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Review

Maximising fitness in the face of parasites: a review of host tolerance☆

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ABSTRACT

Tolerance, the ability of a host to limit the negative fitness effects of a given parasite load, is now recognised as an important host defence strategy in animals. Together with resistance, the ability of a host to limit parasite load, these two host strategies represent two disparate host responses to parasites, each with different predicted evolutionary consequences: resistance is predicted to reduce parasite prevalence, whereas tolerance could be neutral towards, or increase, parasite prevalence in a population. The distinction between these two strategies might have far-reaching epidemiological consequences. Classically, a reaction norm defines host tolerance because it depicts the change in host fitness as a function of parasite load, where a shallow negative slope indicates that host fitness slowly deteriorates as parasite load increases (i.e., high tolerance). Despite the fact that tolerance was only recently acknowledged to be an important component in an animal's immune repertoire, it is frequently referenced, so our aim is to emphasise the current advances on the topic. We begin by summarising the ways in which biologists measure the two components of tolerance, parasite load and fitness, as well as the ways in which the concept has been defined (i.e., point and range tolerance). It is common to test for variation in host tolerance according to intrinsic, innate factors, where variation exists among populations, genders or genotypes. Such variation in tolerance is pervasive across animal taxa, and we briefly review some of the mechanistic bases of variation that have recently begun to be explored. Three further novel advancements in the tolerance field are the appreciation of the role of extrinsic, environmental factors on tolerance, host tolerance in multi-host–parasite systems and individual-based approaches to tolerance measures. We explore these topics using recent examples and suggest some future perspectives. It is becoming increasingly clear that an appreciation of tolerance as a defence strategy can provide significant insights into how hosts coexist with parasites.

Contents

1. Introduction 282
2. The x- and y-variables and their statistical relationships 282
   2.1. Resistance measures and their implications for tolerance 282
   2.2. Measuring fitness 283
   2.3. Statistical analyses 284
   2.4. Point vs. range tolerance 284
3. Mechanistic bases of tolerance 284
4. Environmental (extrinsic) factors influencing tolerance 286
5. Tolerance in multi-host–parasite populations or species combinations 287
6. Individual-based approaches 287
7. Outlook and conclusions 287
   Acknowledgements 288
   References 288

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1. Introduction

A host has two broad counter-strategies that it can employ when it encounters a parasite. The first strategy, resistance, can reduce the risk of parasitic infection by avoidance, improve host recovery time by clearing an infection, or control an infection by targeting a parasite's rate of replication (Best et al., 2008). However, animal researchers now recognise the importance of a second strategy, host tolerance. In the context of ecology and evolution, tolerance is the ability to limit the negative fitness effects of a given parasite load (Råberg et al., 2007; Best et al., 2008) without controlling the infection, and it results from the slope of the relationship between parasite load (x-axis; see Section 2.1) and fitness (y-axis; see Section 2.2) (Roy and Kirchner, 2000; Råberg et al., 2007; Graham et al., 2011) (Fig. 1A). On the other hand, resistance is described as an organism's ability to reduce parasite load (quantitative resistance) (Råberg et al., 2009; Graham et al., 2011; Schmid-Hempel, 2011) or to reduce the probability of infection (qualitative resistance) (Restif and Koella, 2004; De Roode and Lefèvre, 2012). Quantitative resistance is usually measured as the inverse of parasite load (Råberg et al., 2009) (Fig. 1A; see Section 2.1). In evolutionary terms, Darwinian fitness, an organism's ability to pass on its genes to the next generation, is what determines an organism's success over its lifetime. In light of this, a more resistant host that is better able to reduce parasite load is not necessarily the fittest and, conversely, a host with a relatively high parasite load can be comparatively fit (Råberg et al., 2009).

A particularly striking property of resistance and tolerance strategies is that they are predicted to have different evolutionary outcomes. Resistance interferes with within-host parasite survival and will reduce parasite prevalence in a host population. As parasite prevalence is reduced in a population, the fitness advantage of having the resistance gene will go down, resulting in a negative feedback loop (Roy and Kirchner, 2000), which can lead to antagonistic coevolution between host and parasite (Råberg et al., 2009; Graham et al., 2011; Schmid-Hempel, 2011). Tolerance, however, does not directly affect within-host parasite survival and its effect on parasite prevalence is predicted to be dictated by whether the host evolves mortality or fecundity tolerance (Best et al., 2008, 2010, 2014; Vale and Little, 2012). If tolerance means that hosts live longer (mortality tolerance), the parasite's infectious period is prolonged and parasite prevalence increases in a population. Therefore hosts with a gene conferring tolerance will have a fitness advantage and this gene will spread through a population, leading to a positive feedback loop (Roy and Kirchner 2000; Best et al., 2008). In contrast, fecundity tolerance is predicted to be neutral with respect to parasite fitness, but if fecundity tolerance comes at the cost of a reduced host lifespan, the parasite's infectious period will be reduced (Best et al., 2008).

Tolerance has a number of potentially important consequences for applied research in the veterinary, public health and medical sciences, and for conservation (Rohr et al., 2010; Doeschl-Wilson et al., 2012; Medzhitov et al., 2012; Hayward et al., 2014). For example, understanding individual variation in tolerance when infected (see Section 6) may have relevance for livestock breeding (Hayward et al., 2014), where fitness could refer to growth rate or litter size (Doeschl-Wilson et al., 2012). The public health sciences, and epidemiology in particular, can implement the analytical framework used in ecological studies of host tolerance, for example, to understand more about defence against diseases such as human immunodeficiency virus (HIV), which may lead to proposals for non-traditional treatment options or therapies (Regoes et al., 2014; see Section 3). There has been recent interest in designing therapeutics that focus on limiting damage (tolerance) rather than limiting parasite growth (resistance) directly, in the hope that these will not directly select for increased resistance on the part of the parasite, as has happened with some earlier medications (Vale et al., 2014). However, although this approach would have positive individual-level effects, Vale et al. (2014) sound a note of caution because the consequences of targeting damage limitation are not fully understood at the population level. Lastly, a fuller understanding of tolerance may be relevant for vulnerable taxa such as amphibians, where management programmes could select for tolerance, which, in contrast to resistance, is predicted to reduce selection on parasites to counter host resistance, i.e., to not select for antagonistic co-evolution (Rohr et al., 2010).

Our appreciation of the importance of tolerance in different hosts and contexts has grown since Råberg et al. (2009), Little et al. (2010), Baucom and de Roode (2011) and Graham et al. (2011) reviewed the topic. It is our aim in the present review to first provide a brief background on how resistance and tolerance are measured and then to highlight the recent advances in the field, namely, the mechanistic bases of tolerance, the role of extrinsic, environmental factors that contribute to variation in host tolerance, host tolerance in multi-host–parasite systems, and individual-based estimates of tolerance. Lastly, we propose future directions that might give additional insight into factors affecting tolerance.

2. The x- and y-variables and their statistical relationships

2.1. Resistance measures and their implications for tolerance

Resistance can be measured quantitatively or qualitatively (Restif and Koella, 2004; De Roode and Lefèvre, 2012). An individual with a greater parasite load is less resistant than an individual with a lower load in quantitative terms, and an individual that is better able to reduce the risk of an infection than another is more resistant in qualitative terms (Restif and Koella, 2004; De Roode and Lefèvre, 2012). The former is consistent with the majority of tolerance studies, where resistance is typically measured as the inverse of parasite burden (Råberg et al., 2007; Fig. 1A). We here use a broad definition of parasite, including, but not limited to, viruses, bacteria, fungi, protozoans, worms, and arthropods.

How do we measure resistance in practice? Table 1 details some of the resistance measures used within the framework of tolerance. The parasites in these studies vary widely from natural (Blanchet et al., 2010; Hayward et al., 2014; Jackson et al., 2014; Mazé-Guilmo et al., 2014; Regoes et al., 2014) to experimental infections (e.g., Vale and Little, 2012), and from coevolved parasites (e.g., Sternberg et al., 2013) to less well characterised parasites (Vincent and Sharp, 2014). Quantitative resistance quantification methods vary according to the host–parasite system, but quantification always involves a measure of parasite load. If some individuals avoid an experimental infection (qualitative resistance), then we could take advantage of this self-selected group of exposed but uninfected animals by comparing their fitness to that of unexposed controls because they might provide information about the cost of parasite exposure (Rohr et al., 2010). Observational field studies present different problems; if the infection dose is unknown and a host has fewer parasites, then we do not know if the host used an avoidance strategy to begin with or if it has superior quantitative resistance (Graham et al., 2011).

A resistance strategy can have three outcomes: (i) a host clears an infection, (ii) a stable infection persists within the host, or (iii) an infection leads to host death (Doeschl-Wilson et al., 2012). These can relate to fitness measurements as well as to interpretations concerning the tolerance strategy in question. For example, survival may be a good indicator of fitness if an infection leads to death within the experimental timeframe, but if fitness measures include fecundity, host mortality will result in the selection of a sub-set of

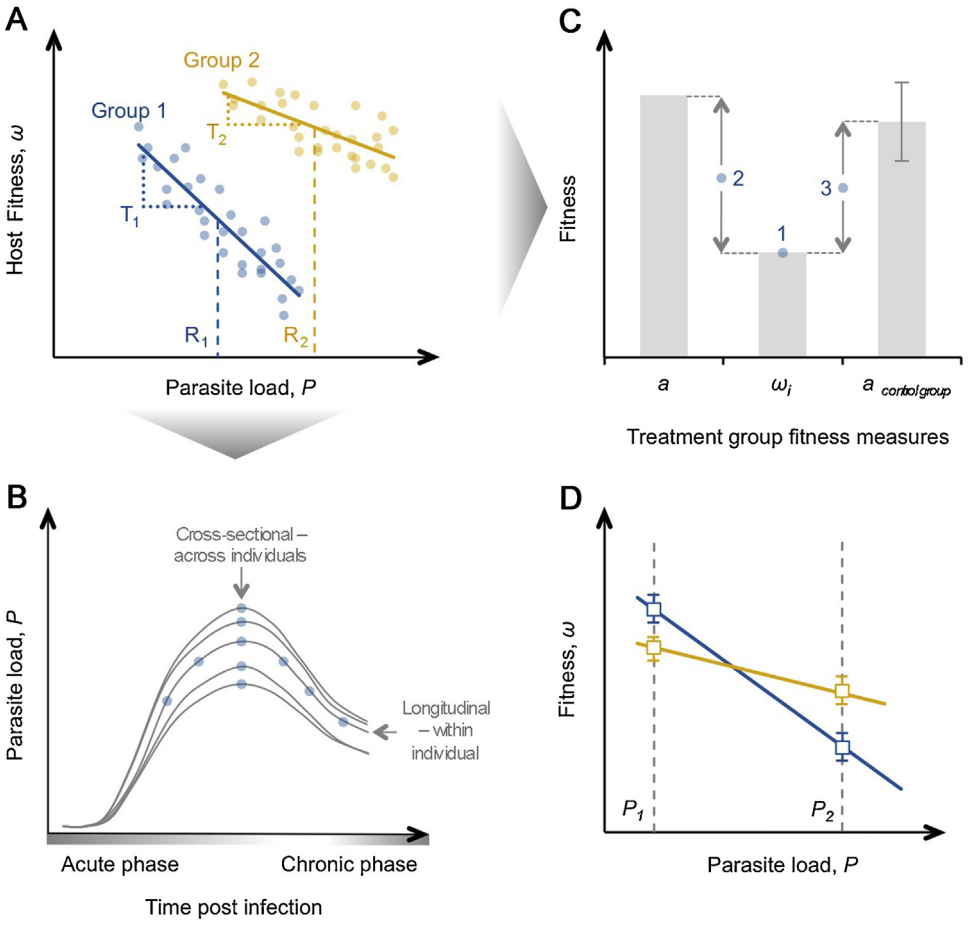

Fig. 1. Measuring tolerance, resistance and fitness. (A) Hypothetical relationships between parasite load (P) and host fitness (ω) for two groups (e.g., genotypes; see Table 1 for examples). The average resistance of group 1, R_1, is higher than that of group 2, R_2, because it has a lower parasite load. However, tolerance of group 2, T_2, is higher than that of group 1, T_1, because of the shallower slope of the reaction norm, meaning a lower loss of fitness for a given parasite load. Illustration modified from Råberg et al. (2007) and Regoes et al. (2014). (B) Parasite load can be measured at different times and in different ways (see Table 1). Each line represents an individual and the hypothetical relationship between time post infection and parasite load. The blue circles represent time points at which measures are taken. In addition to longitudinal studies where repeated measures have been taken from one individual over time, cross-sectional samples have been taken, e.g., during the acute or chronic phase of the infection. (C) Some ways in which fitness has been measured in the context of tolerance: 1, fitness when infected, ω_i; alternatively, the cost of infection ($\Delta\omega$) (Graham et al., 2011) can be measured as 2, $\omega_i - a$ (pre-infection fitness); or as 3, $\omega_i - a_{controlgroup}$ (mean fitness of uninfected control group). See Section 2.3 for statistical considerations. (D) In this example, the solid lines represent range tolerance, where fitness is measured over a range of parasite loads, and the white squares represent point tolerance. Mean group fitness is plotted for a low (P_1) and a high (P_2) parasite load. At P_1, group 1 (blue) has a higher point tolerance and at P_2, group 2 (yellow) has a higher point tolerance. Overall, group 2 has a higher range tolerance. Therefore, inferences about which group is more tolerant can, in some cases, depend upon whether point or range tolerance is measured. Illustration modified from Little et al. (2010).

experimental animals. Choosing the time(s) at which to assay parasite load is not trivial. A cross-sectional assay (Fig. 1B) measures resistance at one point during the infection. However, variation in resistance can be misinterpreted as tolerance if one group is faster to clear an infection than another (Råberg et al., 2009). To address this problem, Råberg et al. (2007) used peak parasite density and total parasite number, which led them to the same conclusions. However, in this mouse–malaria example, peak parasite density and total parasite number are correlated. A similar method may not produce analogous results in a different host–parasite system. Other studies tested different time points, corresponding to the acute and chronic phases of the infection (Fig. 1B), and showed that these can produce different tolerance estimates (Howick and Lazzaro, 2014). However, if the study is an observation-based field study it can be difficult, if not impossible, to obtain information on the particulars of the infection stage in the absence of knowledge about the time point of initial infection. A further complication is that individuals show variation in resistance over time, as demonstrated by individual infection trajectories (e.g., longitudinal study, Fig. 1B; cf. Section 6) (Lough et al., 2015); therefore, acquiring information on disease progression before assaying resistance and, subsequently, fitness, can be particularly useful.

2.2. Measuring fitness

In tolerance studies, the dependent variable, y, describes fitness (Simms, 2000; Fig. 1), and we use this notation herein. From a life history evolution perspective, fitness is "[t]he expected contribution of an allele, genotype, or phenotype to future generations . . ." (Stearns, 1992). However, what constitutes a fitness measure varies across tolerance studies, depending upon the context, question and logistical constraints (Rohr et al., 2010). For example, adult offspring counts are an informative fitness measure in a species with a short generation time like *Drosophila melanogaster*, but may be unrealistic in a long-lived mammal, where it is possible to instead obtain repeated measures of body weight or cell density. Therefore, "health" (Råberg et al., 2007) or "host performance" (Doeschl-Wilson et al., 2012) can also be used to describe the dependent variable. Host performance can include traits that are relevant for production, like growth rate or feed intake, because of the interest

of livestock breeders (Doeschl-Wilson et al., 2012). These health measures often come from long-lived mammals (e.g., Råberg et al., 2007; Hayward et al., 2014; Jackson et al., 2014; Regoes et al., 2014) (Table 1). On the other hand, studies using reproductive fitness as a dependent variable come from comparatively short-lived invertebrates like *D. melanogaster* or *D. magna* (e.g., Graham et al., 2011; Vale and Little, 2012; Howick and Lazzaro, 2014; Vincent and Sharp, 2014; Kutzer and Armitage, 2016) (Table 1). Using survival as a fitness measure (mortality tolerance; see Section 1) is widespread at the genotype level (e.g., Corby-Harris et al., 2007; Ayres and Schneider, 2008, 2009; Lefèvre et al., 2011; Sternberg et al., 2012; Howick and Lazzaro, 2014), but resistance cannot be quantified in a dead animal when the exact time of death is not known. The way in which fitness is measured can have consequences for the conclusions that are made concerning tolerance (see Section 1).

2.3. Statistical analyses

We here give a brief overview of statistical considerations and analyses commonly used in studies on tolerance. A reaction norm (see Fig. 1A) defines the fitness (ω) of group g as:

$$\omega_g = a_g + b_g \mathrm{P},$$

where a_g is uninfected host fitness of group g or the intercept, P is parasite load and b_g is the slope of the relationship between ω and P or the tolerance of group g (modified from Råberg et al., 2009). Variation in tolerance is statistically determined using an analysis of covariance (ANCOVA), where a significant interaction between genotype or experimental treatment and pathogen load indicates variation in tolerance between or among genotypes or treatments (Råberg et al., 2009). Since the definition of tolerance makes no supposition on fitness in the absence of infection (a), it does not need to be included as a covariate in a statistical model of tolerance (Graham et al., 2011). Indeed, in some cases it may be impossible to obtain pre-infection/uninfected fitness, a, for every individual in an empirical study. Rather than ignore uninfected fitness, it is prudent to include an uninfected control group where possible. In this case, the mean of the uninfected group or experimental control can be subtracted from each value of ω so that ω_g becomes $\Delta\omega$ (i.e. $\omega_i - \omega_0$), or the change in host fitness when infected (Graham et al., 2011) (Fig. 1C). In this example, the y-axis represents the cost of infection for a particular group, not infected fitness, and the intercept, a, is inferred from the response variable, $\Delta\omega$ (Graham et al., 2011).

Although widely implemented, the ANCOVA method estimates variation in group tolerance rather than individual variation in tolerance. Random regressions have been proposed as an extension of the ANCOVA method described above (Graham et al., 2011; Kause, 2011; Doeschl-Wilson et al., 2012; Kause and Ødegård, 2012) to assess individual variation in tolerance by including random intercepts as well as random slopes for each individual, in addition to including the fixed population means of slope and pathogen load (Graham et al., 2011; Kause, 2011; Doeschl-Wilson et al., 2012; Kause and Ødegård, 2012). However, random regressions will not work in every host–parasite system because they require repeated measures over time from the same animal. This method is covered in detail elsewhere (Graham et al., 2011; Kause, 2011; Doeschl-Wilson et al., 2012; Kause and Ødegård, 2012) and deserves more attention, as it has the potential to broaden our interpretation of host disease tolerance.

2.4. Point vs. range tolerance

In the present review the in-text examples are generally those concerned with *range* tolerance, where fitness is measured over a range of parasite loads (Little et al., 2010) (Table 1 and Fig. 1D). However, many studies use *point* tolerance (Adelman et al., 2013) (Table 1 and Fig. 1D), where mean group fitness is plotted against a single parasite load (Little et al., 2010). A number of authors highlight the importance of distinguishing between these two measures because they can lead to different conclusions about host tolerance (Råberg et al., 2009; Little et al., 2010; Graham et al., 2011). Little et al. (2010) discuss this problem in detail. In brief, if we measure point tolerance as mean fitness at one parasite load instead of measuring range tolerance, then our conclusion depends upon the point in the reaction norm at which we make the measurement if the reaction norms cross one another or are non-linear (Little et al., 2010; Fig. 1D). Adelman et al. (2013) performed the only study we are aware of to measure both point and range tolerance; house finches (*Haemorhous mexicanus*) were inoculated with a bacterial parasite, *Mycoplasma gallisepticum*, and different inferences could be reached depending on the tolerance measure used. We use the distinction between point tolerance and range tolerance (reaction norms) in Table 1 to discriminate between the two experiment types.

The majority of studies examining range tolerance have inoculated hosts with a single parasite dose. In the case of microparasites, load varies according to a combination of parasite replication rate and host defences (i.e., load is an uncontrolled outcome of the experiment; Graham et al., 2011), and it is these differences in load that provide the variation against which fitness is regressed. However, a few studies have varied parasite load by using different parasite inoculation doses in addition to using the variation in parasite load that arises from each dose (Graham et al., 2011; Lefèvre et al., 2011; Vincent and Sharp, 2014). The advantage of using different inoculation doses is that it gives the experimenter greater control. One does not need to rely on differential resistance, and hence potential variation in parasite load, to measure tolerance. In the interaction between Monarch butterflies and their natural protozoan parasite, *Ophryocystis elektroscirrha*, butterfly families were exposed to four parasite spore doses and spore load was measured in relation to adult lifespan and mass; parasite load and parasite dose produced similar results in the statistical models (Lefèvre et al., 2011). This approach would be useful to apply to other systems to examine whether reaction norms show non-linearity over wider dose ranges.

3. Mechanistic bases of tolerance

More often than not, biologists have characterised host tolerance according to intrinsic, innate factors, where variation exists among populations or genotypes (Table 1). But tolerance also can act as a function of intrinsic factors including sex or age, both of which influence disease susceptibility. It is interesting that biologists in different specialisations continue to uncover genotypic and population level variation in tolerance in a variety of taxa (Table 1), but studies of particular note are those that go beyond intra-population comparisons to identify the genetics or mechanisms underlying variation in tolerance.

It seems that the mechanisms underlying tolerance vary by study system (i.e. they are context-dependent). In *D. melanogaster*, phagocytosis mediates survival tolerance to a *Salmonella typhimurium* infection (Shinzawa et al., 2009), which may be a more generally applicable mechanism conferring tolerance to microparasites in insects. In vertebrates, the inflammatory response in concert with factors such as age or population, which we focus on here, may underlie genetic differences associated with variation in tolerance in natural host–parasite systems (Medzhitov et al., 2012; Adelman et al., 2013; Jackson et al., 2014).

Table 1
Summary of studies examining host tolerance. Studies are arranged by whether the tolerance measure (Tol) was a group mean/point tolerance (PT) or a reaction norm (RN). Experimental factors are categorised as extrinsic (E) or intrinsic (I), study types are categorised as cross-sectional (CS) or longitudinal (L), infections (Inf) are experimental (Exp) or natural (N). * These studies used reaction norms and point tolerance.

Tol	Host	Pathogen	Factor	Study type	Resistance		Fitness	Reference
					Inf	Measure		
RN	Invertebrates							
	Danaus plexippus	*Ophryocystis elektroscirrha*	I: genotype	CS	Exp	Inoculation dose and spore load	Survival	Lefèvre et al. (2011)
			E: diet	CS	Exp	Spore load	Survival	Sternberg et al. (2012)
			I: genotype/ population. E: parasite population	CS	Exp	Spore load	Survival	Sternberg et al. (2013)
	Daphnia magna	*Pasteuria ramosa*	I: genotype	CS	Exp	Spore load	Fecundity	Graham et al. (2011)
			I: genotype	CS	Exp	Spore load, proportion resistant	Fecundity	Vale and Little (2012)
	Drosophila melanogaster	*Pseudomonas aeruginosa*	I: genotype, sex	CS	Exp	Colony forming units	Fecundity	Vincent and Sharp (2014)
		Escherichia coli, Lactococcus lactis	E: diet	CS	Exp	Colony forming units	Fecundity	Kutzer and Armitage (2016)
	Mytilus edulis	*Mytilicola intestinalis*	I: population. E: parasite population	CS	Exp	Infection intensity	Dry weight of mussel flesh corrected for host length	Feis et al. (2016, this issue)
	Vertebrates							
	Bufo americanus, Rana clamitans	*Echinostoma trivolvis, Ribeiroia ondatrae,* plagiorchid species	I: species	CS	Exp	Trematode cysts	Time to death (survival)	Rohr et al. (2010)
	Haemorhous mexicanus	*Mycoplasma gallisepticum*	I: population	L	Exp	Point tolerance: peak parasite density Range tolerance: integrals of pathology and parasite load over time	Weight, eye lesion severity	Adelman et al. (2013)*
	Homo sapiens	Human immunodeficiency virus	I: genotype, age	L	N	Virus load	Change in CD4+ T cells	Regoes et al. (2014)
	Leuciscus leuciscus	*Tracheliastes polycolpus*	I: genotype	CS	N	Parasite load	Fin degradation	Blanchet et al. (2010)
	Microtus agrestis	Macroparasites	I: genotype, age	CS/L	N	Grouped macroparasite load	Body weight, testis weight, survival	Jackson et al. (2014)
	Mus musculus	*Plasmodium chabaudi*	I: genotype	CS	Exp	Peak parasite density	Minimum weight, minimum red blood cell density	Råberg et al. (2007)
		P. berghei, P. chabaudi	I: genotype	CS/L	Exp	Parasitemia	Tissue damage, survival	Gozzelino et al. (2012)*
	Ovis aries	Gastrointestinal nematodes	I: age	L	N	Strongyle burden: fecal egg counts	Weight	Hayward et al. (2014)
PT	Invertebrates							
	Apis mellifera	*Varroa destructor*, deformed wing virus, black queen cell virus, sacbrood virus, Kashmir bee virus	I: colony	L	N	Mite infestation rate, viral titers	Colony population/survival, brood production	Locke et al. (2014)
	D. melanogaster	*P. aeruginosa*	I: genotype	CS	Exp	Colony forming units	Survival	Corby-Harris et al. (2007)
		Escherichia coli, *Enterococcus faecalis, Streptococcus pneumonia, Listeria monocytogenes, Salmonella typhimurium, Burkholderia cepacia*	I: genotype	CS	Exp	Colony forming units	Survival	Ayres and Schneider (2008)
		L. monocytogenes, S. typhimurium, E. faecalis	I: genotype, E: diet	CS	Exp	Colony forming units	Survival	Ayres and Schneider (2009)
		Legionella pneumophila, L. monocytogenes, S. typhimurium, S. aureus	I: genotype	CS	Exp	Colony forming units	Survival	Shinzawa et al. (2009)
		Providencia rettgeri	I: genotype, E: diet	CS	Exp	Colony forming units	Fecundity, survival	Howick and Lazzaro (2014)
		Drosophila C virus, cricket paralysis virus, *Drosophila* X virus, invertebrate iridescent virus 6	I: genotype	CS	Exp	Viral titer and RNA per fly	Survival	Merkling et al. (2015)
	Vertebrates							
	Leuciscus burdigalensis	*Tracheliastes polycolpus*	I: families/ populations	L	N	Parasite load	Fin degradation	Mazé-Guilmo et al. (2014)
	M. musculus	*P. berghei*	I: genotype	L	Exp	Parasitemia	Brain edema, survival	Ferreira et al. (2011)
		Coinfection: influenza virus, *L. pneumophila*	I: genotype	CS/L	Exp	Colony forming units, plaque forming units	Survival, body temp., weight, albumin levels, histological damage, % inflammation per lung	Jamieson et al. (2013)

Logic suggests that parasitism by ticks, fleas and tapeworms might come at a fitness cost, but Jackson et al. (2014) suggest a more complicated relationship governing changes in host fitness in naturally infected field voles (*Microtus agrestis*). There was a dichotomous relationship between age and immune strategy: mature male field voles were less resistant to infection with macroparasites than immature males, but were more tolerant (Jackson et al., 2014). Moreover, older, more tolerant voles tended to carry a greater parasite load, so there was a positive relationship between parasite load and body condition. In addition, expression of GATA3, a transcription factor associated with T helper 2 (Th2) immunity, was positively associated with parasite load, liver condition, and body condition in mature males but was negatively associated with testis weight, a measure of reproductive effort. In mature male field voles, macroparasites drove GATA3 expression, which in turn resulted in improved body condition but decreased reproductive effort, suggesting that Th2 responses may play a role in tolerance to infections (Jackson et al., 2014). The study illustrates that there can be costs associated with tolerance.

Likewise, differences in early inflammatory responses are associated with tolerance to *M. gallisepticum* in two populations of house finches (Adelman et al., 2013). *M. gallisepticum* made the jump from chickens to house finches in the 1990s when it swept through finch populations in the eastern United States, causing population declines. It took ten years before the bacteria spread west of the Rocky Mountains (Adelman et al., 2013) so eastern populations have a longer coevolutionary history with the parasite. The two populations showed no differences in resistance, but birds from the eastern population exhibited higher point tolerance than birds in the western population. Pro-inflammatory cytokine expression was lower in the eastern population compared with the western population and the data suggest a link between increased tolerance and decreased pro-inflammatory cytokine signalling. These differences between populations point to a role for inflammatory signalling in tolerance to parasites in vertebrates and further suggest that tolerance can evolve quickly in a naïve population (Adelman et al., 2013).

Regoes et al. (2014) recently asked whether there is variation in tolerance to HIV in humans. HIV progression is associated with the depletion of circulating $CD4^+$ T (helper) lymphocytes, thus the rate of disease progression is related to the initial, acute infection period when an individual first develops flu-like symptoms in response to rapid viral replication in the plasma (Regoes et al., 2014). Viral load then falls and, after the appearance of specific $CD8^+$ T (killer) cells, stabilises at a certain point (set-point viral load) where it remains for years before $CD4^+$ T cells drop to such a level that symptoms appear (Regoes et al., 2014). In this study, the set-point viral load is denoted as parasite load and the change in $CD4^+$ T cells is denoted as host fitness, since the rate of change in these cells ultimately determines the rate of infection progression. The relationship between set-point viral load and $CD4^+$ T cell decline was non-linear. Age upon infection was a significant predictor of tolerance. Disease progression, measured as $CD4^+$ T decline, was more rapid in older individuals than in younger individuals (Regoes et al., 2014). Heterozygosity of an HLA-B allele combination was also associated with a slower rate of disease progression or greater tolerance (Regoes et al., 2014). Approaches detailing the effects of multiple factors on parasite tolerance, like those employed here, will help to uncover the underlying genetics and mechanisms associated with immune strategies. Additionally, they emphasise that a meaningful analysis of tolerance involves the identification of host immunopathology and parasite infection dynamics that are specific to the system under scrutiny. The existence of a general mechanism that determines host tolerance is unlikely; rather, we expect tolerance to vary as a function of several factors including parasite type, parasite load, age, sex, immunocompetence and various environmental components influencing plasticity.

4. Environmental (extrinsic) factors influencing tolerance

Variation within and between populations can be attributed to environmental or genetic components or a combination of both; thus, extrinsic factors such as diet, inoculation dose, temperature or co-infection with another parasite can modulate host–parasite interactions. To date, diet is the sole extrinsic factor shown to affect disease tolerance in experimental studies on insects (Table 1) (Ayres and Schneider, 2009; Lefèvre et al., 2011; Sternberg et al., 2012; Howick and Lazzaro, 2014; Kutzer and Armitage, 2016).

For example, monarch butterfly larvae become infected with *O. elektroschirra* spores as they feed on milkweed plants. The parasite replicates within the larvae and then forms spores around the developing adults. Ultimately, the spores are shed onto eggs and milkweed during oviposition by adult females (Sternberg et al., 2012). Although the parasite does not replicate on adult hosts, adult longevity and fitness are negatively affected by parasite spore load. Monarchs are dependent on milkweed for food and development. This plant can reduce *O. elektroschirrha* growth, probably due to a plant-produced toxic secondary chemical, cardenolide (De Roode et al., 2008). Therefore Sternberg et al. (2012) hypothesised that milkweed species would confer disease tolerance. They indeed found that cardenolide concentration conferred a fitness advantage to monarchs when ingested at intermediate levels, which mitigated the effects of an infection with *O. elektroschirrha* (Sternberg et al., 2012). This natural system is an elegant example of how food webs can influence tolerance to parasites, but changes in tolerance due to extrinsic factors can also occur in host–parasite systems that are not encountered in the field.

Although *D. melanogaster* is a natural host to many bacterial and fungal species, it is not clear for the majority of the known microbes whether they are parasitic or symbiotic (Keebaugh and Schlenke, 2014). Nonetheless, *D. melanogaster* exhibits plasticity in immune strategies when exposed to different insect and vertebrate bacterial parasites. Dietary manipulation uncovered or exacerbated these effects (e.g., Ayres and Schneider, 2009; Howick and Lazzaro, 2014; Kutzer and Armitage, 2016). When immune-challenged with *S. typhimurium*, flies on a reduced-nutrient diet had similar bacterial loads compared with flies on a standard diet, but were more tolerant to the infection in terms of survival (Ayres and Schneider, 2009). Conversely, diet affected resistance to *Listeria monocytogenes* but had no effect on survival tolerance (Ayres and Schneider, 2009). In this case tolerance was not described as a reaction norm; rather, tolerance was inferred from group survival combined with information on bacterial load (Ayres and Schneider, 2009). In a separate study, wild-type *D. melanogaster* reared on a reduced-protein diet exhibited an increase in fecundity tolerance in response to an *E. coli* infection as adults in a time-dependent manner (Kutzer and Armitage, 2016). The same host population showed no variation in tolerance after infection with the Gram-positive bacterium *Lactococcus lactis* in both dietary treatment groups, highlighting the importance of parasite species, diet, and time after infection in resistance and tolerance assays (Kutzer and Armitage, 2016). Clearly, the combination of resource availability and bacterial species can influence immune strategy utilisation in single populations of *D. melanogaster*, but whether or not these observations hold up in other host–parasite systems remains to be tested.

5. Tolerance in multi-host–parasite populations or species combinations

The tolerance framework may bring novel insights when applied to topics such as host–parasite coevolution. As discussed in Section 1, tolerance is predicted to have a neutral or positive effect on parasite prevalence. There should be no selection pressure on the parasite to overcome the tolerance of the host (Råberg et al., 2009), and tolerance will not result in antagonistic coevolution (e.g., Rausher, 2001). However, despite the lack of a negative effect on parasite fitness, the evolution of tolerance could result in different selection pressures on the parasite (Little et al., 2010). If tolerance increases parasite prevalence, there is a potential for multiple-genotype infections and within-host competition, which may select for increased virulence in the parasite population (Råberg et al., 2009; Choisy and de Roode, 2010; Baucom and de Roode, 2011). It is noteworthy that virulent parasites can be selected for even in the absence of multiple-genotype infections (Restif and Koella, 2003, 2004). Little et al. (2010) reviewed the effect of tolerance on coevolution in a theoretical context, and Sternberg et al. (2013) examined the effect of tolerance on coevolution in a natural host–parasite system. Local adaptation would predict that either hosts or parasites from a sympatric host–parasite population would have higher mean fitness, often measured as infection probability rather than host fitness, than allopatric host–parasite combinations (Kaltz and Shykoff, 1998; Hoeksema and Forde, 2008). In one of two separate reciprocal-cross experiments, monarch butterflies from an eastern North American and a Hawaiian population were infected with *O. elektroschirrha* originating from the sympatric or allopatric populations: neither host population tolerated local sympatric parasites better than allopatric parasites, but the Hawaiian population showed higher tolerance overall (Sternberg et al., 2013). Interestingly, sympatric and allopatric infections of a bivalve host, *Mytilus edulis*, with the macroparasite *Mytilicola intestinalis* showed similar results, i.e., that there was a main effect of host population origin on tolerance; in this example, one population evolved resistance and another population, tolerance towards the parasite (Feis et al., 2016). It may indeed prove useful to apply this framework to other natural host–parasite systems in the context of host–parasite coevolution.

To date, few studies have tested whether tolerance varies across host species towards the same species of parasites, or within host species towards different parasite species. However, Rohr et al. (2010) examined resistance and tolerance of American toads (*Bufo americanus*) and green frogs (*Rana clamitans*) experimentally exposed to three taxa of ecologically relevant trematodes. The hosts showed similar relative tolerance to two trematode taxa but different tolerance to a third taxon; in the latter case the green frog had higher relative tolerance compared to the American toad (Rohr et al., 2010). The authors suggested that green frogs were more tolerant to infection because of their longer larval period which increases the likelihood of trematode exposure in the field (Rohr et al., 2010).

6. Individual-based approaches

The majority of studies to date estimated tolerance based on measurements taken from multiple individuals at one time point during infection (cross-section; Fig. 1B and Table 1). This approach will give a snapshot of host performance, ignoring, for example, the effects of changing parasite load over time (Graham et al., 2011; Kause, 2011; Doeschl-Wilson et al., 2012; Kause and Ødegård, 2012; see Section 2.1). Instead, Kause (2011) proposed a statistical framework to estimate reaction norms on the individual level, with repeated longitudinal parasite load and fitness measures taken from an individual over time. In a longitudinal study, Hayward et al. (2014) estimated variation in tolerance to gastrointestinal nematodes among individual Soay sheep using individual reaction norms, which were modelled using random regressions (see Section 2.3). Body weight, a strong predictor of host fitness in this population, was negatively correlated with increasing parasite load. Furthermore, there was significant individual variation in the tolerance slopes of individual sheep. Therefore, although parasites can put an intense selection pressure on their hosts to evolve resistance mechanisms (reviewed in Schmid-Hempel, 2011), natural selection can also act upon individual variation in tolerance and appeared to be under positive selection, such that individual sheep that lost less weight with increasing parasite density had higher lifetime breeding success (Hayward et al., 2014). This approach allows us to understand more about the selective forces shaping tolerance (Graham et al., 2011; Kause, 2011; Doeschl-Wilson et al., 2012; Kause and Ødegård, 2012).

Reaction norms illustrate a simple linear or non-linear response of fitness regressed against increasing parasite load, but personalised health curves illustrate the entire course of an infection by plotting repeated measures of health and parasite load from a single individual in phase space (Schneider, 2011). Health curves or trajectories consider the direction and velocity of infection progression over time, in addition to the curve's shape (Schneider, 2011; Doeschl-Wilson et al., 2012). Nine general phase curves can explain the trajectory of host–parasite interactions (Schneider, 2011). Doeschl-Wilson et al. (2012) adapted the nine trajectories to a resistance–tolerance context and categorised them according to three infection types: (i) infections that are cleared, (ii) persistent, stable infections, and (iii) infections that lead to death. More recently, Lough et al. (2015) explored individual two-dimensional infection trajectories, showing that resistance and tolerance were co-expressed differently over the course of a *Listeria monocytogenes* infection in mice. The study demonstrated that both resistance and tolerance are dynamic traits that differ at the level of the individual. Each individual infection trajectory was a good predictor of survival in the infected mice. Mice that survived tended to express resistance earlier during the infection than non-survivors and expressed tolerance later during the infection, indicated by an increase in body mass. Moreover, each infection trajectory appeared to be genetically distinct, which, in future, could help to pinpoint the genetics underlying individual variation in resistance and tolerance to infections (Lough et al., 2015).

7. Outlook and conclusions

It is common to take both fitness and parasite load measures in studies that examine host–parasite interactions, but there have been few studies that specifically address tolerance. This is not for a lack of existing data. We suggest that these datasets could be revisited and analysed using a reaction norm (ANCOVA) approach and/or individual infection trajectories where applicable. In particular, longitudinal data collected in naturally coevolving systems will prove invaluable to our understanding of host immune strategies as well as to the counter-adaptations taken by the parasite.

2015 was the warmest year on record. As the world continues to warm at an alarming rate, causing drastic ecological changes, so too will emerging infectious diseases (EIDs) become more common, affecting both humans and wildlife (Van Hemert et al., 2014). If increasing temperatures favour parasite spread and abundance (e.g., in diseases such as malaria, dengue fever, chikungunya fever, and Zika virus infections, where the vector ranges are also favoured), and the secondary host organism employs a survival-tolerance strategy rather than a resistance strategy, the disease reservoir will likewise expand, increasing the chance of further

transmission. Despite the apparent relationship, we are unaware of any research that has addressed host tolerance in the context of climate change and what it may mean for disease transmission and spread.

The study of host tolerance in the context of animal ecology and evolution is an emerging field that continues to be tested in different contexts. Tolerance can be plastic over the lifespan of the infection, over the lifespan of the host, and in response to environmental cues. Just as hosts vary in resistance, variation in tolerance is a pervasive trait, given that the majority of published papers find support for it in some form or another. The existence of variation in tolerance is interesting, given that some theoretical models predict that tolerance will be driven to fixation in a population (e.g., Roy and Kirchner, 2000). Tolerance is proving to be a useful concept, but as others have noted before us, studies use the concept of tolerance differently (Section 2.4). Tolerance does have its limitations. For example, there can be a relatively weak relationship between parasite load and fitness (e.g., as discussed by Regoes et al., 2014), which is perhaps not surprising given the complex nature of intra-specific interactions, therefore it remains a challenge to explain the sources of variance around tolerance reaction norms. In this review we have taken a host-centred view of tolerance, but host fitness depends on a combination of traits from the parasite and the host. Indeed, it can be difficult or impossible to disentangle the two and establish causation (Little et al., 2010; Graham et al., 2011). Nonetheless, tolerance-centred research has the potential to provide insight into the ecology of host–parasite interactions and advances for immunology in general.

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