8.1 Parasites manipulate their hosts

A parasitic infection often spreads without any obvious reaction by the host’s immune system, even when the infection eventually leads to serious damage. This is the case for human anthrax caused by the bacterium *Bacillus anthracis*. The most dangerous form of anthrax results when bacterial spores are inhaled. In the lung tissue these spores are taken up by alveolar macrophages and transported to the lymph nodes, where germination occurs. The bacteria then replicate to high numbers and eventually cause host death. Hosts should, therefore, be selected to forcefully prevent the build-up of the infection in the first place. Yet, the bacteria initially spread inside the host body without a strong immune response. Why?

The case of *B. anthracis* (Box 8.1) is just one of very many examples where molecular immunologists and microbiologists have unravelled an amazing and sometimes bizarre variety of mechanisms by which parasites escape or modulate the host’s immune response in their own favour. In fact, parasites do not simply hold out against the host’s immune defences—they actively evade, sabotage, or manipulate the immune system, and, furthermore, alter the host phenotype in other ways (e.g. by changing host behaviour) for their own benefit. These diverse phenomena are here grouped as mechanisms of ‘immune evasion’ and ‘host manipulation’, respectively. The principle was discovered more than 100 years ago. One of the fathers of immunology, Paul Ehrlich, reported on the ‘disappearance of receptors’ during infections by African trypanosomes in his Nobel Lecture of 1908. This phenomenon is now known as ‘antigenic variation’ (Bloom 1979; Damian 1997) and is one of the mechanisms by which trypanosomes escape recognition by the host’s immune system. In fact, the parasite is under selection to accomplish two major tasks once it has infected a host: avoid being cleared by the host’s immune response in order to survive, and extract the necessary resources to grow, multiply, and reproduce. Virtually every major parasite group possesses mechanisms of immune evasion and host manipulation to achieve these goals (Schmid-Hempel 2008b, 2008a). Some parasites even manage to utilize and feed on the host’s immune response directly. For example, *Leishmania* use host cytokines as growth factors and tapeworms utilize antibodies as nutrients (Damian 1997). As a note of caution, the respective molecular studies have mostly been done *in vitro* and using model systems, such as the mouse. It is, therefore, not exactly known whether the same processes would also work and be effective *in vivo*. However, given the sophistication of these mechanisms and their obvious value for the parasite, we can safely assume that similar things happen in the living host organism in its natural setting. On another note, it is often not clear whether an observed change in the host is beneficial for the parasite in the first place (Box 8.2). To show a presumed benefit in the natural setting is a daunting task for most cases and will necessitate more research than is currently available.
Bacillus anthracis is a Gram-positive bacterium that infects mammals and is a potential bioweapon. It has, therefore, been well studied for bacterial immune evasion. Based on genetic studies, B. anthracis, B. cereus, and B. thuringiensis appear to be the same species or at least very closely related (Helgason et al. 2000). Among these variants, B. anthracis has the ability to produce anthrax toxin (AT), which is encoded by plasmid genes (pXO1) (Mock and Fouet 2001). AT is a major factor that mediates immune evasion. Note that ‘toxins’ are not poisons as the name might imply, but are parasite-produced molecules that, typically, are finely tuned to disarm the host’s immune response repertoire.

Anthrax toxin actually 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 and Leppla 2004). In the process of infection, PA first binds to host cell receptors, primarily those of the immune system, and by complex mechanisms is able to form pores in lipid bilayers (such as in a cell membrane) without provoking an immune response. This aids to transport LF and EF to their targets inside the host cell where they end up in intra-luminal vesicles, where they are 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 anthrax toxin are those of the adaptive immune system, such as phagocytes and antigen-presenting cells (Abrami et al. 2005). At low doses early in the infection, LT evades a number of host immune responses by suppressing pro-inflammatory cytokines, blocking the release of NOx (a toxic molecule) and TNF-α by macrophages, disrupts dendritic cell responses, and by impeding B- and T-cell deployment. LT was also found to lyse macrophages, to induce apoptosis of endothelial cells, and to interfere with antigen presentation by dendritic cells in mice and humans. Similarly, ET suppresses phagocyte functions and modulates cytokine pathways. Suppression of many innate immune mechanisms facilitates initial spore germination and bacterial growth, and is the reason why no evident immune response to the infection is observed. Furthermore, different bacterial strains appear to vary in their capacities and, perhaps, even in the precise mechanisms of immune suppression (Moayeri and Leppla 2004; Abrami et al. 2005). For example, one strain of B. anthracis (strain 9131) has only one extra-cellular secreted protein, an inhibitor of a metalloprotease (InhA1), which is probably involved in the degradation of antibacterial peptides (Gohar 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 sensitization of host macrophages by bacterial waste products, combined with the effect of LT. At this stage LT (at least in the mouse model) knocks out the immune system by destroying the macrophages, and by inducing 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. The entire cascade eventually leads to host death. Furthermore, EF induces the production of an excess amount of cAMP in host cells, which disrupts cells functions and the flow of ions. LT and ET are sufficient to produce such symptoms of anthrax infection. Mutants lacking these elements are attenuated and do not cause such severe damage (Moayeri and Leppla 2004).
Typically, infection by a parasite leads to a number of changes in the expression of the immune defences, at the level of the biochemistry of cells, and in the visible phenotype of the host, e.g. its behaviour, morphology or life-history pattern. Some of these changes represent instances of host defence—the immune system has to respond, the host needs to forage more frequently for more nutrients to sustain the defence, or the host moves to a more suitable place to defeat the infection. However, a large number of observations suggest that infection-associated changes are not primarily due to the host’s own responses but specifically induced by the parasite to manipulate the host in its own favour. In particular, the parasite should be selected to increase its two major fitness components: avoiding clearance by the host’s defences (i.e. increase parasite survival), and increasing the chances of transmission to the next host (i.e. parasite reproduction). But how can we distinguish whether the observed changes are indeed beneficial for the parasite?

In principle, a change in the host phenotype could mean different things: (1) A change is neither beneficial to the host nor to the parasite; it could simply be an unavoidable side-effect of the infection. Selection could maintain the mechanisms responsible for these changes due to other benefits for host and/or parasite. (2) The change is beneficial to the parasite but only as a coincidental side-effect of other, unrelated changes. In this case, selection might eventually lead to the incorporation of this side-effect into the parasite’s repertoire; it would then become an active strategy. The initial trait is, therefore, an ‘exaptation’, i.e. a fortuitous benefit from other adaptations (Gould and Vrba 1982). For example, eye flukes (trematodes infecting the eye) benefit from the fact that the eye’s interior is an immune-privileged site, i.e. a tissue with low activity of the immune system. But at the same time, the host’s visual acuity is impaired and it therefore is more likely to fall victim to a predator. If the predator acts as final host of the fluke, this initial side-effect might be co-opted for the new purpose of increasing the chances of transmission. Moreover, this new function could lead to the evolution of still further mechanisms that affect host behaviour more directly and specifically. Note that the exaptation provides a benefit, no matter how it has been generated. The distinction between exaptation and adaption is, therefore, based on a judgment of what the true ‘meaning’ of a trait is, i.e. what selection pressure maintains it at present and how it has historically evolved. (3) The change is beneficial for the parasite in which case a true adaptive parasite strategy is observed.

To identify true adaptive changes, several criteria have been suggested (Poulin 1995; Lefèvre and Thomas 2008). Among those, two criteria stand out. First, an adaptation is more likely if a similar mechanism has evolved independently in different lineages. For example, infections by acanthocephalans and trematode parasites are both associated with a change in the intermediate host’s behaviour that puts infected individuals at risk of predation. The convergence (an evolutionary analogy) of these traits makes it likely that it is a change benefitting the parasite to reach the final host. Second, measuring the presumed benefits, at least in principle, can reveal its adaptive character. For example, does the changed behaviour of a trematode-infected intermediate host really lead to higher predation rates by the final host under natural conditions? Amphipods on the mudflat get exposed to shorebirds. Data show that amphipods (the intermediate host, Corophium volutator) infected by trematodes (Gynaecotyla adunca) are indeed more likely to be found on the mudflat surface, and the ‘right’ predator (sandpipers, Calidris pusilla) actually eats these amphipods (McCurdy et al. 2000). Similarly, the presumed

(Continued)
8.2 The diversity of immune-evasion mechanisms

Immune-evasion mechanisms occur at the level of the molecular machinery that regulates and constitutes the immune response. The diversity of these mechanisms can be classified by the mode of action (passive or active) and by the target that is addressed by the parasite.

8.2.1 Passive evasion

Evasion can be labelled ‘passive’ when no parasite-borne molecules are secreted that directly target and actively interfere with an immune system component. Passive evasion is achieved in several ways.

8.2.1.1 Hide away
This is accomplished when an immune-privileged tissue is invaded, such as the central nervous system or the eye (Bhopale 2003). Parasitoids can place their eggs inside host tissue that is not well patrolled by the host’s immune system, for example, in the fat body of insects.

8.2.1.2 Becoming ‘invisible’
Parasites can cap and shed antigenic surface components when they become recognized by the host’s immune system (e.g. in Leishmania exposed to antibodies; Bloom 1979). Furthermore, signals that give away the presence of a virus can be camouflaged or scavenged; this is done, for example, by vaccinia virus (Tortorella et al. 2000; Yewdell and Garcia-Sastre 2002; Seet et al. 2003). Bacteria can actively modify or shield their PAMPs and so escape recognition (Hornef et al. 2002).

8.2.1.3 Changing identity
T-cells and antibodies recognize epitopes (the antigenic surface) of a parasite. The individual parasite can escape the consequences of recognition by changing its antigenic surface during the course of infection (this was Ehrlich’s observation). This makes it difficult for the host to track the parasitic infection. Some parasites store different surface variants in an ‘archive’ and these archived variants are successively expressed. Plasmodium falciparum, for example, has about 60 stored variants, and Trypanosoma brucei has several hundreds (Frank 2002; Sacks and Sher 2002; Turner 2002). Antigenic variation is also known from nematodes (Blaxter et al. 1992), and from bacteria and viruses (van der Woude and Bäumler 2004; Barbour et al. 2006). In an infecting population, for example, two different strains of influenza virus might combine to form a new sub-type with a mixture of the antigenic surface characteristics of the two original types in a phenomenon called ‘antigenic shift’.

8.2.1.4 Population escape by mutation
Evasion also results from the mutation of epitopes. This mechanism unfolds its effect at the level of an entire infecting population. In HIV, for example, such mutants arise with sufficient frequency to outpace the immune system (Kent et al. 2005). This phenomenon is called
'antigenic drift'. Note that such antigenic drift is different from antigenic shift, since the former is due to mutation accumulation in a population, and the latter is a more drastic change due to genetic exchange among co-infecting strains.

8.2.1.5 Molecular mimicry
Poxviruses and herpesvirus produce MHC-decoy molecules that interfere with antigen presentation on the infected host cell. This prevents the attraction of helper and killer cells that otherwise would remove the infection (Yewdell and Garcia-Sastre 2002). *Plasmodium*, schistosomes, and nematodes have all been reported to produce competing ligands to interfere with recognition by the host (Blaxter et al. 1992; Locksley 1997). This class of mechanisms might sometimes also be classified as 'active evasion'.

8.2.1.6 Quiescence
The parasite also can temporarily become inactive to escape the immune system, as it then avoids any signal that would reveal the infection. Bacteria, for example, can go quiescent with little or no metabolic activity, and with no cell division and replication. This also evades the effects of antibiotics that typically target the cell-replication step; this step simply does not happen during quiescence (Lewis 2007). Viruses, too, can enter a state of latency during which the synthesis of viral proteins is down-regulated, e.g. as observed in herpes simplex virus that can stay latent for long periods of time (Kapadia et al. 2002).

8.2.1.7 Capsule formation
Prime targets of immune attack against Gram-negative bacteria are the LPS surface molecules (lipopolysaccharides). LPS binds to the host-protein C3, the signal for activation of the host complement, but also binds to C5b, which is needed to form the membrane-attack complex. The complement-attack complex paves the way for the subsequent effector molecules that will eventually destroy the invader (cf. Chapter 4). Some bacteria have evolved simple but effective means to evade this response by forming polysaccharide capsules (sometimes polypeptides, or protein-carbohydrate mixtures are used). Capsules evade complement activation and the subsequent killing by phagocytes (the inflammation response) (Salyers and Whitt 2002) in different ways. For example, *Neisseria meningitidis* attaches sialic acids to its LPS-surface molecules, *Haemophilus influenzae* modifies the O-antigen side of LPS, and *Streptococcus pneumoniae* as a Gram-positive bacterium lacks a LPS-type molecule altogether. Perhaps because of being able to evade this primary immune response, all of these bacteria have evolved into dangerous pathogens. They are thus able to multiply rapidly within the host and will cause much-feared infections, such as bacterial meningitis or pneumonia. In the process, the bacteria release cell wall components. A cascade of events can be triggered following this, leading to septic shock, and eventually host death. Phagocytes recognize and attack the bacteria only if the host has time enough to mount an antibody response against the capsular antigens (Salyers and Whitt 2002).

8.2.2 Active evasion
Active evasion is a general and widespread strategy of parasite manipulation of the immune system. For this purpose, specific molecules are produced by the parasite (or coded for in the case of viruses) that block or modulate specific steps in the host's immune response, or that interfere with basic cellular functions important for host defence, such as cell motility or apoptosis. The modulatory molecules are furthermore deployed in different ways—acting at short-range, or more distantly. Short-range action is illustrated, for example, by bacterial adhesins and invasins that typically are membrane-bound proteins acting by contact with host cells. Examples are several of the membrane-bound adhesins of *Streptococcus pyogenes* that mediate attachment to the host cell (such as M-protein). Secreted proteins can also act at short range by taking effect near, or on, the bacterial surface and in the immediate surroundings of the parasite. In *Streptococcus*, the EndoS-protein is an example. It interferes with host immunoglobulins that bind to the parasite surface (opsonins). Further examples are the Mac-protein that blocks phagocytosis, and the SIC-protein that prevents the docking of an attack-complement complex onto the
bacterial cell membrane of Streptococcus (Mitchell 2003). Furthermore, a number of bacteria inject their modulatory proteins by specialized secretion systems directly into the host cell. This, too, necessitates close contact with the host cell.

In fact, bacteria possess several different secretion systems that allow the transport of proteins from their cell interior across the membrane to the outside (Tseng et al. 2009) (Figure 8.1). The molecular details differ among these systems. Of particular interest in the pathogenic bacteria are the so-called Type III and Type IV-systems. Type III-systems are known from Gram-negative bacteria and are responsible for transporting molecules, such as toxins, to the bacterial surface and into a host cell. The transport only becomes activated upon contact with host cells in a process named ‘contact-dependent secretion’. For example, Shigella directly injects several bacterial proteins (Ipas; invasion plasmid antigens) into the cells of the host gut epithelium via this secretion system. Many of the genes that encode the proteins of the Type III-machinery are similar to genes involved in the flagellar export proteins. It has, therefore, been suggested that the Type III-system evolved from the system responsible for flagellum assembly, which was present before Type III secretion evolved in the pathogenic bacteria (Mescas and Strauss 1996). However, the two systems might actually have evolved independently. Species with Type III-systems experienced frequent lateral transfer of entire

**Figure 8.1** Secretion systems of Gram-negative bacteria. These bacteria have an inner and an outer cell membrane, separated by the periplasm. To shuffle molecules from the inside (cytoplasm) to the outside, several secretion systems exist. *Universal transporters* (Sec, Tat) are found in Gram-positive and Gram-negative bacteria. Sec (general secretory pathway) and Tat (two-arginine pathway) transport molecules into the periplasm from where they are further shuffled to the outside, for example, by secretins. *Type I secretion system*: consists of three major components; ABC-transporters, membrane-fusion protein (MFP), and the outer membrane protein (OMP). The system provides a trans-membrane channel to excrete bacterial proteins. (Examples of excreted molecules: virulence factors, such as metalloproteases in several plant pathogenic bacteria.) *Type III secretion system*: delivers bacterial proteins across the bacterial membranes and across the host membrane into the host cytosol. This injectosome consists of basal rings, spanning bacterial membranes and periplasm, a translocation pore that is inserted into the host membrane (symbolized by the black dots) that tips a needle (e.g. in Versinia), a filament (Salmonella), or a pilus (Pseudomonas); the injectosome so penetrates the host cell membrane. (Example of excreted molecules: Yop-proteins of Versinia.) *Type IV secretion system*: can transport nucleic acids in addition to proteins; the virB-protein is common to all bacteria that have this system. Type IV is also found in Gram-positive bacteria with cell envelopes (Example of excreted molecules: pertussis toxin of Bordetella pertussis.) *Type VII secretion system*: in some Gram-positive bacteria (especially the mycobacteria), the cell wall is rich in lipids (the mycomembrane). This secretion system is specialized to cross these membranes. (Example of excreted molecules: virulence factor ESX-1 in Mycobacterium.) Redrawn from Tseng et al. (2009).
gene clusters that encode for these secretion systems early in their evolutionary history (Gophna et al. 2003). Some pathogenic bacteria use the Type IV-secretion systems that also transport virulence factors across the membrane (Burns 2003). These systems are ancestrally related to those permitting bacterial conjugation (Schröder and Dehio 2005). The differences in these secretion systems are relevant because they determine whether the secreted molecules act at close range or more distantly.

During the course of co-evolution with their hosts, some parasites—the viruses in particular—have ‘captured’ genes from their hosts and integrated them into their own genome to produce molecules that disarm host immunity (Howell 1985; Barry 1986; Damian 1997). These genes code for molecules that are close to the original host molecules, which regulate the host immune response. By gene capture, original host cytokines are, for example, turned into ‘virokines’ that can interfere with the cytokine network of the host (Kotwal and Moss 1988). Host genes originally coding for receptors can become captured and are turned into ‘viroreceptors’, that is, decoy receptors, that are very effective at subverting the host’s immune response (Upton et al. 1991).

### 8.2.3 Targets of immune evasion

An infecting parasite must overcome a series of successive immune responses by the host. In the case of vertebrate hosts, for example, the subsequent steps in the defence cascade have prompted the evolution of corresponding mechanisms of immune evasion by the parasite (Figure 8.2). The targets of parasite immune evasion can be characterized as follows.

#### Parasite interference

- Avoid recognition
- Quiescence
- Block complement
- Avoid phagocytosis
- Block macrophages, neutrophiles
- Manipulate cell surface

#### Infection

- Block inflammation
- Interfere with signalling
- Interfere with RNAi
- Avoid phagocytosis
- Degrad anti-microbial peptides
- Manipulate cell vacuoles
- Manipulate cell cytoskeleton
- Escape from vacuoles
- Block NK cells
- Induce apoptosis
- Interfere with receptors, with signalling
- Interfere with antigen presentation and processing
- Interfere with cell maturation
- Super-Antigens

#### Change identity:
- Antigenic variation
- Phase shift
- Escape mutants

#### Innate immune response

- Recognition
- Non-specific phagocytes (macrophages, neutrophils)
- Activation of complement (alternative pathway)
- Release of cytokines (IL-1, IL-6, IL-8, ...,TNF-α)
- Release of molecules (oxygen radicals, NO, ...)

#### Early induced response

- Inflammation
- Release of cytokines 
- Recruitment of effector cells (leukocytes, monocytes, neutrophils, NK cells)
- Differentiation to effector cells (macrophages)
- Acute-phase response effectors
- Activation of complement
- Recruitment of effector molecules
- Release of IFN-α, IFN-β (viral infection)

#### Adaptive immune response

- Transport of antigen to lymph nodes
- Recognition by B- and T-cells
- Lymphocyte maturation
- Clonal expansion
- Production, release of antibodies
- Differentiation to effector cells (cytotoxic T cells, etc.)
- MHC I expression
- Antigen presentation
- Activation of NK cells

#### Protective immunity (Memory)

- B-cells differentiate into memory cells

#### Host immune response

- Recruitment of effector molecules
- Release of IFN-α, IFN-β (viral infection)

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**Figure 8.2** Host defence and immune evasion. The figure shows the steps in the vertebrate immune defence (bottom) and the corresponding interference by parasites (top). Redrawn from Schmid-Hempel (2008b) with permission from Elsevier.
Table 8.1 Immune evasion by parasites in vertebrate hosts (typically mouse, rats, humans).

| Type of evasion and observations                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Source                                                                                                                                                                                                 |
|---|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| (a) Avoidance of recognition:                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                        |
| (b) Interference with regulatory networks:                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                        |
| Many viral genes interfere with MHC complex; down-regulate CD4 activity; inhibit NK-cells; inhibit cytokine action; interfere with apoptosis (myxoma virus, adenovirus, vaccinia virus), produce molecules that interfere with the MHC I antigen-presenting cascade, typically by subversion of host's protein degradation or trafficking pathways. Viruses: interfere with interferon signalling of host cell and bypass normal receptors; modulate tumour necrosis factor (TNF)-family, e.g. by producing homologues; block receptor activation in natural killer cells (HIV, HTLV); regulate MHC class I expression (HCMV, HIV, SIV, MCMV). Bacteria: interfere with cytokine secretion and signalling (*Mycobacterium*, *Bacillus*, *Yersinia*), and with antigen presentation (*Mycobacterium*, *Chlamydia*); interfere with effector function of T- and B-cells (*Yersinia*, *Neisseria*) by exploiting signalling pathways; avoid being transported into the lysosome, or escape into cytoplasm of phagocytes by release of pore-forming proteins; prevent cell fusion by retaining crucial host signals. *Mycobacterium tuberculosis* can actively arrest development of phagosome, thus evading anti-microbial effects and maintaining its host for extended periods. Also interferes with the antigen-presenting mechanism. *Mycobacterium*, *Legionella*, *Coxiella*, *Chlamydia* interfere with vacuole maturation in their host cells. *Pseudomonas* produces proteases and lipases that inhibit cell functions (T-cells, NK cells, phagocytes) and inactivate complement. *E. coli* inhibits kinase signalling pathway. *Yersinia* prevents actin polymerization. *Salmonella* prevents delivery of oxidase to its host cell vacuole. *Shigella* induces apoptosis in macrophages. *Entamoeba* manipulate macrophages and T-cells, interfering with transcription of IL-4, IL-10 and transforming growth factor-beta (TGF-β). *Leishmania* manipulates host cytokines and so disable T-cells; prevents induction of IL-12 in macrophages selectively, but leaves other pro-inflammatory cytokine pathways intact; induces IL-10 to avoid clearance; inhibits cytokolytic pathway of the complement cascade by proteolytic degradation of host proteins or by active release of signalling compounds. *Leishmania*: Toxoplasma: down-regulate apoptosis to prolong cell life for prolonged own development, probably by production of homologues of regulatory proteins. *Toxoplasma*: inside cell modifies host vacuole membrane with own proteins to prevent further immune responses; impedes NF-κB pathway in macrophages; down-regulates IL-12. *Trypanosoma*: evades innate response by production of homologue to the regulatory protein, decay-accelerating-factor; suppresses in dendritic cells the induction of IL-12, TNF-α, activates NO-production and so down-regulates immune response in fish hosts. | Ploegh 1998; Tortorella et al. 2000; Guidotti and Chisari 2001; Benedict et al. 2002; Orange et al. 2002; Hewitt 2003; Seet et al. 2003; Lalani et al. 2000; Guidotti and Chisari 2001; Chatterjee et al. 2002; Orange et al. 2002; Yewdell and Garcia-Sastre 2002; Hahn 2003; Seet et al. 2003; Pieters 2001; Hornef et al. 2002; Young et al. 2002; Portnoy 2005; Underhill and Ozinsky 2002; Young et al. 2002; Flynn and Chan 2003; Kharazmi 1991; Hilbi et al. 1997; Underhill and Ozinsky 2002; Campbell and Chadee 1998; Reiner and Locksley 1995; Launois et al. 1997; Mosser and Brittingham 1997; Nunes et al. 1997; Young et al. 2002; Portnoy 2005; Sacks and Sher 2002; Norris et al. 1991; Sacks and Sher 2002; Saeij et al. 2002. |
Plasmodium: prevents maturation and function of dendritic cells; releases toxins that induce host cytokine production, which affects expression of receptors in cell, thus escape destruction; presents similar, competing ligands and prevents maturation of memory cells.

Nematodes express IFN-γ like molecule that changes lymphoid cells probably to delay immune response. Nematodes, schistosomes express C-type lectins from genes with sequence similarity to mammals; presumably compete for receptors and so inhibit leukocyte binding

Kersh and Allen 1996; Hommel 1997; Plebanski et al. 1997; Sacks and Sher 2002

Grencis and Entwistle 1997; Loukas and Maizels 2000

(c) Interference with effector molecules:

Malaria-infected red blood cells form rosettes that are hard to attack. Cytoadherence to host endothelia, auto-agglutination to prevent attack and clearance (e.g. *Plasmodium falciparum*, *Babesia bovis*).

Hornef et al. 2002; Flynn and Chan 2003

Bacteria: degrade anti-microbial peptides or reduce their efficacy, e.g. by reducing negative electric charge of cell membrane (*Staphylococcus*). Such modifications correlate with virulence (*typhimurium*). Prevent being phagocytosed, e.g. by capsule formation, induction of host cell apoptosis (*Salmonella, Shigella*); disorganize host cytoskeleton (*Yersinia*); escape from vacuole (*Listeria*); evade toxicity from immune system-produced reactive intermediates by catabolising these molecules (*Mycobacterium tuberculosis*).

Hornef et al. 2002; Flynn and Chan 2003

Leishmania evades lysis by preventing insertion of host attack complex into its membrane with extended surface molecules and cleavage of crucial host proteins.

Sacks and Sher 2002

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**Table 8.2 Immune evasion by parasites in invertebrate hosts, mostly insects.**

<table>
<thead>
<tr>
<th>Type of evasion and observations</th>
<th>Examples</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>(a) Avoidance of recognition:</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hiding in privileged sites that are not patrolled by immune system (passive evasion).</td>
<td>Parasitoids</td>
<td>Schmidt et al. 2001</td>
</tr>
<tr>
<td>Molecular mimicry and masking to avoid recognition in snail hosts.</td>
<td>Trematodes</td>
<td>Van der Knaap and Loker 1990</td>
</tr>
<tr>
<td><em>(b) Interference with regulatory networks:</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calyx fluid contains serine-protease inhibitor, which blocks haemocyte activity transiently and locally.</td>
<td>Parasitoids</td>
<td>Schmidt et al. 2001</td>
</tr>
<tr>
<td>Produce proteins affecting cytoskeleton of blood cells to prevent normal functioning. Prevent anti-microbial response and induce haemocyte apoptosis</td>
<td>Symbiontic viruses of parasites</td>
<td>Schmidt et al. 2001</td>
</tr>
<tr>
<td>Prevents acidification of cell vacuoles, which is a prerequisite for cell destruction.</td>
<td><em>Metarhizium</em> (fungus)</td>
<td>Vilcinskas and Götz 1999</td>
</tr>
<tr>
<td>Actively and selectively suppress synthesis of lysozyme and cecropins. Suppress haemocyte motility, cytoskeleton formation, filopodia formation, cell spreading. Fungal proteins and secondary metabolites interfere with sub-cellular structure in host immune cells and thus suppress effective response. Cyclosporin A released by fungi probably interferes with haemolymph proteins and may cause specificity. Interference with motility and aggregation of host defence cells in snail hosts.</td>
<td>Fungi</td>
<td>Vilcinskas and Götz 1999</td>
</tr>
<tr>
<td>Trematodes</td>
<td>Van der Knaap and Loker 1990</td>
<td></td>
</tr>
<tr>
<td><em>(c) Interference with effector molecules:</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Releases inhibitors against anti-microbial peptides of moths.</td>
<td><em>Bacillus thuringiensis</em></td>
<td>Edlund et al. 1976</td>
</tr>
<tr>
<td>Suppression of synthesis of anti-microbial peptides, but perhaps only as a side-effect of host tissue destruction.</td>
<td><em>Beauveria bassiana</em></td>
<td>Vilcinskas and Götz 1999</td>
</tr>
<tr>
<td>Production of proteases against lysozymes.</td>
<td>Fungi</td>
<td>Vilcinskas and Götz 1999</td>
</tr>
<tr>
<td>Eggs have surface protein that prevents haemocyte attachment and so impedes encapsulation.</td>
<td>Parasitoids</td>
<td>Schmidt et al. 2001</td>
</tr>
</tbody>
</table>
8.2.3.1 Escape recognition
Recognition is the first step in the host's defence cascade. In addition to passive evasion, active interference impedes recognition in a variety of ways. For example, malaria parasites present competing ligands to the receptors of the immune system that interfere with the ones that actually would ensure recognition (Locksley 1997). Schistosomes produce C-type lectins that can sequester the host's recognition tags, i.e. destroy the opsonization molecules that the host deposits on the parasite's surface (Loukas and Maizels 2000). Mouse cytomegalovirus codes for molecules that can bind to the host's MHC I class molecules and so puts them out of action; this blocks 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 (Murphy 1993; Yewdell and Garcia-Sastre 2002).

8.2.3.2 Avoid complement attack
The complement is the part of the innate immune system that is activated as a first line of defence (see Chapter 4). Parasites interfere with the formation of the complement-attack complex by preventing its binding to the parasite membrane—as in Streptococcus (Mitchell 2003) or Staphylococcus aureus (Rooijakkers et al. 2005). The protozoan Leishmania inhibits the complement cascade by degradation of 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). Three different biochemical cascades can activate complement (cf. Chapter 4) and all of them are targeted by parasites.

8.2.3.3 Avoid being killed by polymorphonuclear cells (PMNs)
PMNs, such as neutrophils, are also in the first line of vertebrate defence and are targeted by parasites. For example, S. aureus inhibitory protein (CHIPS) binds to receptors of neutrophils and blocks their engagement. Furthermore, groups of neutrophils sometimes form 'nets' to physically trap pathogens. Pneumococci have evolved mechanisms that allow them to escape such trapping and to spread into tissues and the bloodstream (Beiter et al. 2006). Many parasites prevent the recruitment of polymorphonuclear cells to the site of infection by interfering with the respective signalling pathways (Urban et al. 2006).

8.2.3.4 Avoid being killed by macrophages and phagocytes
Macrophages are important cells with phagocytic activity. To evade macrophages and other phagocytic immune cells, parasites have evolved a variety of tricks. An effective way is to modulate the attacking cell's cytoskeleton such that the host cell can no longer accomplish the necessary changes in shape and movement to actually perform phagocytosis. This is done, for example, by the Yops proteins deployed by the bacterium Yersinia pestis (Hornef et al. 2002). The bacterium Shigella induces apoptosis in macrophages and so avoids being attacked (Hilbi et al. 1997). Many viruses, such as myxoma, adenovirus, or vaccinia virus, code for molecules that interfere with host cell apoptosis and thus abort the action of the respective cells (Tortorella et al. 2000; Guidotti and Chisari 2001; Benedict et al. 2002; Seet et al. 2003). The defending cells also need to transport the parasite into their specialized cell vacuoles (lysosomes, phagosomes) where the invader is degraded by specialized enzymes (cf. Figure 4.4a). Bacteria have evolved several ways to block their transport into the lysosome. Should they nevertheless end up there, bacterial pore-forming proteins are released to escape from the lysosome into the cytoplasm of phagocytes, where the bacteria can survive. Such trickery is found, for example, in Listeria monocytogenes that produces a lysin to escape from the vacuole into the cytoplasm. Moreover, L. monocytogenes spreads in this manner from cell to cell (Portnoy et al. 2002).

Many bacterial parasites manipulate the internal organization of cell vacuoles to disrupt host defences. For example, Mycobacterium tuberculosis can actively arrest the development of the phagosome, which would be needed to complete the killing process of the parasite. M. tuberculosis thereby can persist in host cells for extended periods of time. M. tuberculosis additionally interferes with the antigen-presenting mechanisms of the adaptive immune system (Flynn and Chan 2003). Legionella, Coxiella, or Chlamydia, also interfere with
maturation of the cell vacuole in which they reside and block the associated destruction mechanisms (Underhill and Ozinsky 2002; Young et al. 2002). Toxoplasma modifies its host vacuole membrane with own proteins to block further immune responses (Sacks and Sher 2002).

To prevent the proper trafficking of molecules into a given vacuole is another strategy to evade host immunity. For example, Salmonella induces the formation of a vacuole that it utilizes as a self-made refuge within the host cell. In addition, Salmonella has evolved ways that prevent the delivery of (toxic) oxidases to its vacuole (Underhill and Ozinsky 2002).

Leishmania and Toxoplasma, rather than stimulating, actually down-regulate the mechanisms of apoptosis of the host cell, perhaps by the production of homologues of regulatory proteins; thus they prolong cell life and time for their own development (Sacks and Sher 2002). Another important function for the cell-based host defence is fusion between cells to destroy an invader. Parasites also can prevent this cell fusion by retaining crucial host signals (Pieters 2001; Young et al. 2002).

8.2.3.5 Manipulate the signalling network

The immune system depends on a signalling network to coordinate the various processes in an efficient way. For this purpose, signals such as cytokines, chemokines, or interferons are produced by immune defence cells, or interferons produced by immune defence cells, either in response to an infection or in response to signals by other cells. Parasites interfere with signalling in many ways and this target seems to be a major evasion strategy more generally. Examples can be found in many viruses that modulate the TNF-receptor family by producing homologues of signals (Seet et al. 2003), and by modulating the cytokine pathways in natural killer cells (Orange et al. 2002). Many other examples have been described for viruses (Guidotti and Chisari 2001; Chatterjee et al. 2002; Seet et al. 2003), but also for bacteria (Hilbi et al. 1997; Hornef et al. 2002; Portnoy 2005), or nematodes (Grencis and Entwistle 1997), and protozoa (Sacks and Sher 2002). In the bacterium Yersinia, for instance, the Yops-proteins down-regulate the expression of TNF-α, one of the most important pro-inflammatory cytokines. The trypanosome, Leishmania inhibits specifically the cytokine IL-12 in dendritic cells and macrophages, but leaves other pro-inflammatory pathways intact, and induces IL-10 to impede its clearance (Reiner and Locksley 1995; Sacks and Sher 2002).

8.2.3.6 Interference with the antigen presentation and processing pathways

An important principle in vertebrate immunity is the presentation of antigens by antigen-presenting cells (cf. Chapter 4). For this mechanism, dendritic cells (DCs) are especially important. DCs stimulate T-cells and release chemokines and cytokines to recruit further defence cells. This complicated process is targeted by parasite interference. For example, two highly dangerous bacterial pathogens, Francisella tularensis and Coxiella burnetii, suppress the release of cytokines. This prevents the maturation of DCs, which would be necessary for stimulating T-cells and producing a full response (Maurin and Raoult 1999; Bosio and Dow 2005). Yersinia infects the DCs and reduces their cytokine production, which also prevents the orchestration of a proper response (Brubaker 2003). MHC class II expression, which normally is responsible for the presentation of parasite peptides endocytosed and generated by the presenting cell, is similarly attacked by various bacteria. Moreover, viruses code for molecules that cause MHC class I molecules to remain inside an infected host cell, rather than being exported to the cell surface. As a result, an infected cell cannot signal its status by external presentation of the MHC–peptide complex that passing T-lymphocytes would recognize. This blocking is achieved by the subversion of the host’s protein degradation or trafficking pathways. The infecting viruses so cause down-regulation of the activity of T-cells and NK-cells and thereby also inhibit cytokine action (Ploegh 1998; Tortorella et al. 2000; Guidotti and Chisari 2001; Hewitt 2003).

8.2.3.7 Avoid being killed by the effectors

When an infection has been recognized, the immune system not only deploys cellular defences (killer cells, phagocytes) but also responds by the production of humoral effector molecules, such as reactive oxygen species, anti-microbial peptides, or metalloproteases (Knorr et al. 2009). Parasites deploy a variety of measures to neutralize these effectors. One mechanism is to degrade
host molecules, as is the case for *Mycobacterium tuberculosis* that catabolizes the reactive, toxic molecules (Flynn and Chan 2003). Many other bacteria degrade antimicrobial peptides or are able to reduce their efficacy, for example, by reducing the negative electric charge of their cell membrane (*Staphylococcus*), or by modifying surface molecules needed for attachment (*Salmonella*). Such modifications are furthermore known to correlate with virulence (e.g. in *S. typhimurium*; Hornef et al. 2002). Moreover, host immunoglobulins are cleaved and degraded by the production and release of proteases and lipases (e.g. *Pseudomonas*; Kharazmi 1991). A particularly interesting evasion strategy is illustrated by *Staphylococcus aureus*. These bacteria produce so-called super-antigens. They have an overwhelming effect of stimulating inflammatory cytokines and hence cause large systemic effects. But far from leading to a better defence, this binds T-cells too strongly and stops them from proliferating, thus eventually also impairing antibody production (Maillard et al. 1997).

In all, different parasite groups show a remarkable degree of parallel evolution in their evasion mechanisms. For instance, trypanosomes and fungi use equivalent signals to target the host; they also deliver modulating factors in similar ways (Haldar et al. 2006). Unrelated bacterial pathogens mimic the same host proteases (Sikora et al. 2005) and target the same elements (e.g. the Rho protein) of the regulatory cascade (such as the Kruppel-like transcription factors, KLF), which affects the expression of pro-inflammatory cytokines, induces phagocytosis, and cell proliferation (O’Grady et al. 2007). Similarly, immune evasion mechanisms are known from vertebrate and invertebrate hosts alike (Tables 8.1, 8.2).

### 8.3 Manipulation of the host phenotype to increase transmission

Beyond the evasion of the immune system, parasites are known to affect their host’s behaviour, morphology, life history, and so forth, in many ways. Natural selection will favour mechanisms that induce such changes of host phenotype when they increase components of the parasite’s fitness. Two components stand out: parasite survival that depends on the lifespan of the infection, and parasite fecundity that depends on the rate of transmission to the next host (Figure 8.3). Here, we first consider what changes in the current host might increase the chances to encounter a next host. For every transmission mode that parasites use—from direct contact between hosts to vector-borne transmission—there are examples where changes in the host phenotype occur.

#### 8.3.1 Manipulation of host behaviour

Changing the behaviour of the host is one of the most effective means to increase the chances of finding a new host. This can happen in many different ways (Table 8.3).

##### 8.3.1.1 Site of transmission in space and time

Many parasites rely on durable propagules to encounter or wait for the next host. For example, *Bacillus anthracis* forms spores that can wait in the soil or in dead bodies for decades and still remain infective (Guillemin 2001). Similarly, spores of *Clostridium* can withstand very adverse conditions and remain infective for years. *Clostridium* causes severe human diseases, such as tetanus (*C. tetani*), botulism (*C. botulinum*), or gangrene (*C. perfringens*). Durable stages are also known from many other parasitic groups.

Especially for parasites that adopt a ‘sit-and-wait’ strategy, being in the right place is of the essence. Hence, the relevant element of host manipulation is increasing the chances to reach a suitable site for transmissions. For example, *Lambornella clarki* is a protozoan parasite (Ciliophora: Tetrahymenidae) that infects mosquitoes but is not transmitted by the blood meal. Rather, *L. clarki* infects new host larvae in the water. Infected mosquitoes show reduced blood-sucking behaviour (Egerter and Anderson 1989) and instead return to water where they display oviposition behaviour. Rather than depositing eggs, the mosquito thereby spreads the parasite, which can be ingested by the mosquito larvae developing in the water (Egerter et al. 1986). Hence, the behavioural change brings the parasite to the right place for transmission. A similar pattern is observed for the spectacular nematode, *Sphaerularia bombi*, which exclusively infects bumblebee queens in their winter quarters, which are holes.
Figure 8.3 Parasite-induced changes in host phenotype. The cartoon shows how changes could increase fitness of the parasite. In this hypothetical example, a snail intermediate host is infected by a manipulating parasite (a trematode) that needs to become transmitted to a final host (a bird). (a) Manipulations to increase transmission: parasites change behaviour so that the host crawls on surfaces, or to places where the risk of predation is higher; losing fear of predators, or getting closer to predators due to increased foraging activity; leave a group of individuals that offers protection; change to conspicuous colour, or preference for the ‘wrong’ background. (b) Ways to extend the lifetime of the infection: reduce host fecundity and re-route resources into host growth and maintenance; gigantism (often associated with castration) increases survival and might reduce predation risk; manipulate host escape behaviour to avoid times and places of high predation risk.
Table 8.3  Parasite-induced changes in host behaviour with different transmission modes.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Host</th>
<th>Observation</th>
<th>Transmission and possible benefits</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Direct transmission:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabies virus</td>
<td>Mammals</td>
<td>Approach other animals more often and biting more frequent (aggressiveness).</td>
<td>Direct contact and transfer via saliva of the virus to next host.</td>
<td>Rupprecht et al. 2002</td>
</tr>
<tr>
<td>Ciliate (Lambornella)</td>
<td>Mosquito (Aedes sierrensis)</td>
<td>Infected mosquitoes return to water and show false oviposition behaviour.</td>
<td>Dissimilation of ciliate parasite that can infect new mosquito larvae in pond.</td>
<td>Egerter et al. 1986; Egerter and Anderson 1989</td>
</tr>
<tr>
<td>Fungi Phycomycetes</td>
<td>Black flies (Simuliidae)</td>
<td>False oviposition behaviour.</td>
<td>Parasites reside in ovaries and get transmitted.</td>
<td>Undeen and Nolan 1977; Yeboah et al. 1984</td>
</tr>
<tr>
<td>Nematomorpha, Mermithidae (nematodes)</td>
<td>Arthropods</td>
<td>Host moves into water.</td>
<td>Spread of parasite larvae to other hosts in water.</td>
<td>Thomas et al. 2002</td>
</tr>
<tr>
<td>Nematode (Gasteromermis)</td>
<td>Mayfly (Baetis bicaudatus)</td>
<td>Feminization of male behaviour (and morphology); infected males join females upstream and show oviposition behaviour.</td>
<td>Parasite can spread from upstream and in water.</td>
<td>Vance 1996</td>
</tr>
<tr>
<td>Fungus Entomophthora spp.</td>
<td>Ants (Formica spp.)</td>
<td>Infected ants stay at grass top where they become fixed by the growing fungal hyphae.</td>
<td>Spores can disperse more widely from elevated position.</td>
<td>Marikovsky 1962; Loos-Frank and Zimmermann 1976</td>
</tr>
<tr>
<td>Microsporidia</td>
<td>Shrimp</td>
<td>Feminization of behaviour and sex ratio distortion.</td>
<td>Parasites transmitted via females.</td>
<td>Dunn et al. 1993</td>
</tr>
<tr>
<td>Nematode (Sphaerulina bombi)</td>
<td>Bumblebee queens (Bombus spp.)</td>
<td>Spring queens seek hibernation sites instead of nests.</td>
<td>Deposition of nematode offspring in soil to infect next generation of hibernating queens.</td>
<td>Madel 1973</td>
</tr>
<tr>
<td>Monogenea, trematode (Gyrodactylus bullatarudis)</td>
<td>Guppies (Poecilia reticulata)</td>
<td>Infected individuals become sluggish and attract other guppies.</td>
<td>Parasite eggs can spread to neighbouring fish.</td>
<td>Scott 1985</td>
</tr>
<tr>
<td>Acanthocephala (Pomphorhynchus laevis)</td>
<td>Amphipod (Gammarus pulex)</td>
<td>Infected intermediate host approach predators more often, based on olfactory cues.</td>
<td>More likely to be eaten by final host, a fish.</td>
<td>Baklauf et al. 2006</td>
</tr>
<tr>
<td>Microsporidia</td>
<td>Shrimp</td>
<td>Feminization of behaviour and sex ratio distortion.</td>
<td>Parasites transmitted via females.</td>
<td>Dunn et al. 1993</td>
</tr>
<tr>
<td>Parasitoid (Conopid flies)</td>
<td>Bumblebee (Bombus terrestris)</td>
<td>Bumblebee hosts dig themselves into soil before being killed by parasite.</td>
<td>Protects developing parasite from cold (during winter) and from hyperparasitoids.</td>
<td>Müller 1994</td>
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<tr>
<td>(b) Intermediate hosts:</td>
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<tr>
<td>Trematode, liver fluke (Dicrocoelium lanceolatum)</td>
<td>Ants</td>
<td>Infected ant stays at grass top.</td>
<td>More likely to be eaten by final host, a herbivorous mammal.</td>
<td>Carney 1969; Schneider and Hohorst 1971</td>
</tr>
<tr>
<td>Acanthocephala (Polymorphus spp)</td>
<td>Gammarids</td>
<td>Changed behaviour towards light and disturbance.</td>
<td>More likely to be eaten by final host, ducks.</td>
<td>Moore 1984a</td>
</tr>
<tr>
<td>Cestode (Schistocephalus solidus)</td>
<td>Stickleback (Gasterosteus aculeatus)</td>
<td>Sticklebacks as intermediate hosts approach predatory fish more closely than usual.</td>
<td>Predator is final host of parasite.</td>
<td>Milinski 1990</td>
</tr>
<tr>
<td>Coccidia (Toxoplasma gondii) Mouse, rat</td>
<td>Infected mice are less fearful. Infected rats do not avoid cat odours.</td>
<td>Mouse or rat more likely to be eaten by final host, a cat.</td>
<td>Hutchison et al. 1980; Berdo et al. 2000</td>
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<tr>
<td>Cestode</td>
<td>Ant (Leptothorax nylanderi)</td>
<td>Ants do not show escape behaviour when disturbed.</td>
<td>More likely to be caught by final host, a woodpecker</td>
<td>Plateaux 1972</td>
</tr>
<tr>
<td>Cestode (Hymenolepis diminuta) Beetle (Tribolium spp.)</td>
<td>Beetles are more often found close to surface of medium (flour).</td>
<td>More likely to be eaten by final host, a mouse or rat.</td>
<td>Yan et al. 1994</td>
<td></td>
</tr>
<tr>
<td>Trematode (Euhaplorchis californensis) Killfish (Fundulus parvipinnis)</td>
<td>Infected fish surface more often and show conspicuous jerking movements.</td>
<td>More conspicuous, and eaten 30-times more frequently by the final host, a bird.</td>
<td>Lafferty and A.K. 1996</td>
<td></td>
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<tr>
<td>Trematode (Microphallus papillorobustus) Snail (Potamopyrgus antipodarum)</td>
<td>Feeding time changes and overlaps with predator activity.</td>
<td>More likely to be caught by final host, a bird.</td>
<td>Levri 1999</td>
<td></td>
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<tr>
<td>Trematode (Gynaecotyla adunca) Amphipod (Corophium volutator)</td>
<td>Host crawls on surface of intertidal mud flat.</td>
<td>More likely (up to 50-times) eaten by final host, a shorebird (sandpiper).</td>
<td>McCurdy et al. 2000</td>
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<tr>
<td>(c) Vectors:</td>
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<tr>
<td>Apicomplexa (Plasmodium gallinaeum) Mosquito (Aedes aegypti)</td>
<td>Mosquito takes more but smaller blood meals but only when parasite stage can be transmitted. Parasites modify enzyme (apyrase) that normally prevents blood clotting.</td>
<td>Parasite is spread among more hosts.</td>
<td>Koella et al. 2002</td>
<td></td>
</tr>
<tr>
<td>Bacterium (Yersinia pestis) Fleas</td>
<td>Bacteria in large numbers block feeding apparatus of flea. As a consequence, the flea more often regurgitates its blood meal.</td>
<td>Bacteria are more readily transferred from flea to vertebrate host.</td>
<td>Moore 2002</td>
<td></td>
</tr>
<tr>
<td>Trypanosome (Leishmania major) Sandfly (Phlebotomus duboscui)</td>
<td>Feeding behaviour changed so that more hosts (mice) are attacked.</td>
<td>Higher transmission rate is likely.</td>
<td>Beach et al. 1985</td>
<td></td>
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</tbody>
</table>
and crevices at specific sites. By its own, the nematode would never be able to reach these sites. In fact, the behaviour of an infected queen emerging in spring from the winter quarters is altered. Instead of searching for a nest site to start her colony, she will search and inspect other hibernation sites (Lundberg and Svensson 1975). In the process, the juvenile nematodes leave the host and reach the soil where they wait for the next generation of hibernating queens to arrive in autumn. In both of the cases (mosquitoes, bumblebees) the parasite manages to co-opt a behaviour that is well defined and useful for the host (oviposition, searching for a hibernation site, etc.) but is now expressed out of context and, therefore, benefits the parasite. Remarkable cases of altered host behaviour are also reported from infections by mermithid nematodes, and from nematomorphs (a group of nematode-like worms). These parasites have a free-living stage that is able to move around. Nevertheless, they often depend on being in water to survive and infect a next host. Correspondingly, infected hosts change their behaviour and seek out water bodies such as small pools and ponds. The infected hosts then literally drown themselves in the water, whereupon the offspring worms emerge and disperse to find a new host (Maeyma et al. 1994; Thomas et al. 2002). Parasites that depend on transmission by dispersal in air, such as fungal spores, have evolved various ways to force their hosts to climb to exposed locations from where the spread of the parasite’s propagules is facilitated. For example, entomophagous fungi cause their ant hosts to march to the tip of grass leaves and to fix themselves there by clasping the leaf with their mandibles. Some fungi additionally start to ‘tie’ the ant down to the leaf surface by hyphae that grow out of the host’s body. A fruit body then develops out of the immobilized ant and spreads the propagules over a wide area (Loos-Frank and Zimmermann 1976; Roy et al. 2006).

The behaviour of intermediate hosts, in particular, seems very often manipulated by parasites. Intermediate hosts have to be consumed by the final host for the completion the parasite’s life-cycle. Many example show that the behaviour of an infected host is changed to increase the probability that the final host can prey on the intermediate host. Classical examples are acanthocephalan parasites (such as *Polymorphus paradoxus*) that infect gammarids as intermediate hosts and use waterfowl as their final host. If infected, these gammarids no longer prefer the dark and do not hide in vegetation or at the bottom of a water body. Instead, they are attracted to light and cling to floating objects. As a result, they are much more frequently found and consumed by foraging ducks that act as final hosts (Bethel and Holmes 1973; Moore 1984a, 1984b). Furthermore, different species of acanthocephalans induce slightly different behaviours in accordance with what species of waterfowl is used as final host. *P. paradoxus*, for example, utilizes surface-feeding ducks, such as mallards, as their final hosts, whereas *P. marilis* uses diving ducks, such as golden eyes or scaups. In line with the different zones of depth in the water column used by the ducks, gammarids infected by *P. paradoxus* move to the water surface, whereas gammarids infected by *P. marilis* prefer to stay in the open water column where they are exposed to diving ducks (Moore 1984b). These responses are quite specific and not a simple consequence of being infected by a parasite, since the behavioural change is associated with a developmental stage of the parasite that is actually infective and does also not occur with infections by other parasites that do not use this transmission route (Bethel and Holmes 1973).

Comparable behavioural changes are associated with infection by a wide range of other parasites, too (see Table 8.3). Digenean trematodes in intermediate hosts show a remarkable pattern. Their metacercaria encyst in the host’s nervous system and await predation by the final host. Typically, however, one of the metacercariae will leave the common site and migrate to the protocerebral ganglia to encyst there, thus becoming the ‘brain worm’. This single metacercarium seems able to affect the host’s behaviour such that the host becomes more prone to move towards the light, rather mowing than towards the dark as usual (Carney 1969; Schneider and Hohorst 1971; Moore 2002). An interestingly similar situation occurs in infections by fungi, where some hyphae grow near to the central parts of the host’s nervous system and probably affect host behaviour in such close proximity (Loos-Frank and Zimmermann 1976).

Parasites that are dispersed by vectors, mainly by blood-sucking arthropods, such as mosquitoes, lice, and ticks, obviously gain by manipulating their transport vehicle in
their own favour, too. For the vector, taking up a blood meal serves the purpose of garnering resources to develop eggs. At the same time, staying on a host is dangerous because of the risk of being killed when the host defends itself, for example, by squashing the blood-sucking insect. For the parasite inside the host this would mean death, too. To minimize the risk of being killed, and to have more opportunities for transmission to new hosts, parasites should manipulate their vector so as to reduce the size of the blood meal, to stay shorter on each host, but to visit more hosts instead. Indeed, many vectored parasites cause their host to take up smaller blood meals and to leave earlier, as compared to uninfected controls (Table 8.3) (Moore 2002). Plasmodium infecting mosquitoes, for example, accomplishes this by blocking the enzyme that normally interferes with blood-clotting (allowing for a large blood meal before the host blood stops flowing; Rossignol et al. 1984). Also, the DNA analysis of blood samples from mosquitoes shows that infected mosquitoes must have visited more hosts than uninfected ones (Koella et al. 1998), thus presumably spreading the parasite over more hosts as well. Blocking the blood meal, in fact, seems to be a common strategy of parasites to manipulate blood-sucking arthropods (Moore 2002).

8.3.1.2 Transmission from host to vector

To increase the chances for the reverse transmission step, from the vertebrate host to the arthropod vector, the likelihood of attracting a vector to the infected host and of getting a blood meal must be increased (Hamilton and Hurd 2002). Manipulation by parasites could explain why infected hosts show decreased vigilance and defence against blood-sucking insects (Rossignol et al. 1985; Scott et al. 1988). Experimental mice, for example, show the weakest defence behaviour against mosquitoes when the gametocytes of Plasmodium circulate in the peripheral blood, i.e. at the time when they can be most easily taken up by a bite (Day and Edman 1983). Similarly, infected hosts seem to be more attractive to blood-sucking insects, at least in some cases (Mahon and Gibbs 1982; Scott et al. 1990; Ferguson et al. 2002; O’Shea et al. 2002; Lacroix et al. 2005). Vectors, such as mosquitoes, do not bite hosts at random but respond, for example, to olfactory cues emanating from the individual hosts (Mukabana et al. 2002). Such olfactory cues might be used where infected hosts are found to be more attractive (Lacroix et al. 2005; Nacher 2005). Similar phenomena as in mosquitoes seem to occur in other vectors, such as ticks or lice.

The parasite could also make it easier for the blood-sucking vector to take up a blood meal in other ways. This might be the reason that blood characteristics of vertebrate hosts infected by vector-borne parasites (such as malaria, dengue, or trypanosomes) are often different, in characteristic ways, from that of non-infected hosts. In particular, the number of erythrocytes (a possible side-effect of parasite-induced anaemia) and platelets is lower, which is associated with reduced blood viscosity and thus with a faster uptake of the blood meal (Rossignol et al. 1985; Taylor and Hurd 2001). Similarly, dilatation of blood vessels facilitates the uptake of a blood meal (Moloo et al. 2000) and might represent yet another way in which parasites can increase their transmission success to a vector.

Manipulation might also affect the risk-taking of the host. Normally, prey animals are very reluctant to expose themselves to their predators. One element of avoidance is to keep a sufficient distance to the predator, allowing for flight if necessary. However, infected prey behave differently and are much more prone to approach a predator than healthy animals. For example, sticklebacks (Gasterosteus aculeatus) infected with cestodes (Schistocephalus solidus) not only venture closer to the water surface but, in experimental tests, are also less intimidated by stimuli that indicate a predator’s presence (Milinski 1990; Ness and Foster 1999). This effect is less pronounced in sticklebacks infected with a parasite (the microsporidian Glugea anomala) that is not transmitted by predation, showing again that the change is not a simple response to infection but tuned towards increased predation where necessary (Milinski 1985). Very likely, the loss-of-avoidance effect results from infected fish simply being hungrier, as this gut parasite drains their food resources more than what microsporidia would do. Infected fish, therefore, forage more and take higher risks to meet their demands (Milinski 1990).

8.3.1.3 Time of transmission

All organisms show a certain degree of daily, monthly, or annual rhythms. This is not different for parasites, and they might have evolved to utilize a favourable time window
for transmission (Tinsley 1990; Moore 2002). Schistosoma mansoni (the cause of human bilharzia or schistosomiasis) illustrates the problem of timing. The parasite is contracted via its cercariae, which are shed into the water by the intermediate host, a snail. Interestingly, in areas where humans are the major host, most cercariae are shed from the snails over midday. But where rats—a nocturnal species—are the main host, the peak of shedding occurs later in the afternoon (Théron 1984). Crossing experiments show that this difference has a genetic component (Théron and Combes 1988). Similarly, trematodes infecting desert toads can only be transmitted when the host enters water and thus meets other toads during their daily routines. The trematodes show peaks of shedding every 24 hours coinciding with the visit to water by their hosts (Tinsley and Jackson 1988).

8.3.2 Change of host morphology

Parasitic infections often change the externally visible host morphology, for example, body size or sexual morphology (Table 8.4). Some of these changes are probably in the service of increasing transmission success, whereas others are more likely to affect the lifespan of the infection (e.g. gigantism). Classical examples are changes of body colour induced by acanthocephalan or cestode infections. In the case of acanthocephalans that infect amphipods as intermediate hosts, the parasite themselves can be colourful, leading to conspicuous red and orange dots under the outer integument. Experiments show that conspicuous colouration increases transmission success, in combination with parasite-induced behavioural changes that put the host at an increased risk from predation (Bakker et al. 1997).

A change in host body colour often also contrasts with the normal background of the habitat where the hosts live (Figure 8.3a). The conspicuous contrast is likely to attract a predator that is the final host. For example, cestode-infected workers of the ant, Leptothorax nylanderi, not only become more sluggish in their behaviour, but also develop a yellow colouration, which makes them highly visible among their brownish nestmates (the cestode infects the worker larvae). The final host of the cestode is a bird that typically feasts on ants, such as the green woodpecker. A conspicuous contrast between host and background is also achieved when an infected host shows an altered preference for the background. Amphipods, Armadillidium vulgare, infected by the acanthocephalan, Plagiorhynchus cylindraceus, for example, prefer to stay on a lighter substrate and so become more visible that on their normal, dark background substrate (Moore 1983). A similar change of substrate preference towards lighter backgrounds is observed in cockroaches infected by Moniliformis moniliformis (acanthocephala).

The altered morphology of a host is most obvious to a human observer if it concerns visible elements, such as size or colouration. However, in the natural context, other changes might be even more important. For example, red grouse infected by cestodes, Trichostrongylus tenuis, emanate a different odour than healthy animals; such changed odour is noticed by hunting dogs. This indicates that conspicuous odours might render infected birds more vulnerable to natural predators that use other cues in addition to visual ones (Hudson et al. 1992b). There is likely to be an entire array of parasite-induced modifications of host 'morphology' that is not immediately noticed by human observers, but that are relevant in the natural context. These might include altered pheromones, isolating an infected host from its social context, colour changes in the UV spectrum of light (for predators able to see this wavelength), body temperature (affecting heat-sensing predators such as snakes), and so forth.

8.3.3 Affecting transmission routes

Some parasites use transmission routes that guarantee transfer into a next host with unusual certainty. This is true for vertical transmission, where the next host is the offspring of the current host and, therefore, easy to reach. Examples include parasite transmission via breast milk (trans-mammary transmission), such as found in trematodes (Shoop 1988) and nematodes (Anderson 1992). An important further category is trans-ovarial transmission from mother into her eggs. This transmission route is used by many groups of parasites, such as viruses (Watts and Eldridge 1975), bacteria (Perlman et al. 2006; Duron et al. 2008), or microsporidia (Smith and Dunn 1991; Dunn et al. 2001). Sexually transmitted parasites also exploit a secure path of transmission, and in these cases might manipulate the host to intensify its mating behaviour.
Table 8.4 Parasite-induced changes of host morphology.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Host</th>
<th>Observation</th>
<th>Possible function for parasite(^1)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungus (Entomophthora muscae)</td>
<td>House fly (Muscus domestica)</td>
<td>Abdomen extended with white spots of fungal conidia on outside.</td>
<td>Attracts males to approach dead fly, presumably mistaken for a fecund female.</td>
<td>Møller 1993</td>
</tr>
<tr>
<td>Cestode</td>
<td>Ant (Leptothorax nylanderi, and many other species)</td>
<td>Infected ants have yellow instead of normal brown colouration (cestode infects larval stage).</td>
<td>More conspicuous for predator</td>
<td>Plateaux 1972; Buschinger 1973; Passera 1975; Stuart and Alloway 1988</td>
</tr>
<tr>
<td>Trematodes (Leucochloridium, Neoleucochloridium)</td>
<td>Snails</td>
<td>Sporocysts of parasite are brightly coloured. Snail tentacles swollen, colourful and pulsating</td>
<td>Attracts attention of final host, a bird.</td>
<td>Lewis 1974; Lefèvre and Thomas 2008</td>
</tr>
<tr>
<td>Trematode (Schistosoma solidus)</td>
<td>Stickleback (Gasterosteus aculeatus)</td>
<td>Distended belly, larger pectoral fins, lack of pigment on integument.</td>
<td>Host becomes more conspicuous and attract potential hosts to the vicinity. Mussel more likely to stay on mud flat surface. Five to seven times more likely to be eaten by final host (oystercatchers).</td>
<td>Brønseth and Folstad 1997; Ness and Foster 1999; Thomas and Poulin 1998; Mouritsen 2002</td>
</tr>
<tr>
<td>Trematode (Curtuteria, Acanthopharyphium)</td>
<td>Mussels (Austrovenus stutchburyi)</td>
<td>Parasite migrates to foot of mussels, replace muscle tissue and cause foot to become smaller</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cestode (Schistocephalus solidus)</td>
<td>Stickleback (Gasterosteus aculeatus)</td>
<td>Colouration change with visible spots.</td>
<td>Host becomes more conspicuous and attracts predators.</td>
<td>LoBue and Bell 1993</td>
</tr>
<tr>
<td>Acanthocephala (Polymorphus spp.)</td>
<td>Gammarid</td>
<td>Orange colouration by carotenoids.</td>
<td>Experimental tests show no effect of colouration on increased predation by final host.</td>
<td>Kaldonski et al. 2008</td>
</tr>
<tr>
<td>Crustacean (Cymothoa exigua)</td>
<td>Fish (snapper)</td>
<td>Parasite attaches to tongue, grows and eventually replaces tongue by its own body.</td>
<td>parasite can easily secure its own share of food. 'Tongue' can be used otherwise normally by fish. Fecundity compensation to limit effect of later castration or early death. Unclear for case of parasitoid infection.</td>
<td>Brusca and Gilligan 1983; Adamo 1999</td>
</tr>
<tr>
<td>Bacteria (Serratia marcescens)</td>
<td>Cricket (Acheta domestica)</td>
<td>Earlier reproduction by females infected by bacteria, but not when infected by fly larva.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Function has rarely been tested in the same system and therefore often remains conjectural.
Vertical transmission typically runs from the female to her offspring. Vertical transmission is affected by a number of host reproductive traits that can be exploited by the parasite. Several corresponding phenomena have been described, depending on whether the parasite finds itself in a male or female host.

**Manipulating a female host**
When parasites are transmitted via mother’s eggs (transovarial transmission), the likelihood of transmission can be increased in various ways:

1. **Increase of host fecundity.** More eggs are produced and can carry the parasite. Increased fecundity likely comes at the cost of future female survival.

2. **Eliminate competing females that do not carry the parasite.** Cytoplasmic incompatibility factors ensure that non-infected females are killed and, hence, the relative frequency of the infection increases in the population. This strategy is observed in *Wolbachia* (Duron et al. 2008) and other bacteria (Duranton et al. 2008).

3. **Induction of (thelytous) parthenogenesis.** This increases the fraction of purely female-derived infected hosts in the population. Examples are *Wolbachia* (as well as *Rickettsia* and other bacteria; Duron et al. 2008) that induce parthenogenesis in hosts with haplo-diploid sex determination systems (such as hymenopterans). In this system, unfertilized eggs normally would develop into (haploid) males, and fertilized eggs into (diploid) females. In infected hosts, the parasite prevents chromosomal separation during the early development of the unfertilized egg, thus turning the male into a genetically diploid female (Huigens and Stouthamer 2003).

**Manipulating a male host**
In this case, the parasite is in the ‘wrong’ sex and can increase likelihood of transmission by turning its host into a female mimic:

1. **Feminization of males.** Feminization of the host by parasites is quite widespread, i.e. genetic males are turned into morphological, and sometimes functional females (Dunn et al. 1993; Bouchon et al. 2008). Feminization is known from *Wolbachia* (Hurst 1993) and microsporidia, primarily in crustacean hosts (Dunn et al. 2001). During development of an infected individual, the parasite suppresses the proper functioning of the male androgenic gland (Rodgers-Gray et al. 2004). Also, male behaviour can become feminized. In these cases, the parasite induces female elements in males, such as oviposition behaviour. This transports the parasite to a favourable location, as in the example of mayflies infected by nematodes. Mayfly males infected by nematodes show ‘oviposition’ behaviour and thereby deposit the nematode larvae in upstream waters (Vance 1996).

2. **Eliminating males.** The male egg or embryo is killed, which frees resources to produce female offspring instead. *Thelohania* and *Ambylostosa* (microsporidia) that infect mosquitoes, for example, show alternating transmission cycles with vertical and horizontal transmission. For vertical transmission from infected mothers, developing males are killed; such killing can come early or late in development. For horizontal transmission, the host larva is killed and spores released into the water (Dunn et al. 2001).

3. **Distorting the primary sex ratio.** A female-biased offspring sex ratio benefits the parasite by an increased frequency of next generation hosts of the right sex. Sex-ratio distortion is generated by various mechanisms (Hatcher and Dunn 1995) (Dunn et al. 2001).
The trans-ovarial transmission route is naturally restricted to the female host, but even when in male hosts, parasites use mechanisms to tip the balance towards their own favour (Dunn et al. 2001) (Box 8.3). Feminization of genetic males, for example, is a strategy often found in parasites of crustacean hosts. In several other host-parasite systems, the formation of intersex morphs (i.e. partly male and partly female) is observed. The significance of intersexes for transmission is still unclear (Moore 2002), but, perhaps, intersexes result from incomplete feminization (Kelly et al. 2006). To find a new host, a parasite can also induce dispersal behaviour (Boulinier et al. 2001). For example, honeybee workers from nests infected by mites (Varroa) have been reported to drift more frequently to foreign nests, than their counterparts from uninfected nests. With drifting, a worker ends up in a foreign nest where it can become accepted. This is likely to facilitate the spread of the parasite (Sakofski 1990).

8.3.4 Affecting social behaviour

Socially living animals offer a lot of opportunities for parasites to get transmitted (Schmid-Hempel 1998). This is suggested by a commonly observed positive association between the size of a social group and infection loads in birds (Brown and Bomberger Brown 1986; Poulin 1991a), fish (Ranta 1992), mammals (Hoogland 1979), or social insects (Schmid-Hempel 1998; but for counter-examples see: Hughes et al. 2002). In the social context, the benefit of manipulation for the parasite depends strongly on its transmission mode. Whereas directly transmitted parasites might gain from close proximity among (genetically related) individuals, vectored parasites, or those needing a final host, might benefit from isolating the current host from its social context, e.g. making it more vulnerable to predation than when in its normal social group.

Consider shoaling behaviour in fish where close proximity among hosts favours transmission. At least under experimental conditions, fish infected by mobile ectoparasites tend to shoal together more often, which should increase the chances of an ectoparasite to reach a next host nearby (Barber 2000). On the other hand, being in a group reduces the risk of predation for each host individual (the ‘selfish herd’ effect; Hamilton 1971), and thus decreases the chances for the parasite to transmit from its current host in the group to the final host, such as a predatory fish. Parasitized host fish, in fact, often show impaired swimming abilities that make them unable to fully participate in shoals, e.g. they often swim in risky peripheral positions (Barber 2000). Furthermore, healthy individuals seem to be reluctant to join shoals with infected fish, in particular, those carrying contagious ectoparasites. Hence, shoaling decisions are certainly affected by parasites. Viewed from another perspective, shoaling might in turn reduce the per capita risk of the host of becoming infected (Barber 2000). Such a dilution effect is likely for herding ungulates where the number of bites by blood-sucking insects per capita decreases with herd size (Rubenstein and Hohmann 1989). Infected individuals that abandon the current social group are observed in colonially nesting birds. Often, such behavioural changes in birds are associated with infections by blood-sucking ectoparasites, such as lice, and might serve to spread the parasite to new breeding colonies (Boulinier et al. 2001).

A change in aggressive behaviour also seems to be commonly associated with infection by directly transmitted parasites. Examples are viruses, such as rabies or hanta virus, that need to get in contact with the next host, and that can enter a new host via wounds that might be inflicted during aggressive encounters between the current and next host (Klein 2003). Alternatively, rodents infected with parasites that rely on a final host, e.g. Toxoplasma gondii, show increased aggression and exploratory behaviour that puts them in jeopardy of being noticed by a predator, such as a cat, the final host (Klein 2003).

8.4 Manipulation of the host phenotype to increase infection lifetime

8.4.1 Fecundity reduction

In addition to transmission, the lifespan of the infection is a major fitness component of the parasite, as it allows for longer growth and development, and the production of more transmission stages. A number of manipulations could prolong the duration of the infection (Table 8.5). For example, full or partial castration (fecundity reduction)
Table 8.5  Parasite-induced changes of host life-history.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Host</th>
<th>Observation</th>
<th>Possible function for parasite(^1)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trematode (Schistosoma)</td>
<td>Snail (Biomphalaria glabrata, and many others)</td>
<td>Infection leads to castration and gigantism.</td>
<td>Prolongs host life by re-allocating host resources from reproduction.</td>
<td>Minchella 1985; Mouritsen and Jensen 1994</td>
</tr>
<tr>
<td>Trematode (Gyrodactylus turnbulli)</td>
<td>Guppy (Poecilia reticulata)</td>
<td>Females infected by parasite are less selective in mate choice.</td>
<td>Perhaps more proximity to potential new hosts.</td>
<td>Lopez 1999</td>
</tr>
<tr>
<td>Cestode (Hymenolepis diminuta)</td>
<td>Beetle (Tenebrio molitor)</td>
<td>Fecondity reduced (castration). Host survival increased.</td>
<td>Prolongs host life and frees resources for parasite.</td>
<td>Hurd et al. 2001</td>
</tr>
<tr>
<td>Cestode (Hymenolepis diminuta)</td>
<td>Beetle (Tribolium confusum)</td>
<td>Reduced mating vigour and fewer matings with females.</td>
<td>Frees resources for parasite.</td>
<td>Rai and Yan 2003</td>
</tr>
<tr>
<td>Cestode (Spirometra mansonioides)</td>
<td>Many rodents</td>
<td>Gigantism.</td>
<td>Prolongs host life and frees resources for parasite.</td>
<td>Mueller 1963; Phares 1996</td>
</tr>
<tr>
<td>Cestode (Schistocephalus solidus)</td>
<td>Stickleback (Gasterosteus aculeatus)</td>
<td>Infected fish grow faster and are in better condition. Gonad development reduced, but does not explain full effect.</td>
<td>Higher reserves might buffer host against starvation over winter. Fatter fish more vulnerable to predation by final host, a bird</td>
<td>Arnott et al. 2000</td>
</tr>
<tr>
<td>Microsporidia</td>
<td>Shrimp</td>
<td>Feminization.</td>
<td>Higher transmission via female functions.</td>
<td>Dunn et al. 1993</td>
</tr>
<tr>
<td>Nematode (Sphaerularia bombi)</td>
<td>Bumblebees (Bombus spp.)</td>
<td>Infected queens emerging in spring are castrated.</td>
<td>Freeing of host resources for parasite.</td>
<td>Madel 1973</td>
</tr>
<tr>
<td>Nematode (Mermithids)</td>
<td>Mayfly (Baetis bicaudatus)</td>
<td>Feminization of males: secondary sexual characters modification results in intersexes or complete sex reversal. Behaviour feminized so that males show oviposition behaviour.</td>
<td>Freeing of host resources for parasite. Dispersal of parasite from upstream locations,</td>
<td>Vance 1996</td>
</tr>
</tbody>
</table>

\(^1\) Function has rarely been tested in the same system and therefore often remains conjectural.
curtails the host’s investment into reproduction and can lead to re-allocating resources to host survival and parasite development instead (Baudoin 1975). A spectacular example of host castration is provided by the crustacean, *Sacculina*, parasitizing shore crabs (*Carcinus maenas*, cf. Figure 3.4). Castration in this case occurs through interference with the host’s hormones. At the same time, both sexes of the crab behave like females during the time when the parasite matures and reproduces. In particular, infected crabs move to deeper water and start fanning the parasite eggs (as they would normally do with their own brood), thus ensuring favourable development of parasite propagules (Rasmussen 1959). Host castration is also notorious for snails acting as intermediate hosts (e.g. *Biomphalaria*) for trematodes (e.g. *Schistosoma*). Because castration typically only occurs some time after infection, the host has the chance to accelerate reproduction and so to compensate for some of the expected loss of fecundity. Such ‘fecundity compensation’ has been discussed as a general strategy of host defence (Minchella and Loverde 1981; Minchella 1985) (see Chapter 14).

When host’s resources are routed away from reproduction to maintenance and growth, the host may grow to a large body size (Table 8.5). Such ‘gigantism’ is different from cases such as human ‘elephantiasis’, caused by infections of the filarial worm, *Wuchereria bancrofti*. This is because gigantism reflects a different allocation pattern of host resources rather than a simple tissue effect. Such a special role for gigantism is suggested by the observation that an increased body size reached with gigantism is not always a simple additive effect of re-routing host resources. In sticklebacks infected by a cestode, for instance, the additional growth is not explained by the amount of resources not invested into gonad development (Arnott et al. 2000). Rather, resources are strategically re-allocated beyond a simple saving effect. Castration and gigantism are thought to prolong the lifetime of the infection by extending host survival. The empirical evidence on this point is quite mixed, however. In many cases, no difference is found between the lifespan of infected and healthy individuals, such as for malaria-infected mosquitoes (Ferguson and Read 2002), or tsetse flies infected by trypanosomes (Moloo and Kutzua 1985; Maudlin et al. 1998). In tsetse flies, other studies have found that infected males flies live longer but females shorter (Makumi and Moloo 1991). In line with the hypothesized benefits, longer host lifespans are found in beetles (*Tenebrio*) infected by cestodes (*Hymenolepis*; Hurd et al. 2001). In contrast, other studies in vectors such as sandflies infected by *Leishmania* (El Sawaf et al. 1994), mosquitoes infected with nematodes (Krishnamoorthy et al. 2004), and ticks infected by *Rickettsia* (Niebylski et al. 1999) or apicomplexa (Watt and Walker 2000), show a shorter lifespan of infected individuals.

Given that the parasite draws on the host’s resources, a parasite-induced re-routing of resources away from reproduction towards maintenance and survival should actually increase lifespan despite the infection (Hurd 2003, Table 8.5). As a result, infected hosts might have the same lifespan as their uninfected counterparts, even though without re-routing of resources they would die earlier. Hence, the frequent observation of no difference between the lifespan of infected and healthy individuals might conceal the fact that the parasite nevertheless increases the host’s lifespan by re-allocation of resources (Lefèvre and Thomas 2008). This makes it difficult to test the real effect and its consequences; ideally, infections where the manipulation by the parasite is experimentally eliminated should be compared to infections where the parasite has an intact repertoire. In the future, such tests might become possible with the advent of RNAi-technology.

### 8.4.2 Changes of the social context

A good study subject for this class of manipulations is the social insects (ants, bees, wasp, and termites). In these social animals, infected individuals often desert their colony; for example, social wasps (*Polistes dominulus*) parasitized by strepsiptera (*Xenos vesparum*) stop working, become inactive, and eventually leave the nest. This behaviour presumably favours the completion of the parasite’s life-cycle because inactive workers would normally be aggressively challenged by their nest mates, which increases the risk of host death and thus destruction of the parasite (Hughes et al. 2004a). In some cases though, nest desertion is a defence strategy of the host,
for example, when bumblebee workers infected by parasitic flies stay outside the nest, especially at night, which seems to retard or abort the parasite’s development (Müller and Schmid-Hempel 1993).

8.5 Mechanisms of host phenotype manipulation

As the examples make clear, a wide range of infection-associated changes in the host phenotype is observed. It is not always clear, however, which party—host or parasite—might benefit from a changed phenotype (Box 8.2). Understanding the mechanisms inducing the change might help to clarify this question. For example, if a molecule generated by the parasite is initiating the phenotypic change, an adaptive benefit for the parasite rather than the host is likely (Table 8.6). Unfortunately, little is known about how parasites induce a change in behaviour, morphology, or life history of the host. Yet, it is reasonable to assume that parasites can either produce their own manipulative molecules (modulators; direct effect), or induce the host to produce them (indirect effects; Adamo 2002). Microparasites, for example, are typically much smaller than their hosts and, hence, often might not be capable of producing large enough quantities of modulators to affect the host directly. Hence, tricking the host into producing these modulators might be a more promising strategy for small parasites. Alternatively, collaboration among small parasites, such as in quorum-sensing (West et al. 2006), could achieve a similar result. Macroparasites, on the other hand, might be large enough to produce their own inventory of modulating molecules and to affect the host directly.

To change host behaviour, the neuronal system of an animal host has to be affected. For example, the manipulation of neurotransmitters and hormones is an effective way to change behaviour (Table 8.6). Parasites can infect cells, such as neurons, glia cells, or endothelial tissues to achieve this. Borna disease virus (BDV), which infects sheep and horses, renders hosts more aggressive. This seems to be possible by choice of the infection site (the limbic and cortical brain regions), where the virus modulates neurons and dopaminergic receptors (Klein 2003). A particularly interesting entry point for parasite manipulation are neuromodulators, typically hormones, that affect the activity of the nervous system (Adamo 2002). Parasites can exploit this possibility by subverting the normal function of neuromodulators in their own favour. In fact, parasites often secrete or induce the production of neuromodulators in their hosts. Wasp-derived venom, for example, acts as a neuromodulator that paralyses a cockroach host and makes it unable to walk in a coordinated way except when the wasp ‘leads’ the victim back to its nest. Hormones, such as serotonin, octopamine, noradrenalin, or the neurotransmitter GABA are also suspected to be involved in parasite-induced changes of host behaviour. Serotonin, for example, has been implicated for the clinging behaviour of acanthocephala-infected gammarids to drifting objects in the water (Adamo 2002). Changes that affect life history, such as fecundity reduction, might also be traced back to changes in neuro-active products (de Jong-Brink et al. 1997). For example, intermediate snail hosts infected by the schistosome, *Trichobilharzia ocellata*, cease to produce eggs under the influence of schistosomin, a parasite-secreted protein that acts as a neuromodulator. Schistosomin reduces the responsiveness of certain cells in the snail’s ganglia (the caudodorsal cells) that normally release signals to induce ovulation and egg-laying (Hordijk et al. 1992). The same infection also leads to an up-regulation of other neuromodulators.

In many of these examples, it is suspected that an eventual effect on the host behaviour is mediated perhaps not by direct interference, but through coercion of the immune system for which parasites have evolved countless strategies of evasion (Adamo 2002). For example, inflammatory responses affect chemical signals in the body and so can change behaviour (Klein 2003). The intrinsic link between the immune and neuronal system could thus be a major axis of how parasites manipulate host behaviour indirectly. Infection by rabies, for example, is associated with the onset of aggressive behaviour and loss of fear by infected hosts such as foxes (Tierkel 1975; Niezgoda et al. 2002). These changes increase viral transmission from the saliva of infected animals to a next host that is more likely to be bitten (Rupprecht et al. 2002). Hence, one should expect that the virus somehow induces this behaviour. However, the virus usually lodges in parts
Table 8.6 Physiological mechanisms of manipulation.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Host</th>
<th>Effect on host</th>
<th>Associated mechanism 1</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Life-history changes:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cestode (Hymenolepis diminuta)</td>
<td>Beetle (Tenebrio molitor)</td>
<td>Fecundity reduction, castration.</td>
<td>Vitellogenesis in fat body down-regulated by parasite-produced molecule. In ovary, egg development disrupted by host-derived inhibitor of juvenile hormone.</td>
<td>Hurd 2001</td>
</tr>
<tr>
<td>Cestode (Spirometra mansonioides)</td>
<td>Rodents</td>
<td>Gigantism</td>
<td>Parasite-secreted proteinases (tissue invasion) and growth factor mimicking mammalian host factor suppressing immune system.</td>
<td>Amott et al. 2000</td>
</tr>
<tr>
<td>(b) Behavioural changes:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Borna disease virus (BDV)</td>
<td>Rats (Rattus norvegicus)</td>
<td>Increased aggression.</td>
<td>Loss of dopamine in neurons. Dopamine binding changes, dopamine metabolism increase in cortex. BDV infects neurons in limbic cortical areas and olfactory bulb.</td>
<td>Solbrig et al. 1995, Solbrig et al. 1996a, 1996b; Klein 2003</td>
</tr>
<tr>
<td>Herpes simplex virus</td>
<td>Mouse (Mus musculus)</td>
<td>Increased aggression.</td>
<td>Increased synthesis of serotonin and dopamine.</td>
<td>Lycke and Roos 1974; Klein 2003</td>
</tr>
<tr>
<td>Tick-borne encephalitis virus</td>
<td>Mouse (Mus musculus)</td>
<td>Increased aggression. Increased sexual interests.</td>
<td>Increased testosterone. Virus infects central nervous system.</td>
<td>Moshkin et al. 2002; Klein 2003</td>
</tr>
</tbody>
</table>

(continued)
### Table 8.6 Continued

<table>
<thead>
<tr>
<th>Par...</th>
<th>Host</th>
<th>Effect on host</th>
<th>Associated mechanism(^1)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxoplasma gondii</td>
<td>Mice, rats</td>
<td>Increased aggression and dominance. More active social exploration, less defensive behaviour.</td>
<td>Increased dopamine and homovanillic acid (HVA) but less norepinephrine in brain. <em>T. gondii</em> infects neurons, glial cells in the limbic areas, frontal cortex and basal ganglia.</td>
<td>Stibbs 1985; Arnott et al. 1990; Webster et al. 1994; Kristensson et al. 2002; Klein 2003</td>
</tr>
<tr>
<td>Trematode (<em>Schistosoma mansoni</em>)</td>
<td>Snail</td>
<td>Changed behaviour.</td>
<td>Parasite secretes endorphins and other peptides. Unclear whether quantity is sufficient to explain observed increase in titres. Secreted molecules affect neuronal and immune functions of the host. Perhaps local immune suppression as main advantage with side-effect on behaviour.</td>
<td>Kavaliers et al. 1999</td>
</tr>
<tr>
<td>Trematode (<em>Euhaplorchis californensis</em>)</td>
<td>Killifish (<em>Fundulus parvipinnis</em>)</td>
<td>Changed behaviour: Infected fish surface often.</td>
<td>Parasite metacercariae secrete fibroblast growth factors known to be associated with neuropathology in vertebrates.</td>
<td>Lafferty and A.K. 1996</td>
</tr>
<tr>
<td>Mite (<em>Ornithonyssus bursa</em>)</td>
<td>Barn swallow (<em>Hirundo rustica</em>)</td>
<td>Decreased song activity and lower mating success.</td>
<td>Increased anaemia.</td>
<td>Møller 1991</td>
</tr>
</tbody>
</table>

\(^1\) These mechanisms are not necessarily causal, since the corresponding studies with defective pathways have not been done.
of the central nervous system that are not directly involved in aggressive behaviour. In addition, two clinical syndromes of rabies exist: the mild form (dumb, paralytic) and the furious (encephalitic) form. Only the latter is associated with aggressive behaviour. Hosts of the furious form are furthermore characterized by being able to mount an intact immune response. It is, therefore, possible that processes associated with an intact immune response towards rabies eventually lead to aggressive behaviour (a kind of immunopathology, cf. Chapter 9) rather than any direct action of the parasite (Thomas et al. 2005a). In fact, the infection stimulates the production of inflammatory cytokines that activates the hypothalamic–pituitary axis, which increases the levels of adrenaline and neurotransmitters that is typical for eliciting aggressive behaviours (Hemachudha et al. 2002). Similar processes might be present in trematodes that affect the behaviour of their snail intermediate hosts (*Lymnaea stagnalis*) (de Jong-Brink et al. 2001). Hence, host manipulation has evolved in many different parasite groups and the mechanisms are often convergent. For example, fungi and trematodes grow hyphae and send metacercariae, respectively, near the central parts of the neuronal system, presumably to induce the host to climb to the tops of grasses and leaves (Moore et al. 2005). Apart from any parasitic manipulation, hosts often respond to parasitic infection by economizing on their resources, such as reducing egg production or increasing foraging activity to acquire more resources. Parasites could exploit this standard response and use it to gain transmission (e.g. becoming more exposed to predators by more frequent foraging), or to prolong survival (e.g. hosts invest more in their own maintenance). Pre-existing compensatory mechanisms of hosts can, therefore, be another evolutionary port of entrance towards parasite manipulation (Lefèvre et al. 2008b).

Any manipulation is likely to be costly for the parasite but these costs are typically difficult to measure (Poulin et al. 2005). Possible costs include energetic expenses, putting the host at risk of being killed at the wrong time, the induction of immunopathology in the host with a serious effect on host condition, or missing the benefits of manipulation. For example, the ‘brain worms’ found in trematodes (*Dicrocoelium dendriticum*) infecting ants that migrate to the central parts of the ant’s brain, do not develop into infectious stages and thus will not benefit from the host being eaten by the final host. The action should, however, benefit related and infective metacercaria that are sitting elsewhere in the same host. A similar situation exists for trematodes infecting mussels. The infection destroys or reduces the size of the mussel’s foot. As a consequence, infected mussels can no longer bury into the substrate, become stranded on the surface, and are eaten by birds and fish, the final host of the trematodes. Those parasitic individuals that cause the loss of the foot are found at the top of the foot, but this part of the body is usually not consumed by the predator (Poulin et al. 2005). Hence, these individuals carry the cost of the manipulation but share the benefits only occasionally.

### 8.6 Strategies of manipulation

The many cases of parasite-induced changes are often spectacular in kind and generate the image of parasites completely taking control over their host. This view is obviously not correct, since any manipulation entails benefits as well as costs. The costs are not only under the control of the parasite, but are also determined by the host. Efficient host defences, for example, would necessitate higher investments by the parasite to succeed in manipulation.

#### 8.6.1 What manipulation effort?

Given costs and benefits, what is the optimal level of manipulation? A simple expectation is that the optimal effort to increase the lifespan of the infection should be higher when otherwise host longevity decreases. Manipulation effort should also increase when otherwise transmission without manipulation is rare, or when the parasite produces only a few transmission stages, or when many hosts in the habitat are already infected. In all these cases, the parasite should invest more into manipulation in order to exploit the host more, or to gain more transmission (Poulin 1994). Models also show that manipulating the host to disperse is advantageous to the parasite (Lion et al. 2006). In this case, the optimal level of the manipulative effort depends on the spatial
PARASITE IMMUNE EVASION AND MANIPULATION OF HOST PHENOTYPE

dynamics of host and parasite. For example, if dispersal is constrained to be local and the spatial dynamics thus not very much affected by dispersal behaviour, the parasite's best strategy is given by the cost–benefit balance similar to a non-spatial situation. If dispersal is long-distance by contrast, the spatial dynamics starts to take effect and the best manipulation effort is to produce medium-range dispersal, even if there are no costs associated with the manipulation. Hence, spatial dynamics is an important determinant of the ESS-strategy (evolutionary stable strategy) for the manipulation effort (Lion et al. 2006).

The parasite might also be in a position to increase the defence costs for the host to a level where it becomes cheaper for the host to tolerate a certain amount of manipulation rather than to fight it at high costs. This parasite strategy is reminiscent of methods of extortion payments in organized crime and has, therefore, been termed the ‘mafia strategy’. As with the mafia, cooperation of the host with the parasite (i.e. no defence) per se is not beneficial. However, the parasite has evolved mechanisms that if hosts fail to cooperate, this will make matters worse. In other words, the parasite steps up its virulence (the damage done to the host) if the host actively defends itself against manipulation. As a result, tolerating the parasite's manipulations might be the better host strategy. For example, cuckoos might force host parents to accept their eggs because, if they reject them, the host's nest is actively destroyed by the cuckoos; rejection is more damaging than accepting the parasitic eggs (Soler et al. 1995). It is still unclear whether the mafia strategy is widespread though. However, given the many ways in which parasites can manipulate the immune system of their hosts (Schmid-Hempel 2008b), the molecular level is a plausible ground for mafia-like strategies. For example, parasites could induce inflammatory cytokines in a strongly defended, but not in a weakly defended, host. The cytokine storm (cf. Chapter 9) in turn eventually causes more serious damage than if the host stepped down the defence and tolerated the infection.

8.6.2 Multiple infections

In the wild, most hosts carry more than one infection: either infections by different strains of the same parasite, or infections by different parasite species. Co-infecting parasites will influence each other, and the combined effect of manipulation is probably more than just the sum of manipulations by the single parasites. Consider the different situations in which the parasite can find itself, as illustrated by the example of intermediate hosts (Lafferty et al. 2000) (Figure 8.4). For example, when two co-infecting parasites are both capable of manipulating a host (‘manipulators’), but each has a different final host or uses different transmission routes, there is a conflict over the optimal manipulation strategy. To avoid such situations, parasites might not infect a host that is already infected; this is an option for female parasitoids that can distinguish infected from uninfected hosts. Avoidance seems less likely for parasites such as viruses—unless the current host avoids other infected hosts; for example, when being induced to behave in this manner by the parasite it already harbours. Another solution of the conflict is to eliminate the competitor. For example, the two cestodes *Hymenolepis diminuta* (the final host is a rat) and *Raillietina cesticillus* (the final host is a chicken) both infect the same intermediate host, the flour beetle *Tribolium*. Both cestodes manipulate the behaviour of the beetle to increase transmission to their final host, but the respective manipulations differ. In this case, *R. cesticillus* is able to prevent the successful establishment of an infection by *H. diminuta*, and so eliminates its competitor from the same host (Gordon and Whitfield 1985). Such cases of interference should be quite common and are, for example, also suspected for competing acanthocephala (Barger and Nickol 1999) that are renowned masters of host manipulation (Moore 1984a). Conflicts over manipulation should notably also be found when one parasite uses the horizontal and the other the vertical transmission route.

An interesting aspect of multiple infections with manipulation is that a non-manipulating parasite might benefit from the manipulation by co-infecting parasites, thus saving the costs of manipulation, yet benefiting from the effects (Thomas et al. 1998, 2005a). This is a case of ‘hitch-hiking’ on the efforts of the manipulating parasite, especially when this happens in a routinely found association between two parasites, or simply...
Figure 8.4 Co-infection by manipulating parasites. The examples illustrate the infection by a manipulating (filled symbol) and non-manipulating (open symbol) parasite of the same intermediate host. (a) The final hosts are different for the two manipulating parasites. The conflict might be solved by killing the competitor, or by avoidance of hosts already infected by the competitor. (b) The final host is the same. The two manipulators might cooperate; a non-manipulator might benefit by hitch-hiking, or when it co-infects by chance (‘lucky passenger’). (c) The final hosts are different and the manipulator wins (solid arrow); the competitor is transferred to the wrong final host (‘unlucky passenger’).
when it results from a chance co-infection, in which case the hitch-hiker is called a ‘lucky passenger’ (Figure 8.4b). A possible example is the case of two co-infecting trematodes, *Maritrema subdolum* (that does not manipulate) and *Microphallus papillorobustus* (that does manipulate), both using amphipods as their intermediate host. *M. papillorobustus* induces the amphipod to be closer to the water surface where they can be eaten by the final host, a bird. *M. subdolum*, in turn, does not manipulate the host, even though the similar final hosts are used. To benefit from the other parasite’s efforts, the cercariae of *M. subdolum* seek out hosts already infected by the manipulating parasites. To do so, cercariae of *M. subdolum* move to the surface and so are more likely to infect an amphipod that is already infected by the other, manipulating parasite (Thomas et al. 1997).

Cooperation among co-infecting manipulating parasites should be affected by the degree of relatedness among them, and unrelated groups of parasite should

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**Figure 8.5** ESS-investment into manipulation (manipulation effort). In this example, a group of co-infecting parasites cooperates to manipulate their host. (a) The relationship of the total manipulation effort of the group and group fitness. In case A, the fitness function saturates, in case B there is a logistic relationship. The value $p$ is parasite fitness without manipulation. (b) The ESS-effort per capita based on the two fitness functions in relation to the number of co-infecting parasites (group size). Below a minimum group size, $n_{crit}$, there should be no manipulation in case B. For case A manipulation always is advantageous. (c) The ESS-combined group effort in relation to group size for the two fitness functions. Redrawn from Brown (1999) with permission from The Royal Society.
• Virtually all parasites manipulate their hosts in various ways. Manipulation affects the immune system, host behaviour, host life-history, and many other host functions.

• Manipulation of the immune system (immune evasion) can be passive or active. Passive evasion does not entail the production of interfering molecules and includes strategies such as molecular mimicry, changing surface identity, or quiescence. Active evasion involves the production of molecules that actively interfere with the host immune system. These molecules can either be secreted into the environment and, hence, act at a distance, or take effect at close range, such as parasite surface-bound molecules or those directly injected into host cells, e.g. by various bacterial secretion systems.

• Immune evasion targets all major steps of the immune response, such as recognition (e.g. blocking opsonization), signalling (manipulating cytokines), and effector systems (inducing apoptosis of phagocytes, destroying anti-microbial peptides).

8.7 Ecological significance of manipulation

Host manipulation by parasites can affect various important elements that govern the ecological interactions between host and parasite. Formally, manipulations affect various variables of the epidemiological equation (see Chapter 11) (Lefèvre et al. 2008a). But ecological characteristics are also affected more directly. For example, a higher rate of predation, and thus changes in the flow of nutrients and energy in the ecosystem, is one obvious consequence of hosts being manipulated to be more often exposed to predators. At the same time, manipulation is reducing competition with other species using the same diet and habitat, perhaps affecting the spatial distribution of organisms. Parasite-induced changes can also seriously alter the pollen flow (Schmid-Hempel and Schmid-Hempel 1990) and thus potentially lead to isolation or hybridization of flowering plants. Manipulation also affects the parasite community itself. Whether or not these effects will significantly alter the functioning of an ecosystem depends on the size of the manipulative effects and the frequency of infected hosts. As of today, the actual impact of manipulation on ecological communities and populations is not known. Given that parasitism is common and that manipulation is suggested by observations in virtually all cases studied to date, the ecological significance of manipulation could actually be quite high (Lefèvre et al. 2008a).
• Manipulation of the host phenotype can increase transmission probability for the parasite, by affecting host behaviour, host morphology, or host social behaviour to put the host at the right place and time. Changing host sexual characteristics is especially important for vertically transmitted parasites.
• Manipulation of the host phenotype can also increase the duration of the infection by increasing the resources allocated to host maintenance and survival. Examples include parasite-induced host fecundity reduction and gigantism. Changing host behaviour in the social context protects hosts from predation and prolongs the lifespan of the infection.
• The physiological mechanisms underlying host manipulation include parasite-induced effects on the neuronal system, e.g. influencing neuromodulators, or by exploiting side-effects of the immune response.
• The effort that parasites should invest into manipulation varies with several factors, including the defence level of the host. Parasites might force hosts into cooperation, i.e. not to defend themselves too strongly, if they can increase damage in strongly defending hosts (the mafia strategy).
• The manipulation strategies of co-infecting parasites can be in conflict. Solutions of this dilemma include avoidance of already infected hosts, or the elimination of competitors. Furthermore, non-manipulating parasites might exploit the efforts of manipulating parasites.