

*Review*

# Immune defence, parasite evasion strategies and their relevance for ‘macroscopic phenomena’ such as virulence

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The discussion of host–parasite interactions, and of parasite virulence more specifically, has so far, with a few exceptions, not focused much attention on the accumulating evidence that immune evasion by parasites is not only almost universal but also often linked to pathogenesis, i.e. the appearance of virulence. Now, the immune evasion hypothesis offers a deeper insight into the evolution of virulence than previous hypotheses. Sensitivity analysis for parasite fitness and life-history theory shows promise to generate a more general evolutionary theory of virulence by including a major element, immune evasion to prevent parasite clearance from the host. Also, the study of dose–response relationships and multiple infections should be particularly illuminating to understand the evolution of virulence. Taking into account immune evasion brings immunological processes to the core of understanding the evolution of parasite virulence and for a range of related issues such as dose, host specificity or immunopathology. The aim of this review is to highlight the mechanism underlying immune evasion and to discuss possible consequences for the evolutionary ecology analysis of host–parasite interactions.

**Keywords:** virulence; immune evasion; dose; transmission; pathogenesis

## 1. INTRODUCTION

Most living species are parasitic (Windsor 1988). Hosts, in turn, deploy their immune system to prevent infections or keep the parasites in check. The immune system is one of an organism’s most complex systems and shows many signs of coevolution with parasites. It is well tuned to its task, as otherwise long-lived multicellular organisms would probably not be able to survive and outpace their short-lived and numerous parasites. Nevertheless, some of the major questions in the field of evolutionary ecology have been to understand why immune responses are not always maximally efficient; in other words, why immune responses vary among host species and vary with many other factors, such as environment, stage of the host’s life cycle or infection by different parasite types. Furthermore, the corresponding question has also been asked with respect to parasites: why are they not always maximally infective or inflict maximum damage? These questions can obviously not all be answered here; instead, I focus on a particular aspect of the core issue: the role of parasite actions against the host’s defence systems.

Research over the last decade has provided evidence for the importance of a range of factors that account for such variation or less than maximal responses. With regard to immune defences, there are fitness costs of deploying or evolving a strong response (Schmid-Hempel 2003). Similarly, parasites cannot always exert

maximum virulence because there are costs of doing so. For example, the host may be killed before the full potential of transmission has been exploited (Bull 1994; Frank 1996). Additional variation results from the ‘matching’ of host and parasite genotypes, i.e. only some parasite genotypes are able to infect a particular host genotype and, vice versa, only some host genotypes are resistant to a particular set of parasite genotypes. The effect of genotype–genotype interaction is well documented (Schmid-Hempel & Ebert 2003); however, this description is a proxy for the deeper, underlying mechanisms that allow a parasite to infect and cause harm, or a host to be resistant to a parasitic infection. These mechanisms turn out to be highly interesting and relevant for a renewed discussion of these major topics.

Given that parasites interact at close range with the host’s immune system, that is, at the level of the molecules involved in defence, we should expect that parasites have evolved ways to interact with the host at this same level. This does not mean that other host defence mechanisms, such as behaviourally choosing a site to live or being active at certain times of day and season to avoid parasites, are not relevant, too. In fact, parasites have evolutionarily countered such strategies by, for example, adapting their transmission pathways to increase the chances of an encounter with the next host. In many cases, the behaviour of intermediate hosts is actively rigged by the parasite to meet the next host in its life cycle (Moore 2002). But one of the most exciting insights of recent years is the gathering evidence for the generality and multitude of

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mechanisms by which parasites evade the host's immune responses, gain entrance to tissues or by which they manipulate the signalling network of the immune system. Indeed, the mechanisms of immune evasion are mind-boggling in their subtlety and diversity, and have been described for all major parasite groups, such as viruses (Tortorella *et al.* 2000; Benedict *et al.* 2002; Orange *et al.* 2002; Yewdell & Hill 2002; Alcami 2003; Hewitt 2003), bacteria (Pieters 2001; Hornef *et al.* 2002; Young *et al.* 2002; Portnoy 2005), protozoa (Locksley 1997; Mosser & Brittingham 1997; Sacks & Sher 2002; Turner 2002) or various helminths (Blaxter *et al.* 1992; Grencis & Entwistle 1997). Here, I first illustrate these mechanisms and then discuss their relevance with respect to some of the major questions in the evolutionary ecology of host-parasite interactions.

## 2. THE DIVERSITY OF IMMUNE EVASION MECHANISMS

All immune evasion mechanisms are deeply entrenched in the fine details of the molecular machinery that regulates the immune response. This literature is generally not easy to access for evolutionary ecologists, but studying these details unravels the fascinating molecular war between a host and a parasite. To gain some understanding for this bewildering diversity, it is helpful to classify immune evasion mechanisms by kind and target as is done in the following and illustrated by a few examples.

### (a) *Classification by mode of action*

#### (i) *Passive evasion*

Parasites can passively evade the immune system in a variety of ways. For example, (i) parasites can hide away from the immune system by invading immune-privileged tissue such as the central nervous system or the eye (Bhopale 2003). Also some parasitoids place their eggs inside tissue such as the fat body that is not well patrolled by the host's immune system. (ii) Parasites can become 'invisible' to the immune system. This is, for example, achieved by shielding surface components as soon as they become opsonized by the host's immune system (e.g. in *Plasmodium*; Bloom 1979). (iii) Parasites can change their surface identity as the T-cells and antibodies of the vertebrate's immune system recognize specific epitopes (the antigenic surface of a parasite). The parasite escapes this recognition by changing its antigenic surface during the course of infection. Often, parasites store surface variants that are successively expressed; for example, *Plasmodium falciparum* has approximately 60 stored variants and *Trypanosoma brucei* has several hundred (Frank 2002; Sacks & Sher 2002; Turner 2002). Antigenic variation is also known from bacteria (phase shift; van der Woude & Bäumlér 2004; Barbour *et al.* 2006) and nematodes (Blaxter *et al.* 1992). Parasites can also evade by mutation of their epitopes. In HIV, for example, such mutants arise with sufficient frequency to outpace the immune system (Kent *et al.* 2005). (iv) The parasite can temporarily become inactive and so escape the immune system (quiescence). For example, bacteria can go quiescent with little or no metabolic activity and no cell division. They thereby avoid being blocked by antibiotics that target the cell

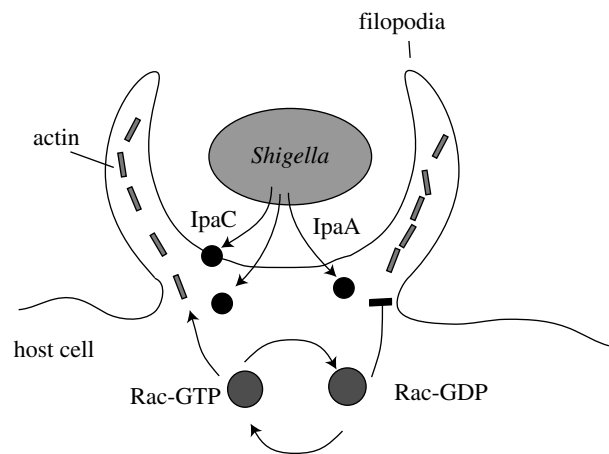


Figure 1. Infection by *Shigella*. *Shigella* (enteropathogenic *E. coli*) preferentially infects M-cells that are specialized epithelial cells overlaying Peyer's patches in the gut. For the infection process, the bacteria use a range of proteins to invade the host cells, with some delivered by a type III secretion system that acts as a needle and syringe. Additional proteins include IpaA (also an injected protein) interfering with host vinculin. IpaB and IpaC become integrated into the host cell membrane (Donnenberg 2000) and (together with another bacterial protein, IpgD) induce cytoskeletal rearrangements, pore formation and surface extensions (filopodia and lamelliopodia), which facilitate cell entry by an endocytic vacuole. This surface change formation can be blocked by Rac-GDP. Later, bacterial proteins induce lysis of the vacuole such that the parasite is released into the host cell cytoplasm (Donnenberg 2000). Inside, the IcsA protein on the surface of the bacterium manipulates the host cell's actin-driven motility apparatus. As a result, the parasite is pulled through the cytoplasm by the host's own apparatus, and eventually reaches another cell wall where protrusions are induced that are engulfed by the neighbouring cell; this allows the further spread to the next cell (a similar system is used by *Listeria monocytogenes*, *Rickettsia* and vaccinia virus; Donnenberg 2000).

replication step (Lewis 2007). Also, viruses, such as herpes simplex virus, are capable of entering a state of latency during which the synthesis of viral proteins is massively downregulated (Kapadia *et al.* 2002).

#### (ii) *Active modulation and interference*

The major group of immune evasion mechanisms implies active interference with the host's immune responses. In particular, parasites commonly interfere with the regulatory network that orchestrates the various arms of the immune defence. But parasites also interfere with basic functions of the host's cells. For such interference, parasites produce or code (in the case of viruses) for molecules that are able to block or modulate specific steps in the host's immune response, as well as general cellular functions that are crucial for host defence (e.g. cell motility). These modulatory molecules are deployed in different ways. For example, bacterial adhesins and invasins that manipulate host cells to facilitate entry of the bacteria can be membrane-bound proteins (e.g. *Streptococcus pyogenes* (*Streptococcus A*); Mitchell 2003). A number of bacteria, by contrast, inject their modulatory proteins directly into the host cell by specialized secretion systems such as the type III secretion system (TTSS, e.g. *Shigella* injects Ipa proteins; Salyers & Whitt 2002; figure 1).

During the course of coevolution with their hosts, some parasites, the viruses in particular, have captured genes from their hosts to produce molecules that disarm host immunity (Howell 1985; Barry & McFadden 1997; Damian 1997). These genes code for 'natural' host molecules that regulate the host's immune response (host cytokines becoming virokines; Kotwal & Moss 1988), or host molecules acting as decoy receptors and thus impeding the immune response (host receptors becoming viroreceptors; Upton *et al.* 1991).

### (b) Classification by targets of immune evasion

Parasites have evolved a variety of mechanisms to overcome immune defences at every step of the interaction (Sansone & Di Santo 2007). Parasites evade the first step of the immune response, i.e. the recognition step, with active and passive mechanisms as already mentioned above. Passive avoidance of recognition is shown by bacteria that modify or shield their PAMPs (parasite-associated molecular patterns)—key surface elements that the immune system recognizes (Hornef *et al.* 2002). Active evasion of recognition is illustrated by schistosomes, which produce C-type lectins that can sequester the host's recognition tags (Loukas & Maizels 2000). Mouse cytomegalovirus generates products that bind MHC I class molecules and therefore block proper recognition (Tortorella *et al.* 2000). Several other viruses also produce decoy MHC molecules that interfere with antigen presentation by the host's immune system and so prevent the attraction of helper and killer cells that would otherwise remove the infection (Murphy 1993; Yewdell & Hill 2002). *Plasmodium*, schistosomes and nematodes have been reported to produce competing ligands to impede recognition by the host (Blaxter *et al.* 1992; Locksley 1997). Finally, signals that give away the presence of the parasite are camouflaged or scavenged (e.g. vaccinia virus; Tortorella *et al.* 2000; Yewdell & Hill 2002; Seet *et al.* 2003).

One of the first reactions of the vertebrate immune system when an infection is recognized is the activation of complement. Complement is part of the innate immune system and consists of serum proteins and cell membrane receptors that act together to kill an invader. It is activated by three different biochemical cascades, which are targeted by parasites. *Leishmania*, for example, inhibits the complement cascade by degrading host proteins or by active release of signalling compounds (Nunes *et al.* 1997). In hepatitis C, specific viral products bind to complement and disable T-cells (Hahn 2003). Parasites also often interfere with the complement attack complex by preventing its binding to the parasite membrane (as in *Streptococcus* (Mitchell 2003) or *Staphylococcus aureus* (Rooijackers *et al.* 2005)). As a vertebrate's immune response proceeds, immune cells (e.g. polymorphonuclear cells such as neutrophils) are activated, which can phagocytose and kill an invader. Again, this step is interfered with by parasites. For example, *S. aureus* inhibitory protein (CHIPS) binds to receptors of neutrophils and blocks their engagement. Pneumococci escape the extracellular nets released by neutrophils to trap and kill bacteria by means of a surface endonuclease that degrades the

DNA scaffold of these nets, and so are able to spread into tissues and the bloodstream (Beiter *et al.* 2006). Furthermore, many parasites prevent the recruitment of polymorphonuclear cells to the site of infection by interfering with signalling, for example, by reducing the production of TNF- $\alpha$  (as in *Blastomyces dermatitidis*) or by inhibitory proteins binding to appropriate receptors of host cells (such as the CHIPS protein of *S. aureus* on the C5a receptor of host neutrophils; Urban *et al.* 2006).

In vertebrates, macrophages are phagocytic white blood cells that are part of the innate and adaptive immune system; they also further activate the cells of the defence system. Parasites have evolved a variety of ways to evade macrophages and other immune cells, for example, by modulating the host's cell cytoskeleton to block proper phagocytosis. As an example, Yops proteins are deployed by *Yersinia pestis* to interfere with macrophages (Hornef *et al.* 2002); *Shigella* induces apoptosis in macrophages with proteins (e.g. IpaB) that also affect host cell shape (Hilbi *et al.* 1997). Similarly, many viruses, e.g. myxoma virus, adenovirus, vaccinia virus, interfere with host cell apoptosis—a process critical for many aspects of defence (Tortorella *et al.* 2000; Guidotti & Chisari 2001; Benedict *et al.* 2002; Seet *et al.* 2003). Bacteria can also prevent being transported into the cell lysosome (where they would be degraded), or can escape from there into the cytoplasm of phagocytes by releasing pore-forming proteins. *Listeria monocytogene*, for example, produces a lysin that allows it to escape from the vacuole into the cytoplasm and to subsequently spread further from cell to cell (Portnoy *et al.* 2002).

Parasites also prevent immune cell fusion by retaining crucial host signals (Pieters 2001; Young *et al.* 2002) and many specifically manipulate the internal organization of cell vacuoles. For instance, *Mycobacterium tuberculosis* can actively arrest the development of the phagosome, thus evading antimicrobial effects and so persist in its host cells for extended periods (it also interferes with antigen-presenting mechanisms; Flynn & Chan 2003). Similarly, *Salmonella* prevents the delivery of (toxic) oxidase into its self-made vacuole (Underhill & Ozinsky 2002), while *Mycobacterium*, *Legionella*, *Coxiella* and *Chlamydia* interfere with vacuole maturation in their host cells (Underhill & Ozinsky 2002; Young *et al.* 2002). *Toxoplasma* modifies its host vacuole membrane with its own proteins to prevent further immune responses (Sacks & Sher 2002). *Leishmania* and *Toxoplasma* downregulate apoptosis to prolong cell life for prolonged own development, perhaps by the production of homologues of regulatory proteins (Sacks & Sher 2002).

A functioning immune response is dependent on its signalling network. In response to an infection, host signalling molecules, such as cytokines, chemokines or interferons, are produced by various cells. Parasites, in turn, interfere with this signalling in many ways. For example, *Yersinia* Yops downregulate the expression of TNF- $\alpha$ , one of the most important cytokines, and so block inflammation. *Leishmania* inhibits (interleukin) IL-12 in dendritic cells (DCs) and macrophages, but leaves other pro-inflammatory cytokine pathways (such as NF- $\kappa$ B) intact; it also induces IL-10 to avoid



clearance (Reiner & Locksley 1995; Sacks & Sher 2002). Viruses modulate the TNF family of receptors by producing homologues (Seet *et al.* 2003) and modulate cytokine pathways in natural killer cells (Orange *et al.* 2002). Many more examples have been described for viruses (Guidotti & Chisari 2001; Chatterjee *et al.* 2002; Seet *et al.* 2003), bacteria (Hilbi *et al.* 1997; Hornef *et al.* 2002; Portnoy 2005) or nematodes (Grencis & Entwistle 1997).

The immune system of vertebrates is efficient, since the adaptive response, in particular, can target specific infections rather than using a generalized response. In this context, DCs play an important role for innate and adaptive responses by stimulating the proper kind of T-cells and releasing chemokines and cytokines to recruit further defence cells. These cells are targeted by parasites, too. For example, *Francisella tularensis* and *Coxiella burnetii*, two highly dangerous bacterial pathogens, suppress the release of cytokines and prevent the maturation of DCs such that they do not become functional (Maurin & Raoult 1999; Bosio & Dow 2005). *Yersinia* infects DCs and reduces cytokine production (Brubaker 2003). Furthermore, bacteria can impede MHC class II expression (normally responsible for the presentation of antigens on an infected cell's surface). Viruses can retain class I molecules in the cell by the subversion of host's protein degradation or trafficking pathways. Viruses therefore downregulate CD4 activity, NK-cells and thus also inhibit cytokine action (Ploegh 1998; Tortorella *et al.* 2000; Guidotti & Chisari 2001; Hewitt 2003). In all of these instances, the specific adaptive response is inhibited by the parasite. Many parasites can also lodge in phagocytic host cells that would normally engulf and destroy them. For killing, the host's cells produce reactive oxygen radicals, change the pH in the parasite-holding vacuoles or mobilize degrading proteases. Immune evasion factors play a prominent role in allowing the parasites to overcome these defence mechanisms (Sacks & Sher 2002). Such mechanisms are limited to neither animal hosts (e.g. also occur in plant hosts; Nomura *et al.* 2006) nor any one parasite group (e.g. are also deployed in protozoa or fungi; Rappleye & Goldman 2006).

Any immune response will eventually deploy mechanisms that are able to control, contain or kill an invader. Thus, a variety of killer cells, phagocytes, reactive oxygen species or antimicrobial peptides are the eventual means by which a parasite is killed by the host. Parasites, in turn, deploy a variety of means to neutralize these effectors. For example, *M. tuberculosis* evades reactive toxic molecules by catabolizing them (Flynn & Chan 2003). *Pseudomonas* produces proteases and lipases that cleave immunoglobulins (Kharazmi 1991), while many bacteria degrade antimicrobial peptides or reduce their efficacy, e.g. by reducing the negative electric charge of the cell membrane (*Staphylococcus*), or by modifying surface molecules needed for attachment (*Salmonella*); such modifications are known to correlate with virulence (*Typhimurium*) (Hornef *et al.* 2002). Some bacteria, such as *S. aureus*, produce so-called superantigens that cause systemic effects due to their overwhelming effect of stimulating inflammatory cytokines. At the same

time, the binding stops T-cells from proliferating and impairs antibody production (Maillard *et al.* 1997).

Comparing the immune evasion mechanisms of different parasite groups shows a remarkable degree of parallel evolution, for example: trypanosomes and fungi use equivalent signals to target the host and deliver modulating factors in similar ways (Haldar *et al.* 2006); different bacterial parasites are targeting the same elements (e.g. the Rho protein) of the regulatory cascade (Krüppel-like transcription factors, KLF), which affect the expression of pro-inflammatory cytokines, phagocytosis and cell proliferation (O'Grady *et al.* 2007); or different parasites mimic the same host proteases (Sikora *et al.* 2005).

### 3. PATHOGENESIS

Pathogenesis is a process based on physiological, biochemical or molecular mechanisms, and which leads to harmful effects for the host, for example, to the depletion of its resources, tissue destruction, detrimental changes in behaviour, reduced fecundity, premature death and so forth. A general evolutionary ecological definition of virulence is considered to be a reduction in host fitness as a consequence of the infection by a parasite. 'Virulence' in a more narrow but commonly used sense refers to an increase in host mortality. Hence, pathogenesis (as used here) is the major process by which virulence is generated. Therefore, pathogenesis is the mechanism responsible for generating the virulence effects that are of interest for evolutionary ecologists. The respective mechanisms can be very different, however. The biomedical, veterinary or parasitological literature lumps them altogether under the term 'pathogenesis'. Unfortunately, in the discussions on the evolution of virulence, the relationship of pathogenesis to virulence has generally been treated as a black box (Levin & Svanborg Eden 1990; Weiss 2002, but see Graham *et al.* 2005). Yet, this black box may actually hold promise for an improved understanding of the evolution of virulence because pathogenesis is often due to the effects of parasite immune evasion as the following two examples may illustrate. Heuristically, the mechanisms of pathogenesis can be grouped into several categories (table 1).

When considering the mechanisms of pathogenesis, 'virulence factors' are particularly instructive. These are genetic elements, mostly described from bacteria and viruses, which encode proteins or cell surface elements whose presence is associated with pathogenic effects. Typically, these factors help the parasite to invade the host and to spread within its tissue. Depending on their precise function, virulence factors can be adhesins (factors that allow the parasite to attach to the host's surfaces), colonization factors (allowing the parasite to survive in a difficult host environment, such as the acid stomach in the case of *Helicobacter pylori*; Atherton 2006), invasins (aiding the parasite's spread through the host body but outside the cells, such as in *Streptococcus aureus*) or, more generally, are considered to be toxins with a general strong adverse effect on the host (Woolard *et al.* 2007). In many respects, toxins act as enzymes and are, in general,

Table 1. Mechanisms of pathogenesis.

category	mechanisms	example of pathogenetic effect	reference
impairing capacities	presence of a parasite leads to the loss of full functionality for important capacity; associated tissue damage not a main effect; many parasites induce behavioural changes that impair the host's capacity to function normally	mites lodging in one ear of nocturnal moths impair the hearing of hunting bats; trematodes on the gills of fishes reduce water flow and generate respiratory failure; phototactic behaviour that leads to host death by predators acting as the next host of the parasite	Ebert & Herre (1996), Moore (2002) and Reed <i>et al.</i> (2002)
destruction of tissue	a parasite destroys critical or a large mass of tissue, which leads to the failure of organs and eventual host death	parasitoids consuming internal tissues or organs of the host; haemorrhagic viruses cause necrosis and failure of the vascular system	Godfray (1994) and Mahany & Bray (2004)
virulence factors	mainly described from bacteria and viruses, but also known from protozoa and fungi; this general category includes the toxins (below)	various mechanisms, such as disruption of host cell cytoskeleton, cytokine signalling, neutralization of host defences; often associated with severe necrosis of tissues or inflammatory processes; pathogenic effect correlated with the presence and expression of a virulence factor	Wilson <i>et al.</i> (2002), Dustin & Rice (2006) and Rappleye & Goldman (2006)
toxins	secreted proteins (exotoxins) or components of cell walls (endotoxins and enterotoxins) that allow a pathogen to invade and spread within the host; toxins have high biological activity and act as enzymes	cytotoxins lead to apoptosis and tissue necrosis; disruption of cytokine functioning causes fatal septic shock	Wilson <i>et al.</i> (2002), Fukao (2004), Moayeri & Leppla (2004) and Abrami <i>et al.</i> (2005), Lapaque <i>et al.</i> (2005)
proteases	enzymes described from all major parasitic groups, aid in breaking into tissues and across cell membranes	similar role as toxins; proteases are antigenic and can induce inflammation and other severe pathogenic effects	McKerrow <i>et al.</i> (2006)
response exhaustion	parasites deplete the host's immune response in various ways; antigenic variation is a major mechanism by which a pathogen persistently changes epitopes recognized by the host; escape mutations produce new variants in an infecting population; opportunistic infections damage the host	the host is forced to respond to a continuously changing parasite antigenic surface; eventually, defence breaks down and progression to disease follows; in many cases, weakening of the immune response allows secondary infections by other pathogens that lead to severe pathogenesis	Rall (2003), Deitsch & Hviid (2004), Dustin & Rice (2006) and Picker (2006)
self-damage (immunopathology)	a parasite induces a host immune response that causes damage to own tissue; note that this general notion would apply to many of the above cases	immune response with cytotoxic lymphocytes destroys own, uninfected tissue; continuous destruction leads to pathogenesis	Pawlotsky (2004), Graham <i>et al.</i> (2005), Bray (2006) and Guidotti & Chisari (2006)

highly antigenic. Parasite-released toxins are often sophisticated molecules allowing the parasite to invade and spread inside the host. They either attack specific cells of the host (e.g. tetanus or botulinum toxin attacks the nerve cells) or are broadly 'cytotoxic' (e.g. those produced by Staphylococci, *Clostridia*).

#### (a) Example 1: *Bacillus anthracis*

*Bacillus anthracis* has the ability to produce anthrax toxin (AT), coded for by plasmid genes (pXO1) (Mock & Fouet 2001), which also determines host specificity (Gohar *et al.* 2005). AT is also the major factor that mediates immune evasion. AT is a mixture of three components: lethal factor (LF), oedema factor (EF) and protective agent (PA), which are already expressed at the spore stage and by newly germinated spores (Moayeri & Leppla 2004). In the process of

infection, PA first binds to host cell receptors, primarily those of the immune system, and by complex mechanisms forms pores in lipid bilayers without provoking an immune response. This aids the transport of LF and EF to their targets inside the host cell where they end up in intraluminal vesicles, protected from host proteases (Abrami *et al.* 2005). The combinations of PA and LF (also called the 'lethal toxin', LT), and of PA and EF ('oedema toxin', ET), are released into the host cell cytoplasm where they target multiple host functions.

Major target cells of AT are those of the immune system such as phagocytes and antigen-presenting cells (Abrami *et al.* 2005). At low doses early in the infection, LT acts to evade a number of host immune responses by suppressing pro-inflammatory cytokines, the release of NO<sub>x</sub> (a toxic molecule) and TNF- $\alpha$  by

macrophages, DC responses and B- and T-cell deployment. LT has also been found to lyse macrophages, induce apoptosis of endothelial cells and interfere with antigen presentation by DCs in mice and humans. Similarly, ET suppresses phagocyte functions and modulates cytokine pathways. Such suppression of (primarily innate) immune mechanisms facilitates the initial spore germination and bacterial growth, and is the reason why no evident immune response to the infection is observed (Moayeri & Leppla 2004; Abrami *et al.* 2005). Furthermore, different bacterial strains appear to vary in their capacities and perhaps even in the precise mechanics of immune suppression (Moayeri & Leppla 2004; Abrami *et al.* 2005).

As the bacterial population grows, the increasingly higher dose of the toxin causes severe pathogenic effects. This is probably also due to the sensitization of host macrophages by bacterial waste products combined with the effect of LT. At this stage, LT (in the mouse model) knocks out the immune system by destroying the macrophages and induces other as yet poorly known events that eventually lead to vascular leakage, systemic hypoxia and a shock-like collapse, stimulated by an excessive parasite-induced cytokine (IL-1) secretion by the macrophages that leads to eventual host death. Furthermore, EF induces the production of an excess amount of cAMP in the host cells, which eventually disrupts cell functions and the flow of ions. While LT and ET are sufficient to produce such symptoms of anthrax infection, mutants lacking these elements are attenuated (Moayeri & Leppla 2004).

#### (b) *Example 2: human rhinovirus (HRV)*

HRV is a small single-stranded RNA virus (Picornaviridae) and is the cause of the common cold. More than 100 different serotypes have so far been described. Typically, its pathogenic effects are benign and up to 40 per cent of infections are asymptomatic (Heikkinen & Jarvinen 2003). The immunomodulatory effects are thought to be the source of the symptoms of the common cold (Kirchberger *et al.* 2007). HRV infects the epithelial cells primarily of the nose and upper respiratory tract by binding to host cellular receptors. ICAM-1 is the receptor for more than 90 per cent of the serotypes. The binding to ICAM-1 leads to changes in the surface of the viral capsid, which facilitates the uncoating and release of the viral RNA into the host cell. ICAM-1 is also important for the migration and adhesion of leucocytes and for the activation of the T-cell response. The infection step therefore leads to the release of a variety of factors and to a number of changes in the host's immune response—changes that are primarily induced by viral interference with the type I IFN pathway (that normally activates the antiviral defences and requires several transcription factor complexes), the modulation of leucocyte interactions, cytokine production and the targeting of DCs.

More specifically, HRV blocks the activation of the first-line innate defence by preventing the proper assembly of IFN (type I interferon) regulatory factors (IRF-3, which is actually targeted by many viruses) in epithelial cells. HRV binding to epithelial cell ICAM-1

receptors furthermore inhibits T-cell proliferation and T-cell cytotoxic responses (only a few viral particles are needed for this effect). Phagocytes (monocytes) upon interaction with the virus release a different spectrum of cytokines into their environment than normal, and this eventually inhibits the antigen-specific T-cell response. For example, IL-10, an immunosuppressive cytokine, is overexpressed, which notably inhibits the production of pro-inflammatory cytokines (IL-1, IL-6 and TNF- $\alpha$ ) and downregulates MHC II class expression (IL-10 is a common target for many viruses, including the deployment of homologues) (Kirchberger *et al.* 2007). Human rhinovirus (and many other viruses) also impedes the function of DCs. The overall effect of these immune evasion mechanisms is a localized immunosuppression, which may predispose the host to opportunistic secondary infections that cause more severe complications of the infection.

#### (c) *Immune evasion and pathogenic effects*

As the examples of *B. anthracis* and HRV suggest, pathogenic mechanisms can often, but not always, be traced back to molecules that inhibit or manipulate the host's immune defences—suggesting that the mechanisms deployed to evade the host's immune response overlap with those that lead to pathogenesis. For example, a major pathogenic condition associated with bacterial infections is septic shock, which results from the combined action of host cytokines, components of complement and the coagulation cascade as the host's immune system responds and is misguided by the bacterium (Lapaque *et al.* 2005). Gram-negative as well as Gram-positive bacteria (e.g. *S. aureus*, *Staphylococcus epidermalis*) can induce septic shock even though the latter lack the respective endotoxins. Instead, peptidoglycan fragments and other molecules (e.g. teichoic acids) induce the same responses (Woolard *et al.* 2007). Similarly, parasite-derived proteases aid in migration across host tissue barriers, degradation of host blood proteins, direct evasion of host immunity but are also instrumental in inflammation and pathogenesis (McKerrow *et al.* 2006). The capacity of strains of *Escherichia coli* to cause diarrhoea-related deaths (mainly in infants) is tightly linked to the capacity of the bacteria to attach to the cell lining of the gut, which, in turn, is governed by bacterial adhesin factors (Kaper *et al.* 2004; Dean *et al.* 2005). *Pseudomonas aeruginosa* secretes an enzyme (also a toxin) that degrades extracellular molecules and facilitates tissue invasion leading to necrosis. A number of bacterial species have toxins that generate pores in the host cell membranes for the parasite to enter but which then lead to cell lysis and damage (Woolard *et al.* 2007). A different aspect is demonstrated by parasites critically depending on colonization factors. For example, *H. pylori* can cause gastric ulcers and cancer in humans. The bacterium produces the enzyme urease that allows it to survive in the acid environment of the stomach and eventually cause these pathogenic effects. The level of induced virulence is correlated with the production of urease in the various bacterial strains. Even though the main function of urease is not to evade the host's immune responses but to tolerate the hostile environment, the ability of the parasite to cause



virulence is under parasite control (Atherton 2006). A further strategy of parasite evasion is similarly known to create pathogenic effects, capsules. Bacterial capsules provide protection from the host's immune responses by impeding the recognition process through antibodies in vertebrate hosts. The capsules and the aborted process of host phagocytosis lead to enhanced inflammatory reactions, associated with tissue damage and pathogenic effects in the host (Wilson *et al.* 2002).

Immune evasion does not always lead to increased pathogenic effects. For example, immune evasion by helminths leads to a downregulation of the inflammatory response and, hence, to a lower degree of virulence (Schierack *et al.* 2003). Such downregulations are probably most common in parasites depending on long-lasting infections (Locksley 1997). Hence, more generally, immune evasion is a modulator of virulence, yet, as such a core process that affects how virulence evolves.

#### 4. VIRULENCE

##### (a) *Pathogenesis and virulence*

The evolutionary theory of virulence rests on trade-offs in the life history of the parasite. Trade-offs describe the situation where an investment of an organism's resources into one fitness component (e.g. to increase survival) goes at the expense of another fitness component (e.g. the same investment reduces transmission success). The trade-off-based approach has generated a range of successful predictions that match how virulence, generally defined as the loss of host fitness resulting from an interaction with a parasite, varies with different ecological conditions (Bull 1994; Ebert & Hamilton 1996; Frank 1996). Because evolutionary theory is rather general, there are some obvious limitations. For example, the life cycle of parasites that need to kill their host (as is true for most parasitoids) will not match models describing transient microbial infections (Ebert & Weisser 1997). Similarly, parasites that have recently invaded a host population may not yet have been subject to coevolution as assumed by theory (Weiss 2002). Further factors can also lead to a mismatch between prediction and observation, such as the effect of environmental conditions and host status (e.g. nutrition, temperature; Ferguson & Read 2002; Beck *et al.* 2004; Bedhomme *et al.* 2004), or plasticity in the response (Taylor *et al.* 2006). Also, genotypic variation in both parties adds to the variation in infection success and virulence (Ferguson & Read 2002; Kover & Schaal 2002; Schmid-Hempel & Ebert 2003). In some cases, virulence emerges because the parasite enters a different organ with no obvious value to its transmission, e.g. poliovirus entering the central nervous system (Levin & Bull 1994; Lipsitch & Moxon 1997). Finally, problems may also surface because the term virulence is used in various ways. Plant pathologists use virulence to refer to the presence or absence of the infection; virulence is also defined by host mortality (e.g. in epidemiological models; Anderson & May 1979), fecundity (Jaenike 1996) or even to the presence/absence of specific factors in a microbial pathogen (Dussurget *et al.* 2004).

But from what has been described above, taking into account the mechanisms of pathogenesis can improve our understanding of virulence evolution. For example, the host's own immune response causes the damage, as is likely for lymphocytic cytomegalovirus (Buchmeier *et al.* 1980) or malaria (Graham *et al.* 2005). More specifically, taking into account immune evasion into account can add to the scope of the standard theory. As we have seen above, the term 'immune evasion' groups the different processes by which parasites evade, subvert, usurp, avoid or overload the host's immune system. Not all immune evasion leads to pathogenesis and not all mechanisms of pathogenesis result from immune evasion. But immune evasion appears to be the major missing link in theories of virulence evolution and our analysis of host-parasite coevolution. Immune evasion is initiated by the parasite and the host is the responding party. The necessary machinery is part of the parasite's genomic endowment even when the parasite usurps the host's immune defence system for its own benefit (e.g. by using host enzymes (Nomura *et al.* 2006), or by sequestering gene sequences from the host (Seet *et al.* 2003)). At the same time, host and parasite face asymmetrical consequences should immune evasion fail. The parasite will lose all its future prospects while the host may still have a chance to survive and reproduce. This asymmetry, reminiscent of the 'life-dinner' principle (Dawkins & Krebs 1979), suggests that parasites should generally be ahead in the coevolutionary race for the control over the host's immune system.

Furthermore, the mechanisms underlying immune evasion are typically not the same as those underlying resource extraction from the host. Therefore, the parasite may face a trade-off between its investment in mechanisms to control the host by immune evasion (thus causing pathogenesis and virulence) and mechanisms that allow resource extraction and that would lead to growth and/or more transmission propagules (Antia & Lipsitch 1997). Note that resource extraction can, of course, also be associated with virulence effects, e.g. a reduction in fecundity due to resource depletion or resource extraction by destruction of host tissues. However, the current distinction is to emphasize that different mechanisms have different consequences for pathogenesis and often (though not always) may result in different virulence effects. This difference is especially pertinent when virulence effects induced by immune evasion take effect at a different time during the course of an infection (or are of a different magnitude) than those effects generated by resource extraction (see also Frank & Schmid-Hempel 2007).

An illustrative example of this difference in mechanisms is the acquisition of iron by pathogenic bacteria. Iron is a crucial resource for bacteria and must be extracted from the host. For this purpose, some bacteria produce so-called siderophores to sequester iron from the host (West 2002). In addition to producing siderophores, the bacterium also produces toxins, i.e. immune evasion molecules that are not directly involved in resource extraction (e.g. in *B. anthracis*; Cendrowski *et al.* 2004; Abrami *et al.* 2005). While toxins bear on pathogenesis in ways described above, iron extraction by siderophores is

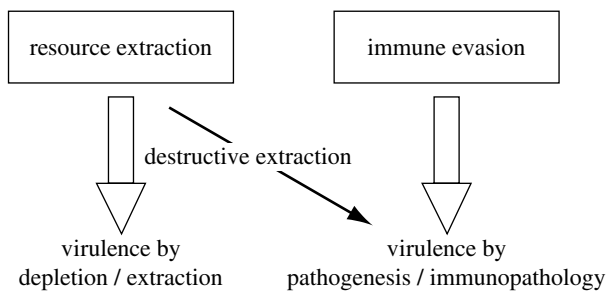


Figure 2. Different components of virulence. Immune evasion causes virulence by pathogenesis, while resource extraction leads to virulence by resource depletion (e.g. loss of fecundity). The immune evasion hypothesis suggests that virulence by pathogenesis is the major factor; the parasite may also face a trade-off with resource depletion.

associated with a reduction of host fitness by way of resource depletion. In some cases though, iron is mobilized from host cells by parasite-induced tissue destruction (Ratledge & Dover 2000), which so causes direct pathogenic effects (cf. table 1). Some parasites even manage to feed on the host's immune response; for example, *Leishmania* uses host cytokines as growth factors or tapeworms use antibodies as nutrients (Damian 1997). In these cases, the two separate processes, resource extraction and immune evasion, have probably converged secondarily. If, for example, pathogenesis by immune evasion dominates the virulence effects, either by magnitude or timing, when compared with those generated by resource extraction leading to high rates of replication) is (at least partially) decoupled from virulence by virtue of the difference in the underlying mechanisms (figure 2)

### (b) Immune evasion and tolerance

Clearance of the parasite from the host is a threshold process—the parasite is either cleared or not. To the extent that this capacity is affected by parasite immune evasion, immune evasion also has threshold properties. By contrast, a gradual control of the infection by the host is often thought to be the cause of long-lasting, chronic infections, where the pathogen is kept at a level below damage. This issue is related to the concept of 'tolerance', which has been primarily developed in plant pathology (Clarke 1984; Kover & Schaal 2002). Tolerance is considered to be the ability of the host to reduce or buffer the effect of an infection on host fitness. It implies no or, more likely, a negative relationship between the levels of infection (e.g. as measured by infection intensity) and of host fitness (e.g. loss of body mass or fecundity). The slope is expected to vary among hosts and may converge to zero host fitness at high levels of infection. Considering the possible involvement of immune evasion, however, tolerance may instead reflect the parasite's ability to persistently evade the host's defences, as to remain inside the host and to achieve eventual transmission (Merrell & Falkow 2004). If so, the host might in turn be selected to bear the negative effects of infection if unavoidable by successful parasite immune evasion. Indeed, a plethora of host immune mechanisms have been described for parasites that cause chronic

infections (e.g. *Trypanosoma*, *Plasmodium*, nematodes, some bacteria and viruses; Locksley 1997; Loukas & Maizels 2000; Sacks & Sher 2002; Turner 2002; Merrell & Falkow 2004). The relationship between immune evasion and tolerance therefore clearly deserves further attention.

### (c) Expanding the standard equation of epidemiology

The standard evolutionary theory of virulence uses the basic epidemiological equation (Anderson & May 1982). How immune evasion affects the evolution of virulence has so far not been well studied in this context (but see Bonhoeffer & Nowak 1994; Frank 1996; Antia & Lipsitch 1997; Day *et al.* 2007). The following basic equation can be used to illustrate the potential consequences:

$$R_0 = \frac{\beta(v)N}{\mu + \alpha(v) + c}. \quad (4.1)$$

In the standard theory (equation (4.1)), the parasite's reproductive rate,  $R_0$ , is supposed to be maximized by the parasite's choice,  $v$ , of the level of virulence (Frank 1996), where  $N$  is the number of susceptibles in the host population;  $\mu$  is the background (parasite-independent) host mortality rate;  $\alpha(v)$  is the parasite-induced mortality rate;  $c$  is the rate of clearance of the parasite from the host; and  $\beta(v)$  is the rate of transmission to new hosts. Only  $\alpha(v)$  and  $\beta(v)$  are assumed to depend on the level of parasite virulence.

To take into account immune evasion, Frank (1996), for example, has considered a parasite-induced change in the clearance rate  $c$ . In a recent treatment of immunopathology, Day *et al.* (2007) expanded the standard epidemiological equation by adding a term that includes the generation of virulence effects,

$$R_0 = \frac{\beta(v)N}{\mu + \alpha(\varepsilon, c) + c(v)}, \quad (4.2)$$

where  $\alpha(\varepsilon, c)$  is a term that includes the virulence effects generated by host resource exploitation,  $\varepsilon$ , and by immunopathology, which is assumed to be some function,  $f(\varepsilon, c)$ , of exploitation and clearance rate,  $c$ . With this formulation, host mortality (virulence) corresponding to the ESS level of virulence from the parasite's point of view should increase as the effect of immunopathology increases. This is true when immunopathology is independent of exploitation, as might be suggested by the difference in the underlying mechanisms illustrated above. By contrast, the ESS level of host mortality should decrease with immunopathology if the latter is fully generated by the exploitation effects (Day *et al.* 2007), as in the example of iron acquisition by tissue destruction mentioned earlier. The reason for this difference is obvious—in the first case, independently generated immunopathology lowers the future value of the host regardless of the parasite's actions, while, in the second case, immunopathology is an effect that is under the parasite's control. The implementation of immunopathology into the framework of the standard evolutionary theory alters the expected level of parasite virulence.



The interpretation of pathogenesis as a side effect of parasite immune evasion outlined in this paper differs from a view of immunopathology as reviewed, for example, in [Graham \*et al.\* \(2005\)](#). The latter ('immunopathology') assumes that the host controls its response but is doomed to incur collateral damage owing to overshooting (e.g. ([Müller & Bonhoeffer 2003](#); [Graham \*et al.\* 2005](#))). With immune evasion, by contrast, the parasite initiates the process and controls (to as yet unclear degree) the pathological process as it is subverting the host's immune responses. This is implemented in the second scenario modelled by [Day \*et al.\* \(2007\)](#), in as much as immunopathology is assumed to be a result of exploitation. Immune evasion can be more fully accommodated in the terms of equation (4.2)—for example, by varying clearance rate or assuming different shapes of functions that relate the terms to each other and to the level of virulence ([Antia \*et al.\* 1994](#); [Frank 1996](#); [Antia & Lipsitch 1997](#); [André \*et al.\* 2003](#); [Ganusov & Antia 2003](#); [André & Godelle 2006](#)). Regardless, an important distinction is the degree by which the parasite can 'control' pathogenesis as a result of its investment into immune evasion. This parameter will be crucial to develop a further understanding of the selection pressures underlying the evolution of virulence ([Frank & Schmid-Hempel 2007](#)).

Empirical tests following from equation (4.2) must still assume that the functional relationships among transmission, immunopathology, exploitation or clearance stay the same in the different tested cases. This may indeed be the case when the same host–parasite pairings are tested under different ecological conditions, e.g. differences in host densities or background mortality rates. Equation (4.2) then predicts that a parasite should evolve to higher or lower levels of virulence depending on conditions. In principle, equation (4.2) might be generalized by considering different functional relationships. For example, for parasite A, an increase in exploitation by an amount  $d\varepsilon$  may lead to a corresponding increase in immunopathology,  $d\alpha_1(\varepsilon)$ , while the same increase may have an effect  $d\alpha_2(\varepsilon?)$  when the host is infected by parasite B. Knowing all these relationships would generate a generally applicable evolutionary theory of virulence. However, as [Frank & Schmid-Hempel \(2007\)](#) argue, it is unlikely or even impossible that these relationships can be known in any particular case. A more promising approach is therefore to analyse the sensitivity of parasite fitness towards a change in any of the relevant pathogenic mechanisms. Combining this analysis with life-history theory ([Day 2003](#)) suggests that, in many cases, a major factor for the evolution of virulence might be parasite immune evasion to prevent clearance of the infection from the host. The reason is that according to life-history theory, parasite fitness is most sensitive to the duration of the infection, i.e. the lifespan of the parasite. With this effect, virulence effects accruing due to the manipulations of the parasite but after most of the transmission has taken place (i.e. post-reproduction) will not be strongly selected against. Hence, parasites will evolve towards high virulence effects if this is associated with reducing clearance early in the life of the infection ([Frank & Schmid-Hempel 2007](#)). As illustrated above, immune

evasion by parasites is often targeted at reducing clearance but might also be associated with massive pathogenic effects appearing at some later stage of the infection. Sensitivity analysis against the background of life-history strategies provides a novel way to analyse parasite fitness and to generate a more general evolutionary theory of virulence. Immune evasion is likely to play a major role in many of the cases and should also elucidate problems such as cross-species infections or invasion by novel parasites into a host population ([Schmid-Hempel 2008](#)). For example, if successful infection and parasite persistence inside the host critically depends on a particular immune evasion mechanism, this mechanism might only work in host A but be ineffective in host B ([van Baarlen \*et al.\* 2007](#)). As this review shows, immune evasion mechanisms are typically tuned towards interfering with specific host molecules or signalling pathways, which are not necessarily conserved across host species. To systematically clarify the extent of such matching of immune evasion mechanisms to their potential hosts will be a major challenge for future comparative molecular immunology with repercussions for the evolutionary ecology of host–parasite interactions.

#### (d) Immune evasion and dose

Immune evasion puts a major selective step within the host. This raises the question of how the dose at which a parasite infects is related to the eventual success of the parasite to infect and establish. For example, would a high dose be required owing to a simple demographic process where a high initial number leads to higher growth rates in absolute number, or is a high dose required to ensure the presence of a few suitable propagules for the particular host in question, each of which may infect at very low numbers? Alternatively, is dose a phenomenon of cooperation in some parasites but not in others?

Before considering the latter question, we might expect that, given the intricate mechanisms at the molecular level, the capacity for immune evasion should critically depend on the host that is encountered by a given parasite. Thus, the immune evasion hypothesis lets us expect that a few evasion-potent propagules, rather than sheer numbers, are crucial. In infection experiments with a happenstance collection of infective cells in the inoculum, a high dose may be required to increase the chances of containing some of these potent types rather than to dominate the subsequent population dynamic process within the host. The latter should still be relevant, of course, within any given infecting type or when types compete. Given the right combination of parasite and host, however, even a few propagules that encounter the next host could establish an infection. In this sense, the widespread observation of genotypic host–parasite interactions might have its main basis in the immune evasion processes, as illustrated by the genetic variation in bacterial virulence factors that associate with differential host–parasite interactions ([Schmid-Hempel 2005, 2008](#)). In turn, the advantage of higher infection doses should saturate very quickly with immune evasion as the major step. A particularly interesting case is given with multiple infections, since an infecting

parasite could benefit from the activity of all other co-infecting strains and, hence, the effect of multiple infections on virulence would become less. In addition, the arrival order of different infections would become important, a pattern that has been found in experimental tests (De Roode *et al.* 2005; Jäger & Schjørring 2006).

We still lack a coherent picture of dose–infection relationships, however. Various studies suggest that the effective dose for successful infection may often be surprisingly small. For example, a dose of only 100–200 influenza viruses per litre of air infected virtually all experimental mice and the dose–infection relationship increased very steeply before that point (Schulman 1967). Infection by low doses (dozens or a few hundreds of infecting cells) and/or rapidly saturating dose curves were also found with experimental exposures to other viruses in mice (Diamond *et al.* 2003; Tibbetts *et al.* 2003), pigs (Billinis *et al.* 2004; Hermann *et al.* 2005) or birds (Reisen *et al.* 2004); and similarly with mice exposed to helminths (Dematteis *et al.* 2003) or humans experimentally infected by the protozoan parasite *Cryptosporidium parvum* (Teunis *et al.* 2002a,b). Remarkably low doses are also often emerging from epidemiological data that statistically infer dose–infection relationships; for example, *C. parvum* in the human water supply (Englehardt & Swartout 2004), gamma-herpesvirus (Tibbetts *et al.* 2003), outbreaks of *E. coli* (Teunis *et al.* 2004) or cases such as foot-and-mouth disease virus in cattle (Schijven *et al.* 2005). Along similar lines, very low doses can be quite effective in generating immune responses (Fattorini *et al.* 2002).

The effectiveness of low doses, of course, does not necessarily contradict the standard virulence transmission models but certainly raises some questions about the exact role of large propagule numbers. Dose–infection curves have rarely been discussed in view of the immune evasion hypothesis. For example, a rapidly saturating effect of dose for adapted but not for foreign parasite strains would be one expected outcome (Ginsberg 1953; Osnas & Lively 2004). In studies of plant diseases, it has furthermore been noted that the probability of infection is independent of the dose when adapted (‘compatible’) parasites are used, but depends on the dose when non-adapted (‘incompatible’) strains are tested (Ercolani 1984).

Returning to the question of cooperative action, parasites have evolved strategies to enhance their success of invasion in various ways. For example, many bacteria produce factors that change the motility and surface of host cells so that the parasite is actively taken up by the host cell, e.g. surface ruffling induced by *Salmonella* (Donnenberg 2000). Most interestingly, some bacteria seem to secrete these factors into their surroundings while others inject them directly into the host cells, typically with a type III secretion system. Taking these differences in factor delivery into account suggests that ‘cooperatively’ acting bacteria (where factors are secreted) tend to have higher infective doses than bacteria where the factors are injected individually (Schmid-Hempel & Frank 2007). Even though this pattern is still suggestive rather than firmly established, this kind of analysis points the way towards a more

general consideration of parasite manipulation strategies and their effects on macroscopic properties of interest such as virulence or infective dose. Note that immune evasion during the infection stage could also mean evasion of recognition. Hence, the host’s ability to recognize either one or thousands of infecting parasite cells might not be the same when manipulated by the parasite.

In summary, consideration of immune evasion by parasites adds a crucial element for the analysis of host–parasite interactions. Even though in evolutionary ecology this issue has been addressed repeatedly in various contexts (Bonhoeffer & Nowak 1994; Frank 1996; Koella & Boete 2003; Graham *et al.* 2005; Bergstrom & Antia 2006), a more comprehensive treatment is overdue and should be enlightening for many questions that have been studied in evolutionary ecology.

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