibody-mediated and parasite product-mediated subtractions of endogenous cytokines to alter immune feedbacks. We will focus on those cytokines because of their demonstrated strong impact on rate of clearing *T. muris* {Hurst, 2013 #7679;Klementowicz, 2012 #7672}, and because the reagents work in many strains of mice, at established dose/timing for delivering reagents. We will infect wild types alongside manipulated mice.

We will titrate these cytokines in and out of the system to test our predictions, as follows. For 3 doses surrounding the observed Th1-Th2, chronic-acute “tipping point” for each host strain (likely around a different dose range for each strain), we will manipulate cytokines in different treatment groups, to promote Th1 responses (+IFN- -IL-4), Th2 responses (-IFN- +IL-4) or to suppress effector mechanisms (-IL-13), independent of the doses of parasites. We will use pharmaceutical grade recombinant cytokines or cytokine complexes (to add murine IFN-, IL-4 or IL-13; e.g., {Schirmer, 2016 #7873}) and pharmaceutical grade monoclonal antibodies to deplete them (e.g., {Berry, 2009 #7874}). In addition, we will use the *T. muris* excretory/secretory molecule p43, which binds to IL-13, interfering with the development of an appropriate effector response against the nematode {Bancroft, 2019 #7863}. Following the protocol under development by collaborator Grencis, we will use purified p43 to mimic parasite suppression of Th2 effector mechanisms, decoupled from the biomass of nematodes.

For each host strain, we hypothesize that we can shift the interaction towards acute infection by strengthening Th2 feedbacks, and shift the interaction towards chronic infection by strengthening the Th1 feedbacks or by decoupling the worm-clearing effector mechanisms (such as mucins {Grencis, 2015 #7699}) from an induced Th2 response. **Whether system dynamics are more sensitive to changes in IFN-, IL-4 or IL-13 concentrations will reveal the relative strength of positive and negative signaling feedbacks in each strain**, providing an important secondary test of the model fitting results from Aim 1 while preparing for the model development below. Finally, we hypothesize that parasite biomass-driven immune feedbacks will manifest as acceleration of Th1ness above and beyond what the cytokine manipulations confer.

**In each experiment, we will collect the data types at the timepoints described under Aim 1.**

***Expected outcomes and potential pitfalls.*** The work under this Aim is relatively low risk and high reward, though the challenges of *in vivo* cytokine manipulation are non-trivial. We do have experience with timing and dosing such manipulations for sustained effect (reviewed in {Long, 2011 #6625}) and the rest of the experimental procedures are familiar from past work (as noted under Aim 1). If we find that cytokine manipulations *in vivo* prove uninformative for testing the hypotheses of **Fig. 2,** we will instead use a key knockout on the C57BL/6 genetic background to dissect the contribution of parasite-driven feedbacks to bifurcation in outcomes. A particular genotype of interest is Muc5ac-/- mice, which make potent Th2 responses but because they are deficient in the key mucin required for *T. muris* expulsion, they nonetheless are highly susceptible to chronic infections {Hasnain, 2010 #7668}. This will mean that immune recognition and signaling will proceed as normal, but parasite clearance will be prevented. Thus we hypothesize that the negative feedback of immunity upon parasites will be broken, fostering parasite growth and accelerating the positive feedback of parasites in enhancing and sustaining a Th1 response. Resulting duration should be long indeed. The experimental work *per se* would be high reward in that multivariate immunological data has not been reported following manipulation of the cytokine network to assay nematode susceptibility.

The models developed here will also represent an important advance. While theoretical immunology has been successful in developing detailed mathematical models for host-microparasite interactions (e.g., HIV {Wodarz, 2002 #7818}, malaria {Wale, 2019 #7816}, dengue {Ben-Shachar, 2015 #7819} & tuberculosis{Marino, 2004 #7820}), there has been considerably less work on modeling within-host dynamics of macroparasites {Thakar, 2012 #7813;Garnier, 2016 #7695}. Thus, the models developed and tested in this Aim will provide a valuable signpost to guide future work in modeling these critically understudied infections.

Again, a potential challenge here is the complexity of the model fitting enterprise. This is why we have proposed the less ambitious model fitting component of Aim 1 first. By undertaking fitting the simpler models, we will gain valuable insights into functional forms that will help guide us here: for example, strategic simplification of the complex model must produce a model that is structurally similar to the verified simple model. Nevertheless, there is a risk that we will not be able to fit the complex model. In such a case, we will simplify our complex dynamical model to a discrete dynamic model {Assmann, 2009 #7821}, which represents the immune system as network of interacting nodes, each of which can take only two states (ON or OFF). This approach is useful when quantitative data are insufficient to characterize the functional relationships between variables {Thakar, 2010 #7822}. Such models have been successfully applied to model host-macroparasite interactions {Thakar, 2012 #7813;Thakar, 2007 #7812}, suggesting that they are a viable alternative pathway to our system as well.

**Aim 3: Titrate in full environmental naturalism, in “rewilded” mice raised outdoors, to quantify effects upon duration of nematode infection.**

With this Aim, we will take an even greater step towards environmental realism, to place duration-driving feedbacks **(Fig. 2)** into a fully natural context and thereby to “field test” our predictions. As noted above, recent work on naturalizing lab mice with “dirty roommates” {Beura, 2016 #7712}, serial infections {Reese, 2016 #7721}, or fecal {Rosshart, 2017 #7728} or *in utero* {Rosshart, 2019 #7801} exposures to the microbes borne by wild mice under otherwise-controlled conditions has shown that lab mice exposed to natural microbes rapidly exhibit shifts in immune phenotype that alter resistance to microbial and inflammatory challenges. Thus. the importance of incorporating environmental realism into immunological experiments is increasingly appreciated {Abolins, 2017 #7747;Abolins, 2018 #7746}. **Furthermore, previous work of others {Scott, 1991 #1389;Scott, 2006 #1388} as well as ourselves in experimental outdoor mesocosms {Leung, 2018 #7710} has shown that mice in a more natural environment exhibit greater duration of nematode infection compared to mice in the lab.**

***Leveraging the real-world environmental context to hone and field-test predictions.*** To test these predictions, we will titrate in a natural environmental context. We will begin with fecal feeding experiments across all host strains, for the refined (possibly strain-specific) dose range – transferring fecal pellets from inbred mice maintained outdoors in a germ-rich but nematode-free environment to lab recipients via coprophagy. **Each summer for the full duration of the project**, we will also field-test dose response experiments outdoors in C57BL/6 and B10.BR (predation-resistant non-albinos) so that we can assess how dramatically dose-response relationships are changed, and to generate feces of worm-free controls outdoors, for subsequent transfer to mice kept back in the laboratory. We will not allow infections go patent outdoors. Our sample sizes outdoors will be larger than in lab experiments but still tractable (in line with our past work that was sufficiently powered in each treatment group {Leung, 2018 #7710}). **We are especially keen to observe whether outdoor living shifts all genotypes towards greater chronicity of infection (and potentially towards greater parasite domination of immune signaling). We hypothesize that infection duration will indeed be enhanced across all host\*dose combinations with Th1 enhanced outdoors.**  We further hypothesize that we will be able to explain those shifts via alterations to the relative magnitude of Th1 feedbacks and parasite biomass-driven Th1 feedback in the more natural environment.

**In each experiment, we will collect the data types at the timepoints described under Aim 1.**

***Quantifying feedbacks in the field*.** From Aim 2, we will have a validated theoretical model for generating predictions of infection duration that incorporates all of the mechanistic detail needed to describe infection dynamics in the lab. That is, the model identifies the key immunological variables and their interactions with one another and with the parasite. Here we test whether this model is sufficiently detailed to predict infection duration in the field. That is**, in Aim 3 we will determine whether moving outdoors simply changes parameter values in the immune-parasite interaction model of Aim 2, or whether entirely new mechanistic detail must be added to the model to capture infection dynamics outdoors.** In particular, microbes found in nature, but not in the lab, may open new channels of immune crosstalk that are not found in the lab {Abolins, 2017 #7747;Abolins, 2018 #7746}. To address this, we will repeat the model fitting process described in Aim 2 using the data from rewilded mice. We will use the validated model of Aim 2 as a starting point, and compare that model to additional models that include immune mechanisms that appear to be important based on observed differences in immunological measures from rewilded mice and lab-reared siblings.

***Expected outcomes and potential pitfalls.*** The experimental work under this Aim is the most challenging of the 3 Aims, yet we have 5 years of experience running mesocosm experiments that will promote our success here. Furthermore, the work will reveal new biological insights into mammalian immune function in a natural environment, even if our central hypotheses about Allee effects within mouse guts are unsupported outdoors. The question of how complex a model of immunity needs to be in order to accurately capture immune dynamics is a critical open question in theoretical and computational immunology {Thakar, 2010 #7817;Fenton, 2010 #2336}. Indeed, we may find that different functional forms will predict infection duration in lab and field. By slowly and systematically building in real-world complexity across the three Aims here, we will have a novel perspective on this question. This process will help to identify a general approach to determining the critical level of complexity needed to predict infection dynamics in the real world.