CHAPTER TWENTY-ONE

Processes generating heterogeneities in infection and transmission in a parasite–rabbit system

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21.1 Introduction

Understanding how host heterogeneities to infection are generated and how they influence the dynamics and spread of infectious diseases remains a challenging issue in disease ecology and evolution. Host–parasite interactions play an essential role in shaping host variation to infections. On one hand, the degree of susceptibility and immunocompetence determines how good a host is in preventing or controlling an infection; on the other hand, the level of virulence and ability to transmit describes how accomplished a parasite is in avoiding the host constraints. All else being equal, we should expect some variation in the host population as part of these inherent, often genetically driven, properties of the two parties. However, individual hosts do age, reproduce, adjust their behaviour or microbiota with time and, in so doing, generate additional sources of variation that can affect host–parasite interactions. A large body of work has examined the contribution of these hosts' characteristics and the general conclusion is that they often modulate host responses and disease outcome in a non-linear manner, adding a further level of complexity to the system (Anderson & Gordon, 1982; Hayes et al., 2010; Cizauskas et al., 2015; Izhar & Ben-Ami, 2015). In natural settings host populations are also exposed to external perturbations, such as changes in climate, infections with other parasite species, or anthropogenic disturbance (Figure 21.1). These perturbations can exacerbate or mitigate the way the host's characteristics affect the parasite as well as directly altering parasite dynamics and traits, such as fecundity, and lead to further variation in infection and transmission among hosts.

A challenge in the ecology of infectious diseases is to disentangle the contribution of these sources of variation, specifically, to understand how external perturbations interact with the hosts' properties to affect parasite dynamics and life history, and the consequences over time and space. A powerful approach is to integrate long-term field studies of natural

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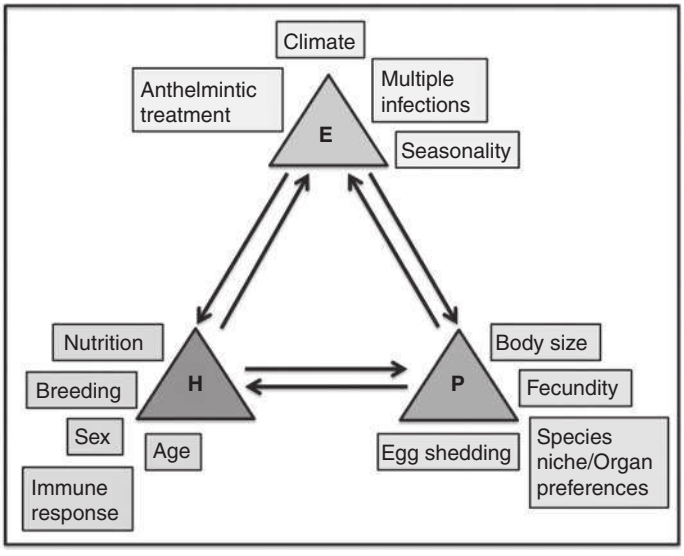

Figure 21.1 Diagram showing the interactions among hosts (H), parasites (P), and environment (E). For each of the three variables we have reported the components that have been considered in the rabbit system and that can contribute to generate host heterogeneity to infection and transmission in this system. Only the impact of external disturbance (e.g. climate changes, drug treatment, and multiple infections) has been addressed in this chapter, although other components have been implicitly included (e.g. host age, immune response, seasonality).

populations with detailed laboratory manipulations; the latter allowing insights into within-host processes while controlling for confounding effects of inherent variation in host exposure or susceptibility commonly found in the field. The rabbit–helminth system (Figure 21.2) has proved to be particularly suitable in that we have detailed historical data on the parasites and the host from wild populations, and have performed laboratory experiments to characterise the fundamental components and responses over the course of the infection.

We take an ecological approach to the study of the rabbit–parasite system and explore how external perturbations alter the trophic relationship between the host and its parasites and contribute to heterogeneity in infection and transmission. We synthesise findings on the role of three disturbances: (i) seasonality and climate changes, (ii) coinfection with a second parasite species, and (iii) anthelmintic treatments. We then outline parsimonious mechanisms, based on mathematical models and analytical approaches, which explain the patterns observed. Findings from this system have far-reaching repercussions for a wide range of ecological and health issues, and inform a variety of host–parasite systems of wildlife, agricultural animals and humans, where experiments and long-term

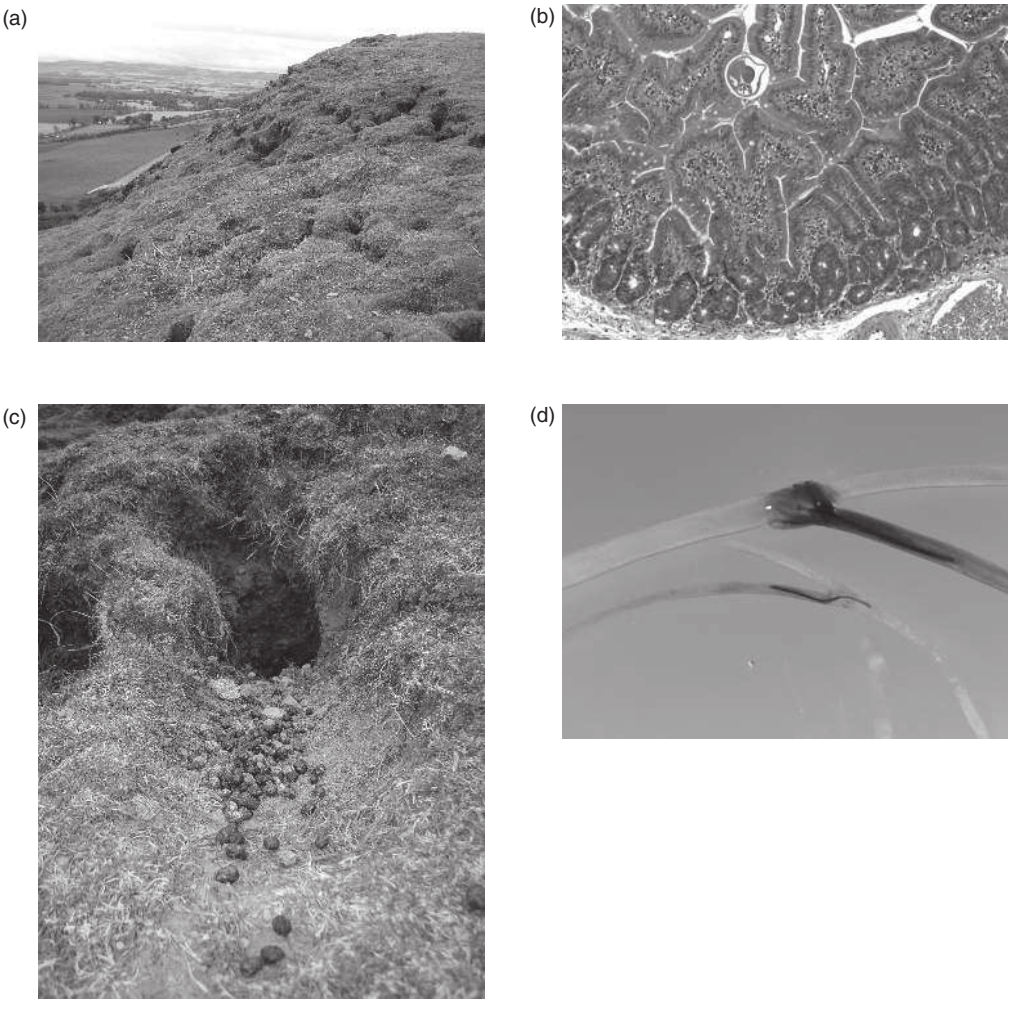

Figure 21.2 The helminth–rabbit system: the semi-natural agro-ecosystem study area (a); adults of *G. strigosum* in the stomach (b); warren entrance and rabbit faeces (c); *G. strigosum* male and female mating (d). (A black and white version of this figure will appear in some formats. For the colour version, please refer to the plate section.)

data are often difficult to conduct or obtain. In this context, we provide future directions, such as how an integrated knowledge of the mechanisms of infection and spread is necessary if we are to understand processes and patterns of parasite dynamics in natural settings.

21.2 The system

The European rabbit (*Oryctolagus cuniculus*) is native to south-west Europe and north-west Africa, but over the centuries has been widely introduced in many

other countries worldwide. Animals construct interconnected burrows where they live in families; dominant males are polygamous, females can also be dominant and, like males, defend their territory. Juvenile males disperse in spring while juvenile females tend to stay with the family. Based on the location and population density, reproduction can be restricted to a few months or occur for most of the year (Thompson & King, 1994; Hoffman & Smith, 2005). Because of the damage caused to biodiversity and farming, the European rabbit is often considered a pest that needs to be controlled.

We have detailed monthly data on hosts and parasites from two rabbit populations sampled in a semi-natural ecosystem at the interface with arable land in Scotland (UK): the first population was monitored from 1977 to 2002 and the second from 2000 to 2014. Both populations were sampled using standardised protocols and details are available on the host (e.g. age, sex, breeding status) and its common infections (e.g. parasite species, prevalence and/or intensity). For the second population we also collected host blood sera and helminth biometrics (e.g. body length and eggs in utero). Details on daily climatic variables were also recorded. The laboratory experiments were performed using the New Zealand white rabbit, a common breed of the European rabbit, commercially available from research suppliers. Parasites for the laboratory work were isolated from our study sites (*Graphidium strigosum*) or kindly provided by Dr Kerboeuf (INRA, France, *Trichostrongylus retortaeformis*) and Dr Harvill (University of Georgia–Athens, USA, *Bordetella bronchiseptica*).

Our work primarily focused on three main parasites, two helminths and one bacterium. *Trichostrongylus retortaeformis* and *Graphidium strigosum* are gastrointestinal helminths that commonly infect the European rabbit. *T. retortaeformis* has a relatively small body size and lower per-capita fecundity than *G. strigosum* (Audebert et al., 2000; Massoni et al., 2011). Infections occur via ingestion of herbage contaminated with third-stage infective larvae; *T. retortaeformis* colonises the small intestine with a preference for the duodenum while *G. strigosum* inhabits the stomach, primarily the fundic region (Murphy et al., 2011). Sexual maturation is much faster for *T. retortaeformis* than *G. strigosum* and after reproduction eggs are shed into the environment with the rabbit's faeces (pre-patency: ~11 and 40 days, respectively). Free-living stages are exposed to the same environmental drivers such as climate and biotic factors. Both helminths have a long history of coevolution with the rabbit (Audebert & Durette-Desset, 2007) and although causing chronic and recurring infections, they exhibit distinct ecological properties (details provided in the next section) that make them an excellent model for exploring trophic interactions both within and between hosts. Moreover, the system has many similarities (e.g. mode of transmission, life cycle, or type of immune reaction) with communities of gastrointestinal helminths of agricultural animals, wildlife, and humans, and provides fundamental insights on disease ecology across taxa.

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The respiratory bacterium *Bordetella bronchiseptica* has been isolated from the respiratory tract of many wild mammal species including rabbits and causes regular outbreaks and chronic infections in domesticated animals kept in crowded conditions (Pathak et al., 2011). By 90 days post initial infection, *B. bronchiseptica* is cleared from the lungs and trachea of laboratory rabbits by an effective immune response but persists in the nasal cavity (Pathak et al., 2010; Thakar et al., 2012). *B. bronchiseptica* is closely related to the human subspecies *B. pertussis* and *B. parapertussis*, responsible for whooping cough outbreaks and notably re-emergent in the USA (Jackson & Rohani, 2014). Given the similar properties and mode of transmission, *B. bronchiseptica* is an invaluable model for studying the dynamics and transmission of whooping cough and, more generally, respiratory bacterial infections of livestock concern (Brogden et al., 1998; Kao et al., 2007).

21.3 Fundamental host–parasite processes

Given that host responses to infections and parasite reactions to host constraints are often highly heterogeneous, it is not surprising that within any host population there is large variation in the intensity of infection among individuals. This intrinsic variation of the host is determined by changes in susceptibility and resistance, namely the ability to control the infection and reinfections, alongside differences in exposure to infective stages (Anderson & May, 1978). For parasitic helminths, resistance commonly engages a 'type 2' immune reaction by the host, with functions and factors that can take weeks or years to reach full effectiveness (Jackson et al., 2009; Yazdanbakhsh & Sacks, 2010). This partly explains the persistence of parasitic infections and the lack or weak life-long protection against reinfections often observed. Parasite immunology has been well described theoretically and experimentally (e.g. Stear et al., 1999; Anthony et al., 2007; Allen & Maizels, 2011), including the defensive properties from hosts with different genotypes or immunocompetence (e.g. Raberg et al., 2007; Stear et al., 2009; McRae et al., 2015), the consequences to the epidemiology of infection and the evolution of resistance (e.g. Bowers, 1999; Restif & Koella, 2004; James et al., 2009), and more recently the molecular and evolutionary outcomes of tolerance to infections (Roy & Kirchner, 2000; Miller et al., 2005; Allen & Sutherland, 2014). From an epidemiological perspective, the extent of the protective immune response to helminths is proportional to the accumulated exposure to infective stages, in addition to the immune status of the host at the time of the infection and the properties of the parasite.

We have characterised the immune response of the rabbit to *T. retortaeformis* and *G. strigosum* from laboratory trials and a wild rabbit population, and below provide some general insights that can guide understanding of the dynamics of infection of our system under perturbations. Laboratory experiments were

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based on rabbits challenged with a single dose or trickle-dosed every week (the total amount of infective stages delivered was the same in the two types of trials); animals were sampled at fixed time points and samples from the small intestine, duodenum, and the stomach fundus (tissue and mucus), as well as blood sera, were used for the analyses. The wild rabbit population was randomly sampled every month and the gastrointestinal tract and blood were collected from every individual for parasitological and immunological work. Three branches of the immune response and associated factors were examined: (i) the type 2 anti-inflammatory response, commonly involved in the removal/control of parasitic helminths; (ii) the type 1 inflammatory reaction, a critical player in protecting against microparasites (viruses, bacteria); and (iii) the regulatory response implicated in tissue repair/regeneration and tolerance to infection (Allen & Sutherland, 2014; McRae et al., 2015). It is important to remember that the same factor might have different functions, including activities specific to different branches of the immune system, depending on the local polarisation of the immune response and the macro-/microparasite involved. Likewise, it has been proposed that the protective type 2 response to macro parasites is also involved in tissue repair and regeneration (Allen & Sutherland, 2014).

For the rabbit–helminth system, we found that *T. retortaeformis* is reduced during the course of the infection, and the pattern is consistent in field and laboratory settings. *T. retortaeformis* stimulates a type 1–type 2 immune response (i.e. inflammatory and anti-inflammatory activities) in that an initially high IFNγ gene expression wanes during the course of the infection to values that are comparable to IL4 and IL10 (Murphy et al., 2011; Thakar et al., 2012). The initial IFNγ inflammatory response is probably caused by local bacterial infiltration into the small intestinal mucosa damaged by the parasites moving into the tissue during colonisation and development (Murphy et al., 2011; Van Kuren et al., 2013). The anti-inflammatory IL4 plays an important role in the clearance of this helminth (Thakar et al., 2012), while IL10 is an anti-inflammatory cytokine that downregulates the IFNγ expression while also contributing to regulatory functions and tolerance (Redpath et al., 2014). We have also quantified the expression of other cytokines and transcription factors and while they were clearly upregulated, values were relatively low except for the anti-inflammatory IL13, which is also important for parasite clearance (Cattadori et al., 2019). The modelling of the immuno-dynamic network of infection, using a discrete Boolean framework, showed that the activation of an anti-inflammatory type 2 response, namely, the relatively rapid recruitment of antibodies (IgA and IgG) together with eosinophils, was responsible for the control but not the complete removal of *T. retortaeformis* from the small intestine (Thakar et al., 2012). The IgA and IgG trends recoded in the laboratory were also observed in the wild rabbits (Cattadori et al., 2014).

Similar studies were performed for *G. strigosum* infections. We found that parasites persist in the stomach with no strong evidence of immune control despite a clear 'type 2' response (Murphy et al., 2011, 2013; Pathak et al., 2012). IL4 and IL13 remain proportionally high through the course of infection, with lower expression of the inflammatory IFNγ and Tbet, and the anti-inflammatory GATA3 and IL10; the remaining immune factors exhibited relatively low values (Cattadori et al., 2019). The movements of *G. strigosum* into the stomach mucosa, both by the establishing larvae and adult worms (Van Kuren et al., 2013), caused tissue damage and local recruitment of immune cells (Murphy et al., 2011). However, and contrary to *T. retortaeformis*, the inflammatory response (i.e. IFNγ and Tbet) did not exceed the defensive anti-inflammatory reaction (i.e. IL4, IL13, and GATA3), probably to prevent immuno-pathology in an environment that is chemically and physiologically relatively extreme. Despite the defensive profile by cytokines and transcription factors, we found consistently low levels of species-specific and total IgA antibody in the stomach mucus (Murphy et al., 2011; Cattadori et al., 2018), supporting the hypothesis that some of the cell-mediated defences against the parasite are relatively weak. However, it should be noted that given the properties of the stomach, it is possible that the protective effectors are down-regulated by the host while it also attempts to repair/regenerate the tissue damaged by the infection (Cattadori et al., 2019). Another possibility is that *G. strigosum* contributes to its own persistence by manipulating or circumventing host immunity. As noted for *T. retortaeformis*, the IgA and IgG responses of wild rabbits against *G. strigosum* were similar to laboratory animals. Overall, whether these studies were from experimental trials or field observations, there was always heterogeneity in the immune response to the infection among rabbits.

At the host population level, a convex relationship between host age and parasite intensity where intensity increases, peaks and then decreases in older individuals, should be indicative of a defensive immune response, which develops proportionately to the force of infection (i.e. rate of accumulated parasite acquisition) and controls the parasite in older hosts (Anderson & May, 1978; Woolhouse, 1992). A convex profile can also be generated by changes in the host and/or parasite properties for instance, differences in the intensity of infection among host of diverse age- or parasite-induced host mortality (Cattadori et al., 2005). On the contrary, the regular accumulation of parasites with host age should be indicative of a weak or no immune control, including parasite manipulation of host defences. Here, parasite dynamics should be driven by ecological processes, such as competition for resources that builds proportionally to parasite intensity. The age–intensity relationship has been widely used in epidemiology to provide fundamental understanding to the processes affecting disease dynamics (Hudson & Dobson, 1989; Woolhouse,

1992; Duerr et al., 2003), including the emergence of host heterogeneity to infection (Lloyd-Smith et al., 2005), and the identification of the individual hosts responsible for the majority of the infection and transmission (Grenfell & Anderson, 1989).

The contrasting immuno-dynamics described for *T. retortaeformis* and *G. strigosum* were confirmed when we applied the age–intensity relationship to rabbit populations. We showed that *T. retortaeformis* infection follows a convex age–intensity profile while *G. strigosum* exponentially accumulates with host age (Cattadori et al., 2005, 2008, 2014; Pathak et al., 2012). To explain these patterns mechanistically, we developed epidemiological models based on an age-structured rabbit population that included the relative contribution of host immune defences and intensity-dependent parasite constraints. Simulations were consistent with these general trends, specifically, *T. retortaeformis* is regulated by an immune response that develops proportionately to the accumulated force of infection, as well as the degree of host immunocompetence (i.e. the ability to develop a successful immune reaction), and is controlled in older rabbits (Cornell et al., 2008; Mignatti et al., 2016). In contrast, *G. strigosum* is mainly regulated by direct intensity-dependent processes, most likely parasite competition for space and food, which build with the accumulated intensity of infection (Mignatti et al., 2016).

In summary, distinctive organ properties and immune functions, combined with the specificity of parasite attributes and host characteristics, create well-defined host–parasite relationships that generate heterogeneities in the responses of both parties and ultimately affect the dynamics of infection. However, external perturbations are expected to alter these relationships, raising the question of whether disturbances enhance or suppress individual variation to infection, and whether the impact affects more heavily some hosts than others. In the following sections we shall examine and discuss the contribution of three different types of perturbations on parasite dynamics: (i) the impact of seasonality and long-term climate changes; (ii) the role of co-infection with a second parasite (either a helminth or a bacterium); and (iii) the effect of anthelmintic treatments.

21.3.1 Climate and seasonality

Most of the predictive models and experimental manipulations investigating climate changes and infectious diseases have overlooked the modulatory contribution of the host on climate impact. Studies have primarily focused on how climate changes can affect host exposure, the risk of infection or disease spread, and the emergence of novel infectious agents (e.g. Molnár et al., 2013; Raffel et al., 2013; Paull & Johnson, 2014). However, as highlighted by our work (Cattadori et al., 2005, 2014, 2018; Murphy et al., 2011, 2013; Thakar et al., 2012) and others (Maizels, 2009; Bourke et al., 2011; Girgis et al., 2013),

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host immunity to infections is an important source of variation among individuals; hence how immunity alters the way climate affects host–parasite interactions needs to be accounted for. Indeed, we might expect different outcomes driven by both the type of interaction and the scale at which these processes are examined. Here we investigate (i) whether an increase in exposure to parasites, caused by climate warming, is associated with a proportional increase in the intensity of infection in hosts with protective immunity, and (ii) how this pattern contrasts if hosts fail to show an effective immune response. In other words, we explore if heterogeneities in the immune response to a parasite exacerbate or suppress the effect that climate changes have on the dynamics of infection.

If parasites are modulated by host immunity then we should expect weak or no long-term changes in the intensity of infection with warming because immunity controls the parasite burden in the host population (Figure 21.3a). At the seasonal scale, however, climate warming is expected to shift the peak of infection towards the younger, less-competent hosts, and increase the variation in infection between individuals (Figure 21.4a). This seasonal change in the host age–parasite intensity relationship caused by climate warming is based on the 'peak shift' concept (Anderson & May, 1978; Woolhouse, 1998). Briefly, this concept assesses the interaction between the force of infection (i.e. parasite acquisition) and the host immune response, and suggests that a high force of infection stimulates a faster immune response that leads to parasite intensities peaking at earlier host age compared to a lower force of infection that results in a lower parasite burden that peaks in older hosts because of an immune response that develops more slowly. The 'peak shift' concept has been empirically described by us and others (Woolhouse, 1998; Cattadori et al., 2005; Blackwell et al., 2011). Here we suggest that this pattern can be exacerbated by the interaction between climate warming and host immunity, specifically, by increasing the force of infection warming can further shift the peak of infection toward the even younger hosts (Figure 21.4a). In contrast, if the parasite is weakly controlled by immunity then it should accumulate with host age and constrained by its own density (Figures 21.2b and 21.3b).

We tested the long-term and seasonal impact of climate warming on our soil-transmitted gastrointestinal helminths, *T. retortaeformis* and *G. strigosum*, from the rabbit population sampled monthly between 1977 and 2002 in Scotland. At this latitude, seasonality regulates the life cycle of the host and the parasites (Cattadori et al., 2005, 2008); more recently, the system has also been under the influence of temperature warming (Harvell et al., 2009; Hernandez et al., 2013) and increasing air relative humidity (Mignatti et al., 2016). As previously described, we also know that the two parasites exhibit contrasting dynamics of infection and distinctive response to host immunity. We developed an age-structured epidemiological model that explicitly

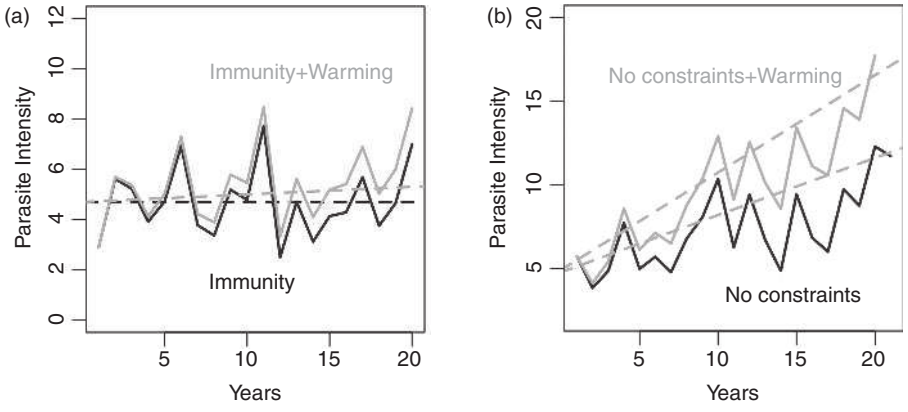

Figure 21.3 Hypothetical scenarios of mean parasite infection in a host population over years with (upper line) and without (lower line) climate warming, and (a) in the presence and (b) absence of immune control.

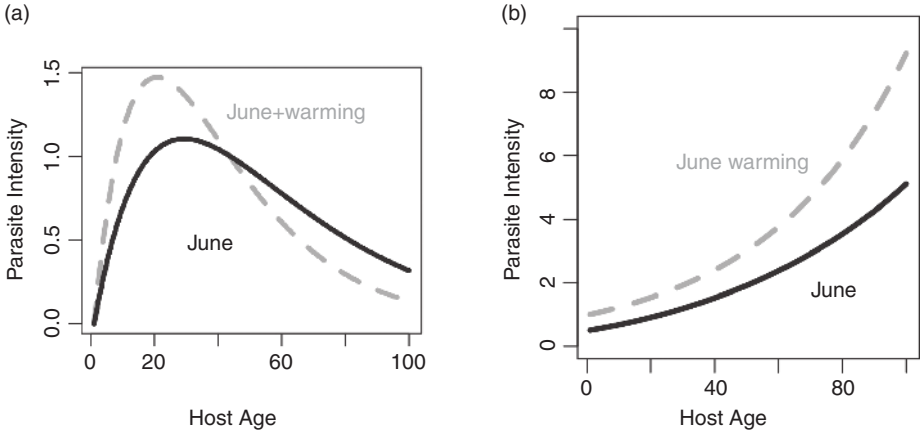

Figure 21.4 Hypothetical scenarios of mean parasite infection by host age in a host population from a month with (dotted line) and without (solid line) climate warming, and in the presence (a) and absence (b) of immune control.

accounted for the daily temperature and humidity available from the study site, and included the regulatory effects of host immunity and parasite density-dependence. Model simulations indicate that *T. retortaeformis* dynamics are affected by host immunity and the linear positive effect of mean air temperature on free-living stages, consistent with our experimental manipulations (Murphy et al., 2011; Hernandez et al., 2013). In the long term,

the mean intensity of infection remains relatively constant in the rabbit population as immunity controls the annual accumulation of parasites (Mignatti et al., 2016). In contrast, *G. strigosum* dynamics are constrained mainly by intensity-dependent ecological forces (i.e. competition for resources) and the linear positive impact of relative humidity on free-living stages. Over the years, *G. strigosum* mean intensity accumulates in the host population proportionately to the force of infection, although within-host parasite regulation and mortality of free-living stages prevent the parasite population from growing exponentially (Mignatti et al., 2016). Overall, these trends are consistent with a scenario where immunity suppresses the long-term positive effect of climate warming on the parasite population (the example of *T. retortaeformis*) (Figure 21.3a), which is observed if immunity has a weak impact on parasite regulation (the example of *G. strigosum*; Figure 21.3b). In other words, over the years, while climate warming increases the survival of free-living stages of both parasites on the pasture and thus, the force of infection, immunity, and to a significantly lesser extent parasite density-dependence, keeps the mean parasite burden and the associated shedding rate under control in the rabbit population.

We also investigated these patterns seasonally, by examining how changes in the host age–parasite intensity relationship were influenced by monthly changes in climate. Simulations were combined and compared between the 'cold' (1980–1989) and the most recent 'warm' (1993–2002) decade recorded in the study site (Hernandez et al., 2013). We showed that for *T. retortaeformis* an increase in the monthly temperature, from the cold to the warm decade, significantly shifted the peak of infection towards the younger hosts for the June and July rabbit cohorts (Mignatti et al., 2016). In other words, in the warmer decade rabbits born in July and June were exposed to a higher force of infection, and parasite accumulated even faster in the younger animals than rabbits of the same age-cohort from the colder period (Figure 21.4a). This faster accumulation means that the peak of infection was reached at younger age in the warmer than colder decade and supports the postulated effect of climate warming on the monthly shift in infection and transmission of *T. retortaeformis*. For *G. strigosum* we found no support for immune-driven changes in the age–intensity relationship among months, consistent with the lack or weak immune regulation of this parasite. *G. strigosum* accumulates with host age and adults consistently carry most of the infection; moreover, higher infections were observed in the summer months and hosts became infected at a faster rate in the warmer and more humid decade (Mignatti et al., 2016). These findings indicate that climate warming increases *G. strigosum* transmission and infection both within and between years, with adults carrying most of the burden (Figures 21.2b and 21.3b).

This work highlighted three main outcomes of the role of climate on the study system. First, intensity-dependence generated through host immunity is more effective in buffering the impact of climate warming on *T. retortaeformis* than is the intensity-dependence in population processes to *G. strigosum*. Indeed, although not fully effective and life-long protecting, immunity keeps *T. retortaeformis* mean infection relatively constant over the years, while the ecological processes of regulation of *G. strigosum* do not. Second, under climate warming monthly changes in the relationship between climate and host immune response to the infection increase parasite burden in younger rabbits, and amplify the variation in infection among individuals. Specifically, warmer conditions increase the survival of free-living stages, which then infect younger and less-immune-protected hosts with higher numbers. This is particularly important because it suggests that younger hosts with lower immunocompetence are at higher risk of infection with climate warming. Third, parasite species that stimulate a weak or no host immune reaction are more greatly influenced by climate changes and, as a result, hosts suffer higher infections across all age groups as conditions get warmer.

These general conclusions can be extended to other host–parasite systems, particularly involving soil-transmitted parasites, where host immunity can mitigate the impact of climate on infection and transmission. This also warrants attention when designing and predicting the success of anthelmintic treatments. Indeed, under climate warming and some level of host resistance, even more consideration should be given to reducing the infection of younger hosts.

21.3.2 Coinfection

The presence of one parasite can increase host susceptibility to a second parasite species, but can also trigger the pathogenicity of a parasite already present that would otherwise cause minor or no disease (Brady et al., 1999; Hudson et al., 2008). Moreover, by impairing the immuno-physiological functions of the host, coinfections can augment among-host variation in infection and shedding, and increase the likelihood of super shedding and/or super-spreading individuals (i.e. hosts that disproportionately shed a large number of parasites or infect secondary hosts) (Lloyd-Smith et al., 2005; Garske & Rhodes, 2008). Hence, the biology of infection is determined both by the nature and status of the primary infection, and the history of current and past coinfections.

Using a theoretical approach where we simulated changes in transmission (or invasion) of parasite species A, by varying the prevalence of parasite species B as well as host susceptibility, we showed that one parasite could potentially facilitate the transmission of a second parasite species, or the invasion by

a novel agent, if there is a positive covariation between infectiousness (i.e. ability of an infectious host to infect naïve individuals) and susceptibility (Graham et al., 2007). The extreme of this scenario is where highly coinfected individuals can lead to super shedding and/or super spreading events. These 'super-cases' have been identified in a growing number of studies of single infections, and are proposed to influence epidemic outbreaks and transmission (Lloyd-Smith et al., 2005; Chase-Topping et al., 2008). However, whether 'super-cases' generated from single or coinfections are responsible for the majority of disease spread remains contentious (e.g. Kao et al., 2007; Pathak et al., 2010; Lass et al., 2013). This is particularly problematic for parasitic helminths because fecundity and shedding could be negatively related to, or exhibit non-linear relationships with, the intensity of infection (Keymer, 1982; Quinnell et al., 1990; Tompkins & Hudson, 1999; Figure 21.5). Coinfections can modify these relationships by altering host responses and parasite traits to the point that we cannot predict the infection–transmission relationship based on the intensity of infection, or by examining these associations in single-infected individuals (Thakar et al., 2012). Tentatively, we suggest that coinfections are expected to modify the infection–fecundity–shedding relationship in parasites that are immune-regulated or in direct competition for resources.

We investigated patterns of infection and shedding in single and dual infections with our two gastrointestinal helminths, *T. retortaeformis* and *G. strigosum*, from our rabbit population sampled monthly from 2000 to 2014 in Scotland (Cattadori et al., 2014). We also report on patterns of infections from laboratory experiments (Murphy et al., 2011, 2013). Our general prediction is that coinfection with the second parasite can increase host heterogeneity in the burden of the focal parasite but can also generate non-linearities between infection and shedding such that the degree of variation in infection

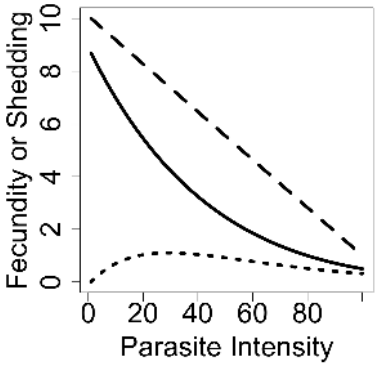

Figure 21.5 Examples of three potential relationships between parasite fecundity (or parasite egg shedding) and intensity of infection in helminths.

might not match the degree of variation in shedding. Field studies showed that compared to single-infected rabbits, coinfected hosts carried higher *T. retortaeformis* intensities, but those worms were shorter and females had fewer eggs in utero (Cattadori et al., 2014). Further investigations showed that the number of eggs in utero increased with parasite intensity in dual-infected but not in single-infected animals, and this was not caused by longer females but eggs that appeared to be produced at a faster rate by females of similar length. We also showed that for every rabbit both potential shedding (the total number of eggs in utero by the population of infecting females) and real shedding (the number of eggs in host's faeces) were comparable between single- and coinfected hosts. Therefore, for *T. retortaeformis*, single- and co-infected rabbits carried different parasite loads but shed a similar amount of eggs. This indicates that the intensity of infection cannot be considered a robust predictor of shedding for helminth coinfections, and that we cannot rely on single infections to predict patterns of infection and shedding in coinfections (Cattadori et al., 2014). Moreover, given the relatively lower parasite fecundity, and despite a positive fecundity–infection relationship, the probability that coinfected rabbits could become supershedders for this helminth appears relatively weak. This general trend is also highlighted when examining the host age–parasite intensity (or shedding) relationship. Indeed, while there are heterogeneities both in the intensity of infection and shedding among rabbits of different age classes, only intensity of infection was significantly different between single and coinfections, once host age was taken into account. This further supports the mismatch between infection and shedding, where the rabbits that carry the majority of the infection are not necessarily the ones that shed the most or can lead to supershedding events.

In the laboratory, rabbits were infected with a single dose of one or both helminths, and then sampled at fixed time. Findings showed that *T. retortaeformis* intensities decreased through the course of the trial and were comparable between single and dual infection (Murphy et al., 2013). The lack of a significant difference in the parasite burden between trials was partly caused by variation in the intensity of infection among rabbits both within and between sampling points. Compared to the natural settings and considering that the laboratory doses were estimated from natural infections, it is highly possible that host and environmental dissimilarities (i.e. age, sex, month of sampling, breeding status), besides differences in exposure, might have contributed to the disparities between laboratory and natural findings.

A similar exercise was performed for *G. strigosum.* In the field and compared to single-infected hosts, dual-infected rabbits harboured fewer *G. strigosum* but parasites were longer and females carried more eggs. Both the number of eggs in utero and female parasite body length were negatively related to intensity of infection and this trend was consistent in single- and coinfected animals

(Cattadori et al., 2014). Potential shedding (eggs in utero) and actual shedding (eggs in faeces) increased with the intensity of infection, and this was more pronounced in dual- than single-infected rabbits. Overall, we found that higher numbers of eggs shed in faeces were associated with lower intensities of infection in dual-infected rabbits. This is consistent with our previous observation that there is a discrepancy between intensity of infection and egg shedding, indicating that this pattern holds true also for helminths that are under a weak or no immune control, like *G. strigosum*. This trend was further confirmed when host age was taken into account; specifically, shedding, but not intensity of infection, was comparable among rabbits of the same age from single and dual infection (Cattadori et al., 2014). Therefore, and consistent with *T. retortaeformis*, these results support the hypothesis that we cannot predict shedding based on parasite burden and, as a corollary of this, there is a low probability that rabbits can become supershedders of *G. strigosum.*

In the laboratory, we found that dual-infected rabbits carried higher *G. strigosum* infections than single-infected hosts (Murphy et al., 2013), which is in contrast with field observations (Cattadori et al., 2014). As suggested for *T. retortaeformis*, the discrepancies between laboratory and field results are probably associated with confounding effects caused by variation in susceptibility and exposure of the wild hosts, as well as the impact of environmental factors.

Using two gastrointestinal parasites with contrasting dynamics of infection and host immune responses, we showed that the presence of the second species can increase host heterogeneity to infection. However, while we do see variation in shedding, this does not necessarily reflect the same degree of variation in infection. Previous work has suggested that the presence of a second parasite species can affect parasite sexual maturity, body size, or fecundity (Poulin, 2007). Our work is in line with these studies by showing that coinfections can alter host–parasite and parasite–parasite interactions, and contribute to host heterogeneity in infection and transmission. However, our work also suggests that the risk of supershedding events arising from coinfection by helminths is probably low. This is partly because of the intrinsic properties of helminths (e.g. sexual reproduction, different life stages) and their life-history strategies, and partly because of the complexities in host–parasite regulation.

As part of our work on coinfection, we also examined the role of these two gastrointestinal helminths on the dynamics of *Bordetella bronchiseptica* infection in the respiratory tract of laboratory rabbits. Parasites were dispensed in a single dose at the same time and rabbits were sampled at fixed times during 4-month-long trials. Dual infections with either *T. retortaeformis* or *G. strigosum* did not change the dynamics of *Bordetella* in the lower respiratory tract:

bacteria were removed from the lungs and trachea with no significant delay induced by the helminth (Pathak et al., 2012; Thakar et al., 2012). However, a higher bacterial load was found in the nose of rabbits dual-infected with *G. strigosum*. Modelling of the immuno-dynamics of *Bordetella–T. retortaeformis* infection in the lungs suggested that T helper cell–mediated antibodies and neutrophils led to phagocytosis and clearance of *B. bronchiseptica* (Thakar et al., 2012). This pattern should be also expected in the coinfection with *G. strigosum* (Pathak et al., 2012). The bacterium also affected the dynamics of the two helminths. Compared to single infections, *T. retortaeformis* was removed more quickly from the small intestine while *G. strigosum* persisted with similar high intensities (Pathak et al., 2012; Thakar et al., 2012). These findings reiterate our general conclusions that coinfection with a second species can increase host heterogeneity to infection, and also highlight the overlooked role of bacteria on helminth dynamics and the fact that the same parasite colonising different organs can exhibit different reactions during coinfections, namely, *Bordetella* in the lungs versus the nose.

In summary, in the epidemiology of multi-species infections there are many aspects of the infection–transmission process that remain poorly understood. Efforts should be directed to quantify the relationship between host infectivity and degree of shedding, including the conditions that can lead to supershedders and how immunity influences the duration of infection and, from here, the rate of shedding. The influence of host attributes on coinfection produces additional conflicting results that require resolution. For instance, parasite interactions can vary with host sex (Curtale et al., 2007; Allotey & Gyapong, 2008; Gao et al., 2010) and age (Brooker et al., 2007). However, these effects are likely confounded by group-specific risk factors or differences in exposure, in addition to changes in susceptibility due to the interplay between coinfection and host factors. Finally, there is a fundamental shortage of data on multi-species infections and, more crucially, data on the intensity of infection from specific organs if parasites colonise different parts of the host. Lastly, we need to be able to quantify the intensity of these infections; prevalence tells us how common a coinfection is in a host population but does not provide any insight on the within-host processes of parasite interaction that affect their intensity of infection, virulence, shedding, or disease severity.

21.3.3 Anthelmintic treatment

In areas endemic to helminth infections, anthelmintic drugs can only remove the parasites for a relatively short period of time. By stopping the treatment, parasite intensities can bounce back to pre-treatment levels (Sabatelli et al., 2008; Keiser & Utzinger, 2008; Jia, 2012), because protection is not complete or life-long and hosts can be reinfected if exposed to infective stages. However, while treatments can alleviate the severity of infection for a limited time, they

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can also affect parasite traits, like fecundity and growth, during the recolonisation and expansion of the parasite population between treatments. By disrupting the host–parasite interactions in the pre-treatment, and by impacting the parasite dynamics and life strategy during reinfections, anthelmintic therapies can influence transmission and risk of infection. For example, multiple reinfections following treatments of hosts with some level of resistance can lead to fast expulsion or slow accumulation of parasites but can also cause stunted growth and low fecundity because of a more reactive host immune response developed from previous infections (Stear & Bishop, 1999; Bleay et al., 2007; Luong et al., 2011). Yet, parasites can accumulate at the same rate and grow to the same size between treatments if there are no immune limitations and competition for resources is minimal, especially during the initial phase of recolonisation. Parasites can also develop resistance to drugs and experience minor changes in density (e.g. Geerts & Gryseels, 2000; James et al., 2009; Reynolds et al., 2016), or even adapt growth and fecundity to overcome the drug effects. Ultimately, by affecting parasite intensity and their traits, anthelmintic treatments can contribute to heterogeneities in host infection and parasite dynamics, including evolution.

How parasite traits relate to the intensity of infection, and how these relationships change before and after anthelmintic treatment, is still not completely clear. Two possible scenarios can be predicted (Figure 21.6). If parasites are controlled by the host immune response then we should expect differences in the dynamics of infection before and after treatment because of differences in the development and intensity of the immune reaction (Figure 21.6b). In other words, an immune response already stimulated in the pre-treatment should react faster (due to immune memory) and constrain the parasites more vigorously in the post-treatment. Alternatively, if parasites are regulated by within-host ecological processes, such as intensity-dependent competition for resources, then, all else being equal, the dynamics of infection should follow similar trajectories before and after treatment (Figure 21.6a).

We looked for evidence of these scenarios in laboratory experiments where rabbits were trickle-dosed every week with 400 *T. retortaeformis* infective stages, and treated with an anthelmintic halfway through the experiment before resuming the infection under the same regime a month later. Animals were sampled at fixed points to quantify parasitological and immunological data. We then used these results to develop a within-host state–space mathematical framework of the dynamics of infection that linked an observation model with a dynamical model (Ghosh et al., 2018). The observation model combines cross-sectional data on parasite intensity, body length, and fecundity (i.e. eggs in utero), with longitudinal data on egg shedding in hosts' faeces. The dynamic model describes the unobservable time progression of the

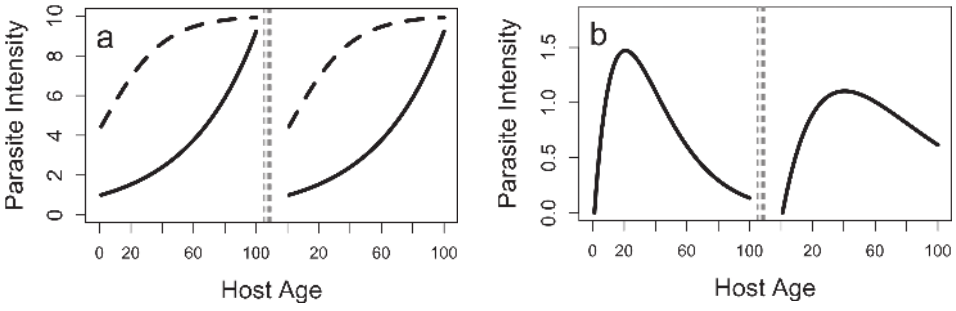

Figure 21.6 Relationship between host age and parasite intensity before and after anthelmintic treatment (shaded grey area), in (a) parasites that are not regulated (continuous line) or are controlled (dotted line) by intensity-dependent parasite constraints, and (b) parasites that are regulated by host immunity. Only the parasites that are immune-constrained show differences in the age-intensity profile before and after anthelmintic treatment.

parasite states. Our goal was to explain how host heterogeneities in the intensity of infection and shedding are generated before and after drug treatment and to identify the parsimonious processes driving parasite dynamics, namely, establishment and expulsion (Ghosh et al., 2018).

Simulations well reflected the empirical data. *T. retortaeformis* mean intensity of infection was comparable before and after treatment, but parasite accumulation was delayed by one sampling point and fecundity was lower in the post-treatment due to shorter worms that carried fewer eggs in utero (Ghosh et al., 2018). Before and after treatment, parasite dynamics were driven by the establishment of infective larvae and the relatively low clearance of adult stages, with both processes affected by accumulated exposure and adult intensities. Importantly, the extent of these processes was very rabbit-specific, to the point that we did not find significant differences between pre- and post-treatment when averages among rabbits were used. This confirmed the high variation in host–parasite interactions and outcomes among rabbits both within and between treatments. The evidence of a convex host age–parasite intensity relationship and slower accumulation of parasites post-treatment, combined with stunted worms and reduced shedding, supported our previous finding that *T. retortaeformis* is controlled by the rabbit immune response (Ghosh et al., 2018). This was confirmed by finding a significant negative effect of antibodies (IgA and IgG) on parasite length. Together, these findings are in agreement with our previous work (Cattadori et al., 2005; Murphy et al., 2011; Thakar et al., 2012; Mignatti et al., 2016). Overall, while it did not affect the mean parasite burden, the anthelmintic treatment modified the dynamics of parasite accumulation and individual growth, which then affected fecundity and shedding. The effect of this perturbation was in addition to the intrinsic variation among individual rabbits observed. Furthermore, the anthelmintic

treatment contributed to generate non-linearities in parasite dynamics, where similar mean intensities of infection before and after treatment were associated with lower shedding in the post-treatment. This mismatch suggests caution when using observations of shedding to predict intensities of infection between treatments, or vice versa. Importantly, while the dynamics of infection can be fundamentally similar between treatments, parasite life history can vary and contribute to heterogeneities in shedding and, thus, risk of infection.

Our work is consistent with previous studies that only reported on specific components of the parasite life cycle and dynamics (Audebert et al., 2000, 2003; Massoni et al., 2011). By combining experimental manipulations and detailed parasitological observations with a modelling approach, we provided a holistic view of the within-host dynamics of *T. retortaeformis* infection and how perturbation with an anthelmintic drug can influence specific components of the parasite life history (Ghosh et al., 2018). The general conclusion from our work is that parasitic reinfection in resistant hosts usually leads to delayed parasite accumulation and stunted parasite growth. Specifically, our modelling approach provides a mechanistic overview of these interactions, and highlights where heterogeneities in parasite dynamics emerge and how the counting on a restricted number of parasite variables (e.g. egg shedding only) might generate inaccurate conclusions on the dynamics of infection and related life-history traits.

Given the growing concern about the development of resistance to drug treatments, understanding the impact of these treatments on the ecology of infection, and how parasite traits adjust to perturbations, is important for providing alternative and possibly long-term approaches to the control of parasites while reducing the risk of infection. The evidence that treatments can have greater consequences on parasite development and/or fecundity than those on parasite numbers could be a possible avenue to explore, particularly given recent progress in molecular biology and diagnostic techniques.

21.4 Future directions

The mechanistic understanding of host–parasite interactions, and the recognition of the critical processes and factors within the host that drive dynamics of infection and transmission between hosts, continue to be the focus of much work on the ecology and evolution of infectious diseases. The challenge is to identify common features that can be captured by a few fundamental host responses and associated dynamics of infection. For example, it is apparent from the rabbit–helminth system that the relationship between intensity of infection and degree of shedding is often non-linear. Although this could be expected, for practical reasons this relationship is frequently assumed to be

linear, and we need to identify general rules that can relate infection to transmission more precisely. One approach would be to use an allometric correction that takes into account parasite size to quantify fecundity and shedding, and then to calculate the degree of host shedding based on the intensity of infection and the parasite sex ratio. Another possibility is to use an empirical approach that estimates the infection–shedding relationship under a range of scenarios, and then apply this information to similar systems. This knowledge is critical when estimating the risk of infection and also when managing helminth infections (e.g. informing on the selection of individuals to treat, or the treatment frequency, based on the degree of shedding).

Our work also emphasises that there are many sources of disturbance that can alter the intrinsic properties of the hosts and their parasites, and the way they interact. The challenge is to understand at what point they should be taken into account and how. For instance, given the widespread prevalence of multi-parasite infections, how parasite species interact with each other and influence their long-term persistence at the host population level remains an underdeveloped topic. Progress has been made in understanding the molecular processes of parasite interaction, particularly the modulatory role of host immunity. However, more work needs to be done to clarify how a second parasite species influences a parasite epidemiology. We showed that during a coinfection we cannot predict the dynamics of infection and shedding based on our understanding of single infection processes; likewise, we cannot predict how coinfections could affect the degree of variation among hosts. To address this, one possibility is to develop theoretical models on the epidemiology of multi-species infection based on parameter ranges that cover a variety of scenarios. More fruitfully, we need long-term data on parasite intensity and degree of shedding, rather than prevalence. These data are not always easy to obtain, especially if the coinfecting parasites are not causing disease or are difficult to quantify. To overcome this issue, one possibility is to base the priority of data collection on the endemicity and prevalence of the parasite, besides its level of disease severity. Laboratory experiments can complement population-level studies by informing on critical parameters and fundamental relationships, such as temporal changes in infection–shedding during the course of coinfection. Ultimately, we need more epidemiological models of coinfections that while describing specific systems can also embrace aspects and mechanisms of broader relevance to other settings and taxa.

Environmental disturbance, such as climate change and habitat disruption, influences host exposure. A large body of work has focused on disentangling how these drivers alter parasite dynamics and risk of infection. However, under the same environmental threats, some individuals are at higher risk than others because of differences in their susceptibility. Therefore, understanding how within-host processes affect host susceptibility and, in turn, how

this interacts with environmental changes is another important topic that needs further attention. The recent rapid advancement and low cost of many molecular techniques have made it possible to quantify many physiological and immunological components of the host, allowing exploration of how susceptibility (and related resistance and tolerance to infections) changes under environmental perturbations or extreme conditions. For systems where host and parasite populations can be experimentally manipulated over multiple generations, many of these interactions can be examined in the laboratory to provide accurate measurements over time and under controlled conditions. In the field, knowing critical molecular properties of the host, and how they vary under environmental perturbations, can inform on infection risk, identify individuals at higher risk, and inform how environmental changes can alter these patterns. In this respect there is no doubt that parasite treatments, like anthelmintic drugs, can be affected by environmental changes but also by the presence of a second infection or an individual's microbiome (Elias et al., 2006; Hayes et al., 2010; Blackwell et al., 2013; Pedersen & Antonovics, 2013). This suggests that many host–parasite processes could be addressed more broadly by embracing a more holistic approach to identifying major sources of variability and from here, better ways to control them. New tools are constantly becoming available to quantify host and parasite data; the general agreement is that we need a stronger combination of empirical and theoretical work to explain processes of infection from within to between hosts.

21.5 Conclusions

We used a rabbit–parasite system and a combination of field observations, laboratory manipulations, and analytical and modelling methodologies to examine how host heterogeneities to infection are generated (specifically, the contributions of climate change, multi-species parasitic infections, and anthelmintic treatments). We showed that the impact of these forces can generate unexpected outcomes, or relationships that tend to be ignored while actually being important sources of variation among hosts. More attention should be paid to these drivers and how they affect the dynamics of infection and persistence at the host population level while not dismissing the contribution of host susceptibility.

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References

Allen, J.E. & Maizels, R.M. (2011) Diversity and dialogue in immunity to helminths. *Nature Reviews Immunology*, **11**, 375–388.

Allen, J.E. & Sutherland, T.E. (2014) Host protective roles of type 2 immunity: parasite killing and tissue repair, flip sides of the same coin, *Seminars in Immunology*, **26**, 329–340.

Allotey, P. & Gyapong, M. (2008) Gender in tuberculosis research. *International Journal of Tuberculosis and Lung Disease*, **12**, 831–836.

Anderson, R.M. & Gordon, D.M. (1982) Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology*, **85**, 373–398.

Anderson, R.M. & May, R.M. (1978) Regulation and stability of host–parasite population interactions I: regulatory processes. *Journal of Animal Ecology*, **47**, 219–247.

Anthony, R.M., Rutitzky, L.I., Urban, J.F., Stadecker, M.J. & Gause, W.C. (2007) Protective immune mechanisms in helminth infection. *Nature Reviews Immunology*, **7**, 975–987.

Audebert, F., Cassone, J., Hoste, H. & Durette-Desset, M.C. (2000) Morphogenesis and distribution of *Trichostrongylus retortaeformis* in the intestine of the rabbit. *Journal of Helminthology*, **74**, 95–107.

Audebert, F. & Durette-Desset, M.C. (2007) Do lagomorphs play a relay role in the evolution of the Trichostrongylina nematodes? *Parasite*, **14**, 183–197.

Audebert, F., Vuong, P.N. & Durette-Desset, M.C. (2003) Intestinal migrations of *Trichostrongylus retortaeformis* (Trichostrongylina, Trichostrongylidae) in the rabbit. *Veterinary Parasitology*, **112**, 131–146.

Blackwell, A.D., Gurven, M.D., Sugiyama, L.S., et al. (2011) Evidence for a peak shift in a humoral response to helminths: age profiles of IgE in the Shuar of Ecuador, the Tsimane of Bolivia, and the US NHANES. *PLoS Neglected Tropical Diseases*, **5**, e1218.

Blackwell, A.D., Martin, M., Kaplan, H. & Gurven, M. (2013) Antagonism between two intestinal parasites in humans: the importance of co-infection for infection risk and recovery dynamics. *Proceedings of the Royal Society of London B*, **280**, 20131671.

Bleay, C., Wilkes, C.P., Paterson, S. & Viney, M.E. (2007) Density-dependent immune responses against the gastrointestinal nematode *Strongyloides ratti*. *International Journal for Parasitology*, **37**, 1501–1509.

Bowers, R.G. (1999) A baseline model for the apparent competition between many host strains: the evolution of host resistance. *Journal of Theoretical Biology*, **200**, 65–75.

Bourke, C.D., Maizels, R.M. & Mutapi, F. (2011) Acquired immune heterogeneity and its sources in human helminth infection. *Parasitology*, **138**, 139–159.

Brady, M.T., O'Neill, S.M., Dalton, J.P. & Mills, K. H. (1999) *Fasciola hepatica* suppresses a protective Th1 response against *Bordetella pertussis*. *Infection and Immunity*, **67**, 5372–5378.

Brogden, K.A., Lehmkuhl, H.D. & Cutlip, R.C. (1998) *Pasteurella haemolytica* complicated respiratory infections in sheep and goats. *Veterinary Research*, **29**, 233–254.

Brooker, S., Akhwale, W., Pullan, R., et al. (2007) Epidemiology of *Plasmodium*–helminth co-infection in Africa: populations at risk, potential impact on anemia, and prospects for combining control. *The American Journal of Tropical Medicine and Hygiene*, **77**(S6), 88–98.

Cattadori, I.M., Boag, B., Bjørnstad, O.N., Cornell, S. & Hudson, P.J. (2005) Immuno-epidemiology and peak shift in a seasonal host-nematode system. *Proceedings of the Royal Society of London B*, **272**, 1163–1169.

Cattadori, I.M., Boag, B. & Hudson P.J. (2008) Parasite co-infection and interaction as drivers of host heterogeneity. *International Journal for Parasitology*, **38**, 371–380.

Cattadori, I.M., Pathak, A.K. & Ferrari, M.J. (2019) Changes in helminth–host interactions under external disturbances: dynamics of infection, parasite traits and host immune responses. Under review.

Cattadori, I.M., Wagner, B.R., Wodzinski, L.A., et al. (2014) Infections do not predict shedding in co-infections with two helminths from a natural system. *Ecology*, **95**, 1684–1692.

Chase-Topping, M., Gally, D., Low, C., Matthews, L. & Woolhouse, M. (2008) Super-shedding and the link between human infection and livestock carriage of *Escherichia coli* O157. *Nature Reviews Microbiology*, **6**, 904–912.

Cizauskas, C.A., Turner, W.C., Pitts, N. & Getz, W.M. (2015) Seasonal patterns of hormones, macroparasites, and microparasites in wild African ungulates: the interplay among stress, reproduction, and disease. *PLoS ONE*, **10**, 0120800.

Cornell, S., Bjørnstad, O.N., Cattadori, I.M., Boag B. & Hudson P.J. (2008) Seasonality, cohort-dependence and the development of immunity in a natural host-nematode system. *Proceedings of the Royal Society of London B*, **275**, 473–591.

Curtale, F., Wahab Hassanein, Y.A., Barduagni, P., et al. (2007) Human fascioliasis infection: gender differences within school-age children from endemic areas of the Nile Delta, Egypt. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **101**, 155–160.

Duerr, H.P., Dietz, K. & Eichner, M. (2003) On the interpretation of age–intensity profiles and dispersion patterns in parasitological surveys. *Parasitology*, **126**, 87–101.

Elias, D., Akuffo, H. & Britton, S. (2006) Helminths could influence the outcome of vaccines against TB in the tropics. *Parasite Immunology*, **28**, 507–513.

Gao, L., Zhou, F., Li, X. & Jin, Q. (2010) HIV/TB co-infection in mainland China: a meta-analysis. *PLoS ONE*, **5**, e10736.

Garske, T. & Rhodes, C.J. (2008) The effect of superspreading on epidemic outbreak size distributions. *Journal of Theoretical Biology*, **253**, 228–237.

Geerts, S. & Gryseels, S. (2000) Drug resistance in human helminths: current situation and lessons from livestock. *Clinical Microbiology Reviews*, **13**, 207–222.

Ghosh, S., Ferrari, M.J., Pathak, A.K. & Cattadori, I. M. (2018). Changes in parasite traits, rather than intensity, affect the dynamics of infection under external perturbation. *PLoS Computational Biology*, **14**(6), e1006167.

Girgis, N.M., Gundra, U.M. & Loke, P. (2013) immune regulation during helminth infections. *PLoS Pathogens*, **9**, e1003250.

Graham, A., Cattadori, I.M., Lloyd-Smith, J., Ferrari, M. & Bjornstad, O.N. (2007) Transmission consequences of co-infection: cytokines writ large? *Trends in Parasitology*, **6**, 284–291.

Grenfell, B. T. & Anderson, R. M. (1989) Pertussis in England and Wales: an investigation of transmission dynamics and control by mass vaccination. *Proceedings of the Royal Society of London B*, **236**, 213–252.

Harvell, D., Altizer, S., Cattadori, I.M., Harrington, L. & Weil, E. (2009) Climate change and wildlife diseases: when does the host matter the most? *Ecology*, **90**, 912–920.

Hayes, K.S., Bancroft, A.J., Goldrick, M., et al. (2010) Exploitation of the intestinal microflora by the parasitic nematode *Trichuris muris*. *Science*, **328**, 1391–1394.

Hernandez, A.D., Poole, A. & Cattadori, I.M. (2013) Climate changes influence free-living stages of soil-transmitted parasites of European rabbits. *Global Change Biology*, **19**, 1028–1042.

Hoffman, R.S. & Smith, A.T. (2005) "Order Lagomorpha". In: Wilson, D.E. & Reeder, D. M. (eds.), *Mammal Species of the World: A Taxonomic and Geographic Reference* (3rd ed.). Baltimore, MD: Johns Hopkins University Press.

Hudson, P.J. & Dobson, A.P. (1989) Population biology of *Trichostrongylus tenuis*, a parasite of economic importance for red grouse management. *Parasitology Today*, **5**, 283–291.

Hudson, P.J., Perkins, S.E. & Cattadori, I.M. (2008) The emergence of wildlife disease and the application of ecology. In: Ostfeld, R. (ed.), *Infectious Disease Ecology: Effects of Ecosystems on Disease and of Disease on Ecosystems*, (1st edn, pp. 347–367). Princeton, NJ: Princeton University Press.

Izhar, R. & Ben-Ami, F. (2015) Host age modulates parasite infectivity, virulence and reproduction. *Journal of Animal Ecology*, **84**, 1018–1028.

Ashutosh Pathak, Department of Infectious Diseases,
The University of Georgia, Athens, GA, USA

Jackson, D.W. & Rohani, P. (2014) Perplexities of pertussis: recent global epidemiological trends and their potential causes. *Epidemiology and Infection*, **142**, 672–684.

Jackson, J.A., Friberg, I.M., Little, S. & Bradley, J.E. (2009) Review series on helminths, immune modulation and the hygiene hypothesis: immunity against helminths and immunological phenomena in modern human populations: coevolutionary legacies? *Immunology*, **126**, 18–27.

James, C.E., Hudson, A.L. & Davey, M.W. (2009) Drug resistance mechanisms in helminths: is it survival of the fittest? *Trends in Parasitology*, **25**, 328–335.

Jia, T.W., Melville, S., Utzinger, J., King, C.H. & Zhou, X.N. (2012) Soil-transmitted helminth reinfection after drug treatment: a systematic review and meta-analysis. *PLoS Neglected Tropical Diseases*, **6**, e1621.

Kao, R.R., Gravenor, M.B., Charleston, B., et al. (2007) *Mycobacterium bovis* shedding patterns from experimentally infected calves and the effect of concurrent infection with bovine viral diarrhoea virus. *Journal of The Royal Society Interface*, **4**, 545–551.

Keiser, J. & Utzinger, J. (2008) Efficacy of current drugs against soil-transmitted helminth infections: systematic review and meta-analysis. *Journal of the American Medical Association*, **299**, 1937–1948.

Keymer, A. (1982) Density-dependent mechanisms in the regulation of intestinal helminth populations. *Parasitology*, **84**, 573–587.

Lass, S., Hudson, P.J., Thakar, J., et al. (2013) Generating super-shedders: co-infection increases bacterial load and egg production of a gastrointestinal helminth. *Journal of the Royal Society Interface*, **10**, 20120588.

Lloyd-Smith, J.O., Schreiber, S.J., Kopp, P.E. & Getz, W.M. (2005) Superspreading and the effect of individual variation on disease emergence. *Nature*, **438**, 355–359.

Luong, L.T., Vigliotti, B.A. & Hudson, P.J. (2011) Strong density-dependent competition and acquired immunity constrain parasite establishment: implications for parasite aggregation. *International Journal for Parasitology*, **41**, 505–511.

Maizels, R.M. (2009) Parasite immunomodulation and polymorphisms of the immune system. *Journal of Biology*, **8**, 62.

Massoni, J., Cassone, J., Durette-Desset, M.C. & Audebert, F. (2011) Development of *Graphidium strigosum* (Nematoda, Haemonchidae) in its natural host, the rabbit (*Oryctolagus cuniculus*) and comparison with several Haemonchidae parasites of ruminants. *Parasitology Research*, **109**, 25–36.

McRae, K.M., Stear, M.J., Good, B. & Keane, O.M. (2015) The host immune response to gastrointestinal nematode infection in sheep. *Parasite Immunology*, **37**, 605–613.

Mignatti, A., Boag, B. & Cattadori, I.M. (2016) Host immunity shapes the impact of climate changes on the dynamics of parasite infections. *Proceedings of the National Academy of Sciences of the United States of America*, **113**, 2970–2975.

Miller, M. R., White, A. & Boots, M. (2005) The evolution of host resistance: tolerance and control as distinct strategies. *Journal of Theoretical Biology*, **236**, 198–207.

Molnár, P.K., Kutz, S.J., Hoar, B.M. & Dobson, A.P. (2013) Metabolic approaches to understanding climate change impacts on seasonal host–macroparasite dynamics. *Ecology Letters*, **16**, 9–21.

Murphy, L., Nalpas, N., Stear, M. & Cattadori I.M. (2011) The role of immunity on the dynamics of chronic gastrointestinal nematode infections of rabbits. *Parasite Immunology*, **33**, 287–302.

Murphy, L., Pathak, A.K. & Cattadori, I.M. (2013) A co-infection with two gastrointestinal nematodes alters host immune responses and only partially parasite dynamics. *Parasite Immunology*, **35**, 421–432.

Pathak, A.K., Boag, B., Poss, M., Harvill, E. & Cattadori, I.M. (2011) Seasonal incidence of *Bordetella bronchiseptica* in an age-structured free-living rabbit population. *Epidemiology and Infection*, **14**, 1–10.

Pathak, A.K., Creppage, K.E., Werner, J.R. & Cattadori, I.M. (2010) Immune regulation of a chronic bacteria infection and consequences for pathogen transmission. *BMC Microbiology*, **10**, 226.

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Pathak, A.K., Pelensky, C., Boag, B. & Cattadori, I.M. (2012) Immuno-epidemiology of chronic bacterial and helminth co-infections: observations from the field and evidence from the laboratory. *International Journal for Parasitology*, **42**, 647–655.

Paull, S.H. & Johnson, P.T.J. (2014) Experimental warming drives a seasonal shift in the timing of host–parasite dynamics with consequences for disease risk. *Ecology Letters*, **4**, 445–453.

Pedersen, A.B. & Antonovics, J. (2013) Anthelmintic treatment alters the parasite community in a wild mouse host. *Biology Letters*, **9**, 20130205.

Poulin, R. (2007) *Evolutionary Ecology of Parasites* (2nd edn). Princeton, NJ: Princeton University Press.

Quinnell, R.J., Medley, G.F. & Keymer, A.E. (1990) The regulation of gastrointestinal helminth populations. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, **330**, 191–201.

Raberg, L., Sim, D. & Read, A.F. (2007) Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. *Science*, **318**, 812–814.

Raffel, T.R., Romansic, J.M., Halstead, N.T., et al. (2013) Disease and thermal acclimation in a more variable and unpredictable climate. *Nature Climate Change*, **3**, 146–151.

Redpath, S.A., Fonseca, N.M. & Perona-Wright, G. (2014) Protection and pathology during parasite infection: IL-10 strikes the balance. *Parasite Immunology*, **36**, 233–252.

Restif, O. & Koella, J.C. (2004) Concurrent evolution of resistance and tolerance to pathogens. *The American Naturalist*, **164**, 90–102.

Reynolds, A., Lindström, J., Johnson, P.C. & Mable, B.K. (2016) Evolution of drug-tolerant nematode populations in response to density reduction. *Evolutionary Applications*, **9**, 726–738.

Roy, B.A. & Kirchner, J.W. (2000) Evolutionary dynamics of pathogen resistance and tolerance. *Evolution*, **54**, 51–63.

Sabatelli, L., Ghani, A.C., Rodrigues, L.C., Hotez, P.J. & Brooker, S. (2008) Modelling heterogeneity and the impact of chemotherapy and vaccination against human hookworm parasite. *Journal of the Royal Society Interface*, **5**, 1329–1341.

Stear M.J. & Bishop, S.C. (1999) The curvilinear relationship between worm length and fecundity of *Teladorsagia circumcincta*. *International Journal for Parasitology*, **29**, 777–780.

Stear M.J., Boag, B., Cattadori, I.M. & Murphy, L. (2009) Genetic variation in resistance to mixed, predominantly *Teladorsagia circumcincta* nematode infections of sheep: from heritabilities to gene identification. *Parasite Immunology*, **31**, 274–282.

Stear, M.J., Strain, S. & Bishop, S.C. (1999) Mechanisms underlying resistance to nematode infection. *International Journal for Parasitology*, **29**, 51–56.

Thakar, J., Pathak, A.K., Murphy, L., Albert, R. & Cattadori, I.M. (2012) Network model of immune responses reveals key effectors to single and co-infection kinetics by a respiratory bacterium and a gastrointestinal helminth. *PLoS Computational Biology*, **8**, e1002345.

Thompson, H.V. & King, C.M. (1994) *The European Rabbit*. Oxford: Oxford University Press.

Tompkins, D.M. & Hudson, P.J. (1999) Regulation of nematode fecundity in the ring-necked pheasant (*Phasianus colchicus*): not just density dependence. *Parasitology*, **118**, 417–423.

Van Kuren, A., Boag, B., Hrubar, E. & Cattadori, I. M. (2013) Variability in the intensity of nematode larvae from gastrointestinal tissues of a natural herbivore. *Parasitology*, **140**, 632–640.

Woolhouse, M.E.J. (1992) A theoretical framework for the immunoepidemiology of helminth infection. *Parasite Immunology*, **14**, 563–578.

Woolhouse, M.E. (1998) Patterns in parasite epidemiology: the peak shift. *Parasitology Today*, **14**, 428–434.

Yazdanbakhsh, M. & Sacks, D.L. (2010) Why does immunity to parasites take so long to develop? *Nature Reviews Immunology*, **10**, 80–81.