

Bacterial strategies to overcome insect defences

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Abstract | Recent genetic and molecular analyses have revealed how several strategies enable bacteria to persist and overcome insect immune defences. Genetic and genomic tools that can be used with *Drosophila melanogaster* have enabled the characterization of the pathways that are used by insects to detect bacterial invaders and combat infection. Conservation of bacterial virulence factors and insect immune repertoires indicates that there are common strategies of host invasion and pathogen eradication. Long-term interactions of bacteria with insects might ensure efficient dissemination of pathogens to other hosts, including humans.

Persistence

The survival of bacteria in large numbers in a host.

Insects are the most diverse group of animals on Earth, with over a million described species — more than all of the other animal groups combined. Insects can be found in almost all environments. Like other metazoans, insects can be infected by viruses, bacteria, fungi and parasites, but surprisingly little is known about the molecular mechanisms of infection¹. Historically, economically motivated studies of sicknesses in domesticated insects, such as silkworm or honeybees, have dominated the field. For example, the French government commissioned Louis Pasteur to find a cure for a silkworm epizootic that was threatening the silk industry in 1865. Pasteur managed to identify the parasite that was causing the epidemic (*Nosema bombycis*, a microsporidia that is found in the haemolymph and is transmitted vertically) and his recommendations saved the silk industry. Another factor that has motivated studies of entomopathogenic microorganisms is the need to develop strategies that are based on microbial pathogens to eradicate pest insects².

The awareness that the dissemination of microorganisms by insects could pose a serious threat to human or animal health revived interest in microorganism–insect interactions. Previously, it had been assumed that insects were passive vectors that could transmit microorganisms by three non-specific interactions: carriage on the body, regurgitation and defecation. Today, most researchers think that dissemination of pathogens by vectors might involve specific interactions between the host and the bacteria. Therefore, studying the mechanisms that ensure persistence and transmission of microorganisms might allow the development of strategies to prevent infectious diseases. More recently, model systems for host–pathogen interactions have been used to dissect

the crosstalk between the partners^{3–5}, and the tractability of *Drosophila melanogaster* to genetic and genomic analyses have made it a popular insect model⁶.

Insects inhabit diverse niches and interact with various bacteria to form relationships that range from mutualistic symbiosis to pathogenesis. Insects have specific features that protect them from pathogens. Their first lines of defence are physical barriers (FIG. 1), including the cuticle, that protect host cells and tissues from the external environment. Although fungi, such as *Beauveria bassiana*, can breach the cuticle⁷, this strategy has not been reported for bacterial pathogens. The introduction of bacteria into haemolymph can occur after wounding by injury, invasive pathogens, parasitoids or predators. Sepsis can also be caused by nematodes that penetrate the insect body through the mouth, anus or the spiracles. Insects can also harbour bacterial pathogens, such as *Wolbachia* and *Spiroplasma* species, that are gamete-transmitted and that colonize only female progeny, killing male hosts. The main route of interaction between insects and bacteria seems to be through the ingestion of contaminated food. Larvae or adult insects feed on decaying fruits or animal cadavers that can contain up to 10¹¹ bacteria per ml. Unlike mammals, pathogen infection through respiratory systems has not been reported in insects.

In this Review we describe the crosstalk that exists between bacteria and insects. In particular we discuss the strategies that are used by bacterial pathogens to persist in insects and the responses of insects to such infections. Positive symbiotic interactions have been reviewed elsewhere^{8,9} and will not be considered here. The strategies discussed here include the interactions between:

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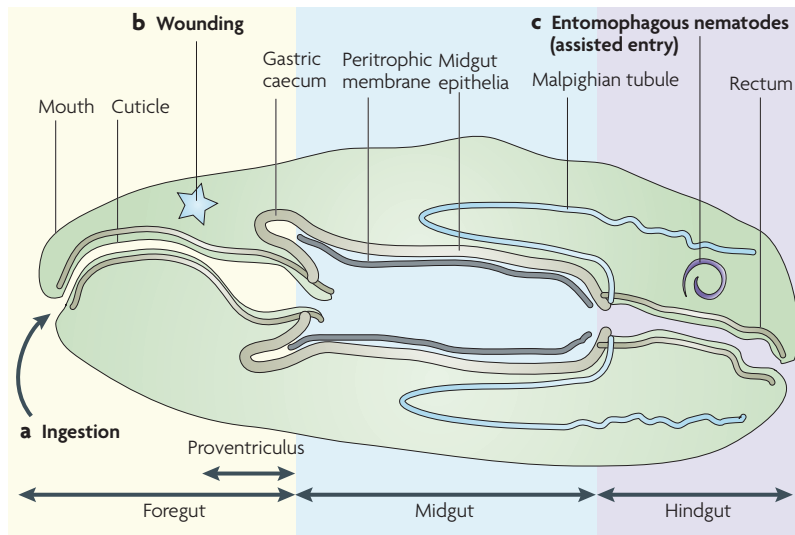


Figure 1 | Main routes of bacterial infection in insects. The different physical barriers that ensure protection from infection by pathogens are shown for a model insect. **a** | Ingestion. The different parts of the insect gut are shown. The foregut and hindgut are covered by cuticle, whereas the midgut epithelium is protected from ingested bacteria by the peritrophic membrane. **b** | Wounding. Direct entry of bacteria in the insect haemocoel can occur after breaching of the cuticle. **c** | Assisted entry. Bacteria, such as *Photobacterium* and *Xenorhabdus* sp., can be delivered into the insect body cavity by entomophagous nematodes.

Yersinia pestis with *Xenopsylla cheopis* (Siphonaptera; fleas); *Photobacterium* species and *Xenorhabdus* species with *Manduca sexta* (Lepidoptera; moths and butterflies); *Bacillus thuringiensis* or *Bacillus cereus* with various insect orders; *Serratia entomophila* with the grass grub *Costelytra zealandica* (Coleoptera); and *Erwinia carotovora*, *Pseudomonas entomophila* and *Serratia marcescens* with *Drosophila* spp. (Diptera). Other interactions (TABLE 1) have been reviewed elsewhere^{1,10,11}, but these infections will not be discussed here because of a lack of detailed molecular or genetic information.

Ingestion of microorganisms

Ingestion of bacteria by insects might have important consequences: several reports have described transmission of bacteria by houseflies¹² (Diptera), sometimes more than two weeks after feeding^{13,14}, and cockroaches (Dictyoptera) might be vectors for nosocomial infections¹⁵. A remarkable case of oral infection occurs when fleas feed on rodents that are infected by *Y. pestis*; the fleas become plague vectors, as *Y. pestis* can colonize the flea gut¹⁶.

The insect gut is a continuous tube that runs from the mouth to the anus¹⁷. The alimentary canal is divided into three regions: the foregut, the midgut and the hindgut. The foregut and the hindgut are of ectodermal origin and are therefore covered by a cuticle that is continuous to the external one. The midgut is of endodermal origin and is not lined by a cuticle; it comprises an epithelium layer that is bordered by a peritrophic membrane on its luminal surface (FIG. 1). The peritrophic membrane forms a barrier between the epithelial layer and the midgut lumen, which contains the food bolus. Circular

and longitudinal muscles are responsible for peristalsis. The insect proventriculus is a remarkable foregut organ that is located at the border between the foregut and midgut. Its morphology is variable, but it can form a simple valve at the entrance of the midgut. In various insect species, such as cockroaches, beetles (Coleoptera) and fleas, the proventriculus is a grinding apparatus with strong cuticular plates that can break up food¹⁷. Circular muscles surround the proventriculus, which can also be an immuno-reactive organ, for example in *D. melanogaster*¹⁸ and Tsetse flies¹⁹ (Diptera). Colonization of this organ by bacteria plays an important role in the interaction of *Y. pestis* with fleas²⁰ (see below).

Elimination of bacteria from the gut. Ingested microorganisms are transferred passively with food through the mouth to the posterior part of the foregut, and then actively transported by peristalsis through the midgut. The conditions of the midgut lumen can inhibit growth or kill ingested microorganisms. Indeed, in most cases, ingested bacteria cannot trigger the production of antimicrobial peptides (AMP) or cause insect death. To persist, it is generally assumed that microorganisms must survive the pH, digestive enzymes, redox potential and ionic strength of the midgut. The epithelial innate immune system might produce sufficient amounts of reactive oxygen species (ROS) to kill ingested pathogens (FIG. 2). Indeed, ROS-dependent protection against ingested microorganisms might constitute the first line of inducible defence in *D. melanogaster*. The ingestion of bacteria results in an increase in ROS that is synthesized by the NADPH oxidase enzyme dDuox²¹. Adult flies in which dDuox expression was silenced by RNA interference showed a marked increase in their mortality rate after ingestion of food that was contaminated with microorganisms; ingested bacteria were shown to persist and proliferate throughout the intestinal tract of flies when dDuox expression was silenced. This indicates that bacteria have to resist the epithelial oxidative burst in order to persist. Moreover, the expression of the antioxidant gene *katN* (a non-haem catalase) in *Salmonella* or *Escherichia coli* spp. allows these bacteria to persist and to trigger AMP production²². Furthermore, as in mammals, an antioxidant system that comprises an extracellular immune-regulated catalase is used by *D. melanogaster* to maintain the homeostatic redox balance that is perturbed by the ingestion of microorganisms²².

Bacterial persistence in the gut. Only a handful of bacteria can persist in large numbers in the digestive tract of insects. It is generally assumed that most ingested bacteria are eliminated by ROS, by peristalsis or by other unknown mechanisms. Some bacteria, such as *Mycobacterium ulcerans*, ensure proliferation by moving from the digestive tract to another organ (BOX 1). The persistence of bacteria can result in established colonization of the insect midgut (FIG. 3), facilitating transmission of bacteria to other hosts.

A striking example of bacterial survival in an insect gut is the interaction between the plague bacillus and the flea¹⁶. *Y. pestis* is endemic among wild rodents, and fleas

Colonization
The ability to multiply in the host.

Innate immunity
Effector mechanisms that control infection and that possess a certain degree of specificity to different classes of microorganisms.

Table 1 | **Examples of interactions between bacteria and insects**

Bacteria	Type of interaction	Host (mode of interaction)	References
<i>Erwinia aphidicola</i>	Pathogen	Pea aphid (ingestion)	113
<i>Dickeya dadantii</i>	Pathogen	Pea aphid (ingestion)	78
<i>Pseudomonas entomophila</i>	Pathogen	<i>Drosophila</i> , <i>Bombyx</i> , <i>Galleria</i> (ingestion)	31
<i>Yersinia pestis</i>	Pathogen	Rat flea (ingestion)	16
<i>Serratia entomophila</i>	Pathogen	Grass grub (ingestion)	38
<i>Serratia marcescens</i>	Pathogen	<i>Drosophila</i> (ingestion)	33
<i>Photorhabdus</i> sp.	Pathogen	Lepidopteran (assisted entry)	53
<i>Xenorhabdus</i> sp.	Pathogen	Lepidopteran (assisted entry)	53
<i>Vibrio cholerae</i>	Pathogen	<i>Drosophila</i> (ingestion)	32
<i>Melissococcus pluton</i>	Pathogen	Honey bee (ingestion)	1
<i>Bacillus thuringiensis</i>	Pathogen	Different orders (ingestion)	111
<i>Bacillus papillae</i>	Pathogen	Scarab larvae (ingestion)	105
<i>Paenibacillus lentimorbus</i>	Pathogen	Scarab larvae (ingestion)	105
<i>Paenibacillus larvae</i>	Pathogen	Honey bee larvae (ingestion)	105
<i>Bacillus sphaericus</i>	Pathogen	Mosquito (ingestion)	105
<i>Bacillus laterosporus</i>	Pathogen	Bee larvae, dipteran (ingestion)	105
<i>Pseudomonas aeruginosa</i>	Opportunistic	Caterpillar (ingestion)	10
<i>Pseudomonas aeruginosa</i>	Opportunistic	<i>Drosophila</i> (direct injection)	58
<i>Bacillus cereus</i>	Opportunistic	<i>Galleria mellonella</i> (ingestion)	114
<i>Erwinia carotovora</i>	Infectious	<i>Drosophila</i> larvae (ingestion)	30
<i>Shigella</i> spp.	Passive vector	House fly (ingestion)	1
<i>Rickettsia</i> spp.	Vector	Cat flea (ingestion)	115
<i>Bartonella</i> spp.	Vector	Cat flea (ingestion)	115

become infected when they feed on these rodents. *Y. pestis* proliferates in the flea midgut and colonizes the proventriculus. This colonization renders fleas unable to pump blood into their midgut (so-called blocked fleas). Starvation that is induced by the proventriculus blockage results in repeated feeding attempts by the flea, thereby inducing multiple flea bites. Regurgitation of contaminated blood into the bite site transmits plague. Ultimately, proventriculus blockage kills the flea.

Survival in the flea midgut, which is an essential step for efficient colonization by *Y. pestis*, is due to a single plasmid-borne gene, named *ymt*²³ (*Yersinia* murine toxin). Introduction of *ymt* into other enterobacterial species enables them to colonize the flea midgut. The *ymt* gene encodes a phospholipase D enzyme that protects bacteria from a blood degradation product. Bacteria that lack *ymt* are eliminated from 80–95% of fleas during the first 24 hours after ingestion. Interestingly, YMT does not have a role in proventriculus blocking, as colonization by *ymt* mutants occurs in the proventriculus of fleas, not in the midgut.

The proventriculus blockage arises because of hemin storage (*hms*) gene products²⁴, which produce an extracellular matrix that is involved in biofilm formation, both *in vitro* and in the flea proventriculus²⁰. Development of a biofilm might protect *Y. pestis* from the immune system²⁵. *Y. pestis* *hms* mutants still proliferate in the flea midgut, but cannot colonize the proventriculus²⁶.

Another gene that is involved in flea blockage, *gmhA*, encodes a phosphoheptose isomerase that is involved in lipooligosaccharide synthesis, an essential component of the outer membrane in *Y. pestis*. Mutations in *gmhA* prevent the formation of biofilms that are required for proventriculus blockage²⁷.

Bacteria also persist in *D. melanogaster*, which has been used as a model to study host–pathogen interactions between insects and bacteria. A few species can persist in the gut, trigger the immune response or are pathogenic^{28–33}. Most of the bacterial persistence mechanisms in *D. melanogaster* are unknown, but some clues have been identified using *Erwinia carotovora* subsp. *carotovora* 15. *Erwinia* species are phytopathogenic bacteria that can use *D. melanogaster* as a vector^{34,35}. *E. c. carotovora* 15 can persist in the gut of *D. melanogaster* larvae and induce both a local and a systemic immune response. Activation of the immune response eradicates *E. c. carotovora* 15 from infected insects³⁰. The *evf* (*Erwinia* virulence factor) gene is required for bacterial persistence³⁶. Transfer of the *evf* gene to other Gram-negative bacteria allows them to persist in *D. melanogaster*, indicating that a single determinant can enable persistence of bacteria in a host³⁶. *Evf* does not seem to be involved in detoxifying or neutralizing molecules that participate in bacterial clearance, such as ROS and AMP, or in reprogramming metabolic pathways that render the bacterial cells refractory to elimination³⁷. The facts that *evf* mutants can survive as well as

Biofilm

Association of bacteria to form a structured community, in contrast to free-living, planktonic bacteria.

Pathogenic

The ability to cause damage to a host.

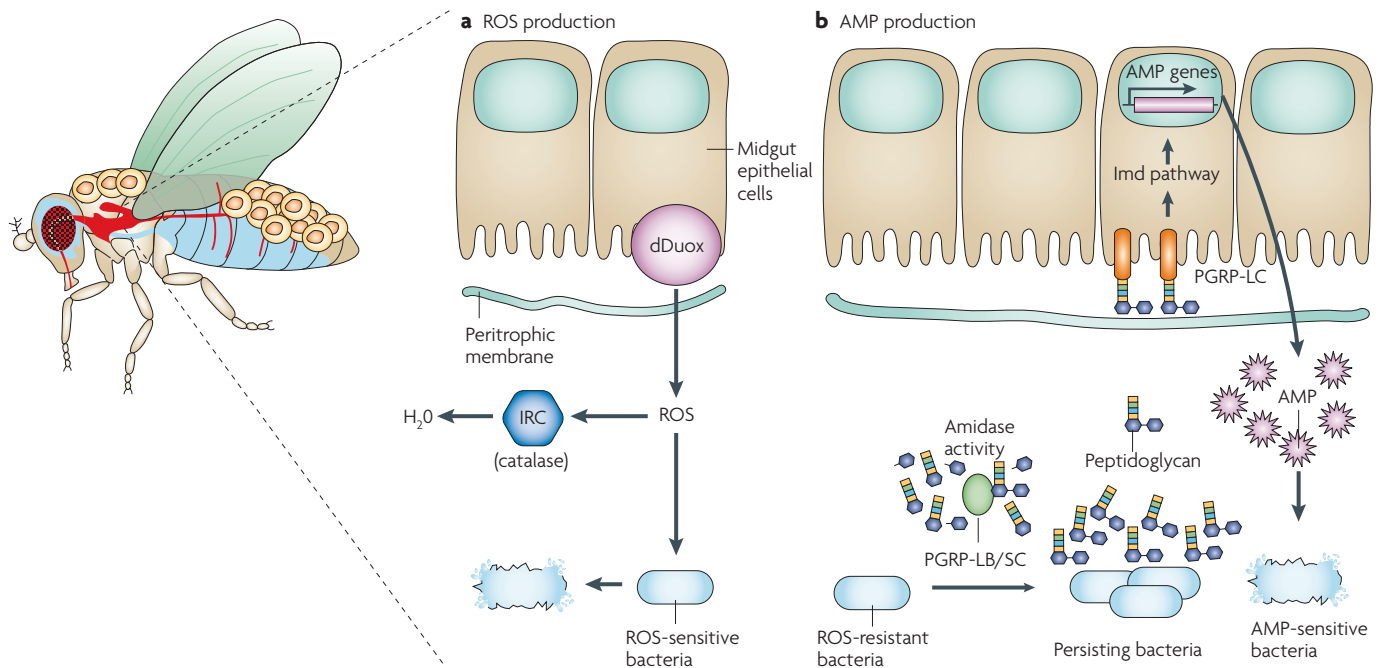


Figure 2 | Schematic diagram of local immune response in *Drosophila*. Bacteria in the gut trigger local production of reactive oxygen species (ROS; panel **a**) and antimicrobial peptides (AMPs; panel **b**), which are both inducible defence mechanisms. ROS are produced by the dDuox protein, and are detoxified by the immune-regulated catalase (IRC). If bacteria can resist the oxidative stress, they may be able to persist in the gut. Persisting bacteria release peptidoglycan fragments that are either detoxified by the amidase activity of some peptidoglycan recognition proteins PGRPs (PGRP-LB for instance), or serve as elicitors of the Imd pathway (upon recognition by PGRP-LC), which leads to AMP production. AMPs contribute to bacterial elimination from the gut. Modified with permission from REF. 93 © (2007) Annual Reviews.

E. c. carotovora 15 in isolated guts, and that *evf*-expressing bacteria allow persistence *in trans* of *evf*-deficient bacteria, supports the hypothesis that Evf perturbs the gut physiology, which would otherwise eradicate ingested bacteria³⁷. Overexpression of *evf* results in massive bacterial accumulation in the gut and insect mortality. Interestingly, no lethality was observed after direct injection of such bacteria into the body cavity, which indicates that Evf-mediated persistence is specific to the physiology and architecture of the *D. melanogaster* larval gut³⁶.

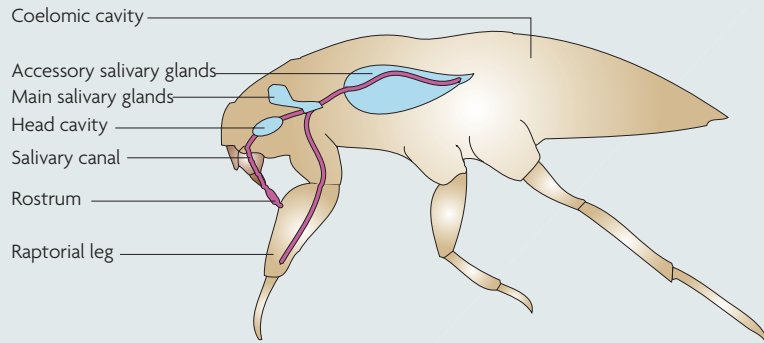
Another example of bacterial persistence in the gut is the 'amber disease', which affects the New Zealand grass grub and is caused by *Serratia* spp.³⁸. Upon ingestion of *S. entomophila*, larvae cease feeding within 48 hours; their gut, which is normally dark, develops an amber coloration that results from gut clearance. In addition, levels of major digestive enzymes in the gut (such as trypsin) decrease sharply³⁸ and larvae enter a long chronic phase during which they stop feeding. They can remain in this state for a prolonged period (1–3 months) before the infecting bacteria eventually invade the haemocoel and cause rapid death^{38,39}. Two loci that are responsible for cessation of feeding and for gut clearance have been identified on a large 115-kb plasmid, pADAP^{40,41}. One locus, comprising 18 genes, is required for cessation of feeding. Sixteen of these genes encode proteins that could form a defective prophage (called Afp for antifeeding prophage)⁴². Expression of the *afp* genes in *E. coli* allows the synthesis of particles

that resemble phage tail-like bacteriocins⁴³. These particles might function as a delivery device for the putative toxins (or effectors) that are encoded by the last two genes. Interestingly, several copies of these prophage-like loci are found in the genome of *Photorhabdus luminescens* and are named PVC (*Photorhabdus virulence cassettes*)⁴⁴. It has been proposed that *Serratia* persistence is ensured by a low toxic activity of Afp. This is supported by the fact that overexpression of the *afp* locus leads to some degree of lethality. The second locus that is involved in amber disease is responsible for the gut-clearance phenomenon⁴⁰ and encodes proteins that are related to the insecticidal toxins (Tc-toxins) that are found in *P. luminescens* and other bacteria.

Gut physiology perturbations and pathogenicity. Afp is not the only persistence factor that might perturb (directly or indirectly) gut physiology through a toxic effect on epithelial cells⁴¹. When the *Erwinia* persistence factor *evf* is overexpressed in *Erwinia* strains, infected larvae stop feeding (suggesting a food-uptake blockage), bacteria proliferate and a strong lethality is observed^{36,37}. A third example of toxicity towards the insect gut is observed with *P. entomophila*, a bacterium that can infect and kill *D. melanogaster* larvae and adults after ingestion³¹. *D. melanogaster* infection by *P. entomophila* is also characterized by a cessation of feeding. Electron microscopy observations showed that infection with *P. entomophila* causes a loss of larval midgut integrity³¹.

Tc-toxin
Insecticidal toxin complex, originally characterized in *Photorhabdus* and *Xenorhabdus* species.

Box 1 | A case of bacterial persistence outside the gut, after ingestion



Bacteria can move out of the insect digestive tract to colonize other organs (see the figure). The figure shows a schematic representation of the median sagittal section of the water bug *Naucoris cimicoides*. *Mycobacterium ulcerans*, the causative agent of Buruli ulcer, colonizes the salivary glands of *N. cimicoides*. Between 5% and 10% of *N. cimicoides* that are captured in endemic areas are infected with *M. ulcerans*⁸⁸. Immediately after ingestion, *M. ulcerans* translocates from the head capsule to the coelomic cavity. In this cavity it is phagocytosed by plasmatocytes that serve as shuttle cells and deliver *M. ulcerans* to the salivary glands, where *M. ulcerans* proliferates without damaging insect tissues^{89,90}. Interestingly, the dermonecrotic toxin mycolactone, which is the only virulence factor that has been characterized for *M. ulcerans*, is required for plasmatocyte evasion and establishment of bacteria in the salivary glands⁸⁹. *M. ulcerans* can proliferate to colonize the salivary canals and the inside cavity of the raptorial legs, where it synthesizes an extracellular matrix that promotes formation of a biofilm-like structure⁸⁹. This matrix is important for bacterial translocation, and its presence enhances colonization of insect vectors and mammalian hosts⁹¹. It has been proposed that *N. cimicoides* can also serve as a vector that contributes to the dissemination of *M. ulcerans*⁹⁰. Figure modified with permission from REF. 89 © (2005) Blackwell Publishing.

Comparative transcriptome analysis of *D. melanogaster* larvae orally infected by *P. entomophila* or *E. c. carotovora* 15 revealed that the expression of 205 genes is altered during a lethal infection compared with a non-lethal infection. Most of these genes are important in gut physiology, which indicates that *P. entomophila* modifies the gut epithelium during the infectious process. One example of this is the activation of insect genes that encode cytoskeleton components that are regulated by the JNK pathway³¹.

The observations made with *E. c. carotovora* 15 (REF. 37) suggest that peristaltic movements of the gut might play an important role in the elimination of bacteria and that entomopathogens have developed strategies to abrogate peristalsis.

Immune defences in the gut

Bacteria that can persist after ingestion are faced with the insect immune response (BOX 2) and can often counteract host defences. The first level of immune response occurs at the site of the infection, in the insect gut (FIG. 2). AMP production in the gut epithelium can be triggered by *E. c. carotovora* 15 (REF. 36), *P. entomophila*³¹ and *S. marcescens*³³. This local response is mediated by the nuclear factor (NF)- κ B signalling pathway called Imd^{18,45}, through the recognition of Gram-negative peptidoglycan by the peptidoglycan recognition proteins (PGRPs), such as PGRP-LC⁴⁶ (BOX 2).

Why commensal bacteria do not generate a state of permanent immune activation in tissues such as the gut is unclear. A central role in gut tolerance to bacteria has been

attributed to amidase PGRPs (PGRP-LB and PGRP-SC) as they abrogate the immunostimulatory activity of peptidoglycan fragments that are released by commensals^{46,47}. It is also interesting to note the existence of specialization in the gut epithelium as only parts of the epithelium are immunoreactive, whereas bacteria are present throughout the midgut after infection^{18,30,31}.

It has been suggested that local AMP production in the gut is a second line of defence, after ROS production, to fight oral ingestion of bacteria. Indeed, in contrast to wild-type *D. melanogaster*, *imd*-deficient flies are highly susceptible to oral ingestion of ROS-resistant *E. coli*. This lethality disappears when a functional *imd* pathway is re-introduced into the intestinal epithelium, or when only the AMP cecropin is expressed in the gut⁴⁸.

The local production of AMP also has an important role in fighting oral infection by pathogenic bacteria. *D. melanogaster* is protected against oral infection by *P. entomophila* by activating the local AMP production in the gut before infection⁴⁹. Furthermore, *D. melanogaster* that has a functional Imd pathway only in the gut was as resistant as wild-type flies to infection by *P. entomophila* or other persisting microorganisms, demonstrating that the local gut response is of prime importance in the fight against infection by the oral route.

Bacteria can fight this local immune response. For instance *P. entomophila* can persist in wild-type *D. melanogaster* larvae as well as in larvae that have been deprived of an immune response³¹, suggesting that this bacterium can evade the *D. melanogaster* immune response. *P. entomophila* might protect itself from immune effectors such as AMP, or it might degrade these effectors. Among the various putative virulence factors that are encoded in the *P. entomophila* genome⁵⁰, the secreted zinc metalloprotease AprA has a pivotal role in the defence of *P. entomophila* against the host gut epithelial immune response⁴⁹. Not only is an *aprA* mutant slightly less virulent, it is also less able to persist in *D. melanogaster* than wild-type *P. entomophila*, and is more sensitive to Imd-mediated defences. AprA also provides specific protection against the dipterin AMP, demonstrating *in vivo* the role of a secreted protease to protect bacteria against insect AMPs⁴⁹.

Systemic immune defences. AMPs are sometimes produced by the fat body cells (the fat body could be considered as an analogue of the mammalian liver) and secreted into the insect haemolymph. This happens when microorganisms are directly injected into the body cavity, but persisting bacteria in the gut can also induce AMP production. Bacterial persistence in the gut is probably the first step that is required for the activation of this systemic AMP response after ingestion.

To analyse the responses that are induced by *E. c. carotovora* 15 and *P. entomophila* oral infection of *D. melanogaster* larvae, a genomic approach was used. Microarrays were used to analyse transcriptomes of *D. melanogaster* larvae that were infected either by ingestion (oral infection) of *E. c. carotovora* 15 or

Peptidoglycan recognition proteins
Peptidoglycan recognition proteins are innate immunity molecules that are present in most invertebrate and vertebrate animals.

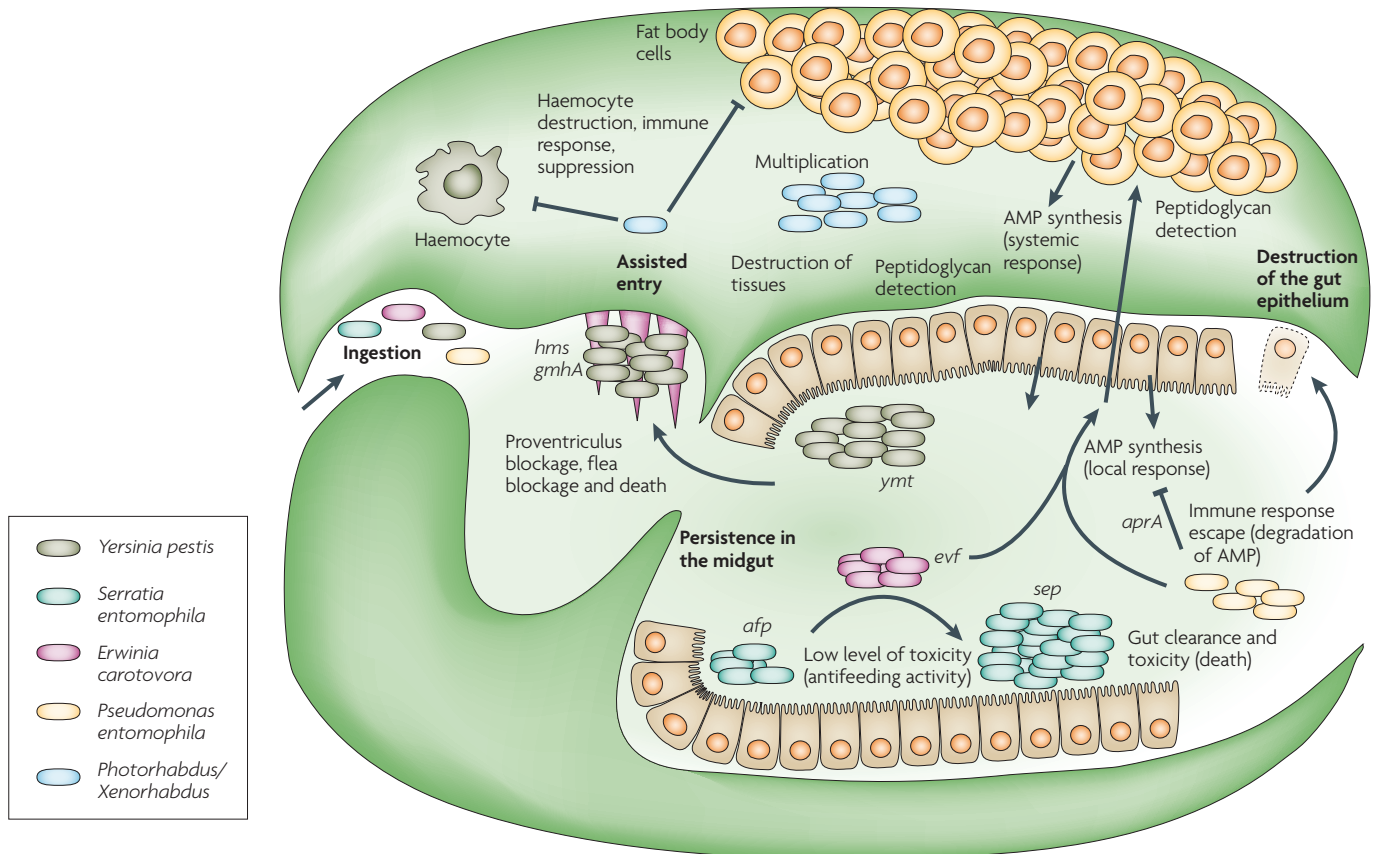


Figure 3 | Examples of bacterial infections in insects. The different bacteria discussed in the text are indicated by different colours, and the names of the genes involved in different steps of infection are indicated. After ingestion or assisted entry, bacteria use different strategies to persist in the insect host. *Yersinia pestis*, *Serratia entomophila* and *Erwinia carotovora* rely on a limited number of genes to persist in the gut. *Photorhabdus* and *Xenorhabdus* sp. suppress the immune response and kill phagocytic cells. Persistence of *Erwinia carotovora* subsp. *carotovora* 15 or *Pseudomonas entomophila* in the gut triggers antimicrobial peptide (AMP) production at both local and systemic levels. It leads to *E. c. carotovora* 15 elimination, whereas *P. entomophila* relies on the AprA protease to neutralize AMPs. *Y. pestis* colonizes the flea proventriculus through the formation of a biofilm-like structure. Upon suppression of the immune response, *Photorhabdus* and *Xenorhabdus* sp. proliferate in the insect body. Persisting bacteria provoke host damage. *P. entomophila* infection is characterized by destruction of the *Drosophila* midgut epithelium. Proventriculus colonization by *Y. pestis* leads to 'blockage', which prevents food uptake and reduces the flea lifespan. Proliferation of *Photorhabdus* and *Xenorhabdus* sp. ensures degradation of insect tissues, which provides nutrients to the nematode vectors.

P. entomophila, or by direct injection of microorganisms into the body cavity (septic injury). Genes that were modulated during infection by the different routes could thus be identified³¹. Oral infection by *E. c. carotovora* 15 or *P. entomophila* induced 72 of the 99 genes that were upregulated after direct injection, indicating that naturally infectious Gram-negative bacteria can induce most of the larval immune-regulated genes. These include AMP genes, genes that are involved in recognition and phagocytosis, genes that encode small immune-regulated peptides and unknown genes. Furthermore, oral infection by *E. c. carotovora* 15 or *P. entomophila* affects the expression of 92 genes whose expression is not modulated by septic injury. These genes mainly encode proteases, constituents of the peritrophic membrane, and gene products that are involved in metabolism, and might constitute the host response to bacterial persistence in the gut. These genes are promising post-genomic targets for a detailed analysis of the gut-infection process.

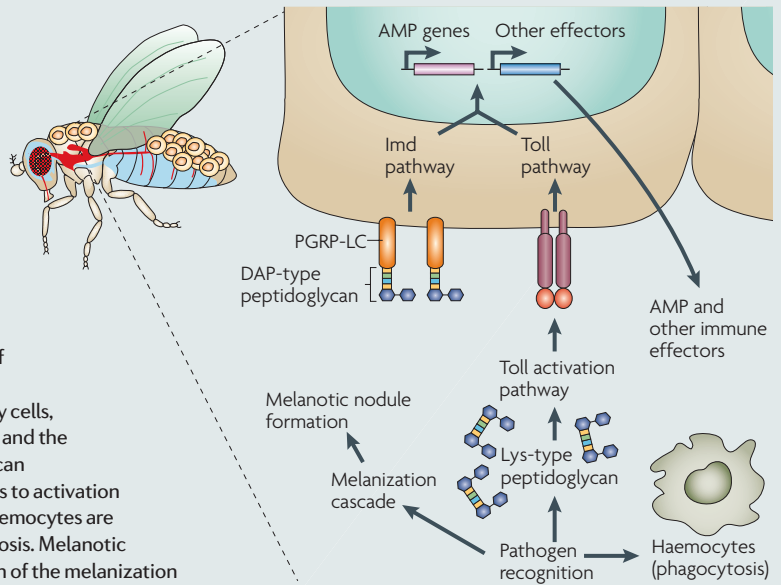
The activation of AMP production by the fat body also relies on peptidoglycan recognition by PGRPs^{51,52} (BOX 2). It is interesting to note that high titres of ingested Gram-negative bacteria, such as *E. coli* or *E. c. carotovora* 15 *evf* mutants, do not induce systemic AMP production, even though they are present in large numbers in the gut several hours after ingestion^{36,37}. Once again, this may be due to the presence of another type of PGRP with amidase activity that establishes a tolerance threshold level of bacteria in the gut by reducing the amount of active peptidoglycan fragments^{46,47}. The absence of systemic AMP production despite a high level of non-infectious bacteria at early time points after ingestion indicates that the triggering of this systemic response by infectious bacteria such as *E. c. carotovora* 15 or *P. entomophila* either requires bacterial persistence and *de novo* synthesized peptidoglycan compounds, or depends on the detection of peptidoglycan molecules in a specific

Infectious

The ability to colonize a host and induce the host immune response.

Box 2 | Insect immune response

To fight infection, insects rely on multiple innate defence mechanisms, many of which are shared with higher organisms⁹². These immune functions have mainly been characterized in *Drosophila melanogaster*, which serves as a paradigm for insect immunity. The figure shows a diagram of systemic immune response in *Drosophila*. Pathogen recognition triggers three major defence mechanisms at the systemic level: production of antimicrobial peptides (AMPs) and other immune effectors by fat body cells, activation of the cellular response, and the melanization reaction. Peptidoglycan detection in the haemolymph leads to activation of the Imd or the Toll pathways. Haemocytes are responsible for bacterial phagocytosis. Melanotic nodules are formed after activation of the melanization cascade. Production of AMPs, cellular response and melanization are the main defence mechanisms.



Recognition of invading bacteria relies on specific molecules that are named peptidoglycan recognition proteins (PGRPs). All PGRPs contain at least one domain that is similar to the prokaryotic peptidoglycan-lytic type 2 amidase, but only some PGRPs have amidase enzymatic activity; other PGRPs have a major role in microbial recognition.

In *D. melanogaster*, the synthesis of immune effectors by the fat body is under the control of the Toll and Imd signalling pathways⁹³. Each of these pathways activates specific nuclear factor (NF)- κ B transactivators, which in turn switch on specific programmes of transcription. The ability of *D. melanogaster* to discriminate between Gram-negative and Gram-positive bacteria is based on the recognition of specific forms of peptidoglycan⁵¹ by different PGRPs⁵². The Toll pathway is mainly activated by Gram-positive bacteria and fungi, and induces the synthesis of several peptides, including the antifungal peptide drosomycin. Recognition of lysine-type peptidoglycans, which are found in many Gram-positive bacteria, is mediated by PGRP-SA and PGRP-SD. The Imd pathway is mainly activated by Gram-negative bacteria through the recognition of the diaminopimelic acid (DAP)-type peptidoglycan by specific PGRPs (such as PGRP-LC). Activation of the Imd pathway induces the expression of different AMPs, such as dipterin, that are active against a range of bacteria and fungi. Moreover, Imd pathway activity in larvae can be modulated by nitric oxide⁹⁴, and this input is mediated by calcineurin, a Ca²⁺-dependent phosphatase⁹⁵.

In addition to the systemic response, insects rely on a local immune response that allows the synthesis of AMPs and reactive oxygen species in surface epithelia, such as gut or trachea, in a tissue-specific manner^{18,45,96}. It has been proposed that the systemic immune response that is induced during gut infection is mediated by the translocation of peptidoglycan fragments from the gut lumen to the haemolymph^{46,47}.

Another major aspect of insect immunity is the cellular immune response. Different types of free and sessile blood cells, named haemocytes, are activated in response to infection, but our understanding of the mechanisms and consequences of this activation is still fragmentary. Mobile plasmatocytes in the haemocoel are responsible for phagocytosis, which is important to fight bacterial invasion⁹⁷.

The melanization process is a humoral response that relies on a proteolysis cascade that results in the activation of the prophenoloxidase. Specialized insect haemocytes can promote melanization, haemocyte aggregation and nodulation, encapsulation or opsonization. Recognition of microbial compounds, such as β -1,3-glucans, lipopolysaccharides and peptidoglycan, ensures that the humoral response is effective^{98,99}. Once activated, phenoloxidase catalyses the formation of melanotic nodules that limit the spread of infecting microorganisms or damaged tissues. Figure modified with permission from REF. 93 © (2007) Annual Reviews.

compartment of the gut. Therefore, the induction of systemic AMP production seems to be a direct consequence of bacterial persistence in the gut.

Direct injection as a route of infection

In nature, ingestion of microorganisms is probably the main route of insect infection. However bacteria can sometimes access the haemocoel directly, either through an accidental breaching of the cuticle, or by assisted transport via an entomophagous host, such as a

nematode. Breaching the cuticle can enable opportunistic bacteria to colonize the insect. This type of infection is readily mimicked in the laboratory by direct injection of bacteria into the body cavity and has been exploited to decipher the recognition and signalling molecules that are involved in the immune response, and to identify bacterial virulence factors⁶.

Assisted transport by entomophagous nematodes has been documented for two bacterial species, *P. luminescens* and *Xenorhabdus nematophila*⁵³. These

Box 3 | Heritable microorganisms

Females of many insect species interact with various different bacteria that are vertically transmitted to progeny⁹. These interactions are complex and the outcomes range from mutualistic to parasitic phenotypes, depending on the host and the bacteria involved. On the one hand, in mutualistic interactions the host relies on the symbiotic microorganism to supply nutrients that are required for viability and fertility. Examples of primary symbionts that colonize a specific organ include *Buchnera* species in aphids (Homoptera) or *Wigglesworthia glossinidia* in Tsetse flies. On the other hand, interactions can lead to different forms of pathogenic 'reproductive parasitism'¹⁰⁰, including parthenogenesis, feminization, male killing or cytoplasmic incompatibility. Two different bacterial genera have been documented in many cases of heritable parasitism: *Wolbachia* and *Spiroplasma* species.

Wolbachia pipientis is an obligate endocellular symbiont with an extremely broad host range¹⁰¹. *Wolbachia* mainly inhabits the reproductive tissues of hosts. However, it can also be found in various somatic tissues¹⁰², and its distribution varies between different *Wolbachia*-host associations. Unlike other persisting bacteria that are described in this Review, *Wolbachia* infection often fails to induce or suppress the host immune response¹⁰³. The absence of effects on host immunity likely results from the stealth of *Wolbachia* cells inside the host cells. In some cases, *Wolbachia* strains are pathogenic¹⁰⁴ and infection leads to rupture of host cells and release of bacteria; thus, induction of the expression of genes that encode the host antimicrobial peptide cecropin C and lysozyme¹⁰¹ might result from the detection of bacterial immune elicitors.

The mollicute genus *Spiroplasma* lacks a cell wall, is vertically transmitted from mother to progeny and kills male embryos — infection is characterized by all-female broods. How *Spiroplasma* kills male embryos is not known. Although the bacterium is widely distributed in different tissues, it accumulates at high concentrations in haemolymph. The presence of *Spiroplasma* does not induce expression of any of seven antimicrobial genes in *Drosophila*⁵⁵. The absence of an immune response does not result from immunosuppression, as the response can be induced by septic injury in *Drosophila melanogaster* that is already infected by *Spiroplasma*⁵⁵. The absence of a response upon *Spiroplasma* infection results from an absence of elicitors; the lack of wall structure, including peptidoglycan, might explain the success of *Spiroplasma* in colonizing many species of insects.

bacteria colonize the gut of specific nematode hosts (*Heterorhabditis* and *Steinernema*, respectively) when they are in the infective soil-dwelling life-cycle stage, named the infective juvenile (IJ) stage. Before the IJ stage, a low number of *Photorhabdus* and *Xenorhabdus* cells colonize their respective hosts using different processes⁵⁴; these bacteria reproduce inside the host to produce a mature population of 50–150 colony forming units per IJ. After entering an insect, IJs migrate to the haemolymph, where they release their bacterial symbionts. Both *Photorhabdus* and *Xenorhabdus* bacteria evade the immune response, kill the insect and supply the nematode with nutrients from the cadaver to produce several hundred thousand IJs⁵³.

Counteracting the systemic immune response

Two main strategies are used by pathogens to escape the systemic immune response: they avoid detection because they lack (or hide) immune elicitors on their cell surfaces, or they suppress the immune response. The first strategy is used by a pathogen from the *Spiroplasma* genus that can infect flies without being detected and also without interfering with the immune response⁵⁵ (BOX 3).

The second strategy is used by the two assisted invaders *P. luminescens* and *X. nematophila*, which can impair both cellular and humoral responses (BOX 2). Several studies have indicated that *X. nematophila* causes immunosuppression at different levels⁵⁶. The cellular response is dampened through the killing of haemocytes within 3 hours of infection, probably by the toxic effects of cytolyisin, lipopolysaccharide, toxins and fimbrial subunits⁵⁶. The humoral response can also be suppressed by repressing AMP gene expression through a mechanism that remains to be characterized⁵⁷. In a similar way, *Pseudomonas aeruginosa* can

suppress *D. melanogaster* defence responses by limiting AMP gene expression when injected into the fly haemolymph⁵⁸, using a mechanism that also remains to be characterized. Additionally, *X. nematophila* and *Photorhabdus temperata* can inhibit phospholipase A2 (PLA2)⁵⁹, which catalyses the first step of eicosanoid biosynthesis. Eicosanoids are important for haemocyte nodulation and prophenoloxidase activation⁶⁰.

The infection of *Manduca sexta* by *P. luminescens* results in the transcriptional activation of several immune-regulated genes. Although *P. luminescens* can overcome this response, if it is absent the bacteria proliferate more rapidly and the insect dies sooner⁶¹. *P. luminescens* also uses a type III secretion system to inhibit phagocytosis⁶². The melanization of pathogens and damaged tissues (BOX 2) is a further insect innate immune defence. *P. luminescens* produces an antibiotic molecule, (E)-1-3-dihydroxy-2-(isopropyl)-5-(2-phenylethyl) benzene (known as ST), that inhibits phenoloxidase⁶³, the main melanization enzyme. *P. luminescens* mutants that cannot synthesize ST are reduced in virulence, replicate more slowly in the host and cause the insect to produce more melanotic nodules than wild-type bacteria.

Virulence factors and toxins

Despite the characterization of multiple virulence factors, how pathogens kill insects is not known. Disease either results from bacterial proliferation or from damage caused by a toxic factor (or factors). Analysis of pathogenesis in different systems has indicated a crucial role for factors that enable persistence (such as Evf, Afp, Ymt and Hms) and factors that counteract the immune response (such as AprA, ST, cytolyisins and haemocyte killing factors). Plus, different toxic factors can have a combinatorial action in pathogenesis.

Type III secretion system
A secretion apparatus that allows direct injection of effectors from bacteria into host cells.

Table 2 | Examples of bacterial toxins that are efficient against insects

Toxin	Organisms identified in	Other producer organisms
Insecticidal toxin complexes (Tc-toxin)	<i>Photorhabdus</i> spp. ¹¹⁶ , <i>Xenorhabdus</i> spp. ¹¹⁷	<i>Serratia entomophila</i> ⁴⁰ , <i>Yersinia</i> spp., <i>Pseudomonas</i> spp., <i>Fibrobacter succinogenes</i> , <i>Paenibacillus nematophila</i> ¹¹⁸
Cyt and Cry toxins	<i>Bacillus thuringiensis</i> ¹¹⁰	<i>Dickeya dadantii</i> ⁷⁸
Binary toxin (BinAB)	<i>Bacillus sphaericus</i> ¹¹⁹	
Makes caterpillar floppy (Mcf)	<i>Photorhabdus</i> spp. ¹²⁰	
Binary toxins (PirAB)	<i>Photorhabdus</i> spp. ⁷¹	

Although virulence factors are often predicted to impair host defences and harm host tissues, these factors can be pleiotropic, as they are also involved in biocontrol⁶⁴.

In contrast to vertebrate and plant pathogens⁶⁵, bacteria that infect insects by ingestion do not seem to rely on type III secretion systems during pathogenesis. This might be due to the architecture of the insect gut, in which the peritrophic membrane separates the food bolus from the epithelial cells, such that direct cell–bacteria contact does not occur in most interactions.

Specific toxins that are active against insects have been identified (TABLE 2). The best studied of these are the Cry and Cyt toxins of *B. thuringiensis*, which are used in biocontrol (BOX 4). Other insecticidal toxins have also been found in *P. luminescens* and *X. nematophila*. They are likely to play an important role in the pathogenic process that leads to insect death upon infection by these bacteria. However, their mode of action is not always known, and some genes that encode similar toxins have been identified in bacteria that are not *bona fide* entomopathogens. Therefore, the exact role of these toxins in the lifestyle of many bacteria is still unknown.

Degradative enzymes, including lipases, proteases and haemolysins, might also contribute to the virulence of entomopathogens. Proteases, especially metalloproteases, contribute to virulence in pathogenic bacteria including *P. aeruginosa*, *S. marcescens* and *B. thuringiensis*^{66–68}. In addition to degrading

AMPs, proteases might be involved in the destruction of cells and tissues to facilitate colonization of the insect body⁶⁹. During symbiotic associations between bacterial entomopathogens and nematodes, proteases might also provide nutrients to their nematode hosts⁷⁰, which multiply in the dead insect body.

In addition to protein factors, several entomopathogens produce toxic secondary metabolites^{50,71} that might either harm the host or out-compete other microorganisms⁶⁴.

Global regulators play an important role in virulence towards insects: the GacS/GacA two-component system controls the *P. entomophila* infectious process⁵⁰, PhoP/PhoQ is the master regulator of virulence in *P. luminescens*⁷², Lrp regulates both mutualism and pathogenicity in *Xenorhabdus*⁷³ and PlcR activates the transcription of genes required for virulence in *B. thuringiensis*^{74,75}. Another striking example is found in *E. c. carotovora* 15, where the *evf* gene is under the control of Hor, a regulator of genes that are involved in plant pathogenesis³⁶. This trend reveals the benefits that bacteria might gain from integrating potential virulence factors under the control of global regulators that activate gene expression when the conditions are right.

Evolving into an entomopathogen

Many bacterial entomopathogens belong to bacterial genera that are not normally associated with insects, which suggests that the ability to interact with insects depends on the acquisition of a limited number of determinants. In simplistic terms, to become an entomopathogen, the bacteria must first occupy the same ecological niche as the insect; second, it must acquire the ability to persist in insects, for example through the acquisition of *evf* or *ymt*; third, bacteria need to evade the host response, by degrading antibacterial effectors or by becoming insensitive to their effects; fourth, persistent infection should enable colonization of insect tissues and/or impair host physiology by the production of toxins. The rapid evolutionary transition of *Y. pestis* from a mammalian enteric pathogen into a blood-borne pathogen of insects suggests that a few events enabled the evolution of this entomopathogen. One important step was the acquisition of two plasmids carrying *pla* and *ymt* genes⁷⁶. Similarly, the

Box 4 | *Bacillus thuringiensis* and other *Bacillus* spp.

Bacillus thuringiensis is a Gram-positive, sporulating facultative entomopathogen that is found in various ecological niches such as soil, plant surfaces, dust from stored products and insects. *B. thuringiensis* spores persist for a long time and can germinate in soil and on plants¹⁰⁵. Other sporulating bacilli that are similar to *B. thuringiensis* are also entomopathogens (TABLE 1). The insecticidal proteins that are contained in the parasporal crystals are the main factors in determining the host range of *B. thuringiensis*. The mechanism of action of the crystal toxins has been reviewed elsewhere^{106–109}. Because of the prime role of toxins in the pathogenesis of *B. thuringiensis*¹¹⁰, other virulence factors have not been well studied. The role of the spores fed to larvae seems to be highly variable and depend on the type, combination and concentration of Cry toxins, the type and density of bacteria, the insect species and the presence of microorganisms in the gut¹¹¹. Several models have been proposed for the pathogenic effect of *B. thuringiensis*: it has been proposed that disruption of the gut epithelium results in a prolonged cessation of feeding and eventual death by starvation. Alternatively, gut cell lysis might provide spores or germinated cells with access to the haemocoel, leading to septicaemia. It was recently shown that *B. thuringiensis* does not kill larvae of the gypsy moth in the absence of indigenous midgut bacteria, which implies that the resident enteric bacteria are responsible for the septicaemia that is associated with *B. thuringiensis* toxicity in gypsy moth¹¹².

ability to colonize and persist in insect species might have resulted from horizontal gene transfer (HGT) of plasmid-borne persistence genes in *S. entomophila* (*afp*-encoding genes) and in *M. ulcerans* (mycolactone biosynthetic genes). A role for HGT in the evolution of entomopathogens is also revealed by the distribution of genes that encode similar toxins in unrelated genera^{50,77,78}, which suggests that the insect gut constitutes an environment that is favourable for gene transfer⁷⁹.

Conclusion and perspectives

The evolutionary success of insects indicates that they have developed strategies to fight bacterial infections. In several cases, constitutive expression of the Imd pathway of AMP genes results in host protection^{49,50,80,81}, which reveals the importance of the race between the establishment of resistant bacterial populations and the activation of the immune response. Furthermore, it demonstrates that the host immune response can be effective against invaders.

Comparison of immune gene repertoires among sequenced insect genomes revealed the conservation of pathways and effectors, at least in Diptera, Hymenoptera and Coleoptera^{82–84}. The lepidopteran insect *Manduca sexta* encodes immune effectors that are similar to those of other insects⁸⁵. Furthermore, insect immune pathways share an overall architecture and specific orthologous components with the innate immune system of vertebrates. We might therefore anticipate that strategies used to fight bacterial infections will be similar in many insect species, at least in holometabolous species that develop through a pupal stage. Using holometabolous insect species, molecular mechanisms of host–pathogen interactions have

been obtained. However, the antibacterial strategies and immune responses that have been developed by hemimetabolous insects remain uncharacterized.

For many years, insects have been considered as vectors of human, animal or plant pathogens. It is becoming evident that persistence of bacteria in insect hosts involves crosstalk that is similar to the crosstalk that is required for bacterial persistence in other metazoan hosts. The acquisition of determinants that counteract insect host defences by bacteria might change the host range of pathogens.

The potential offered by models that are amenable to genetic and genomic approaches is only now starting to emerge. Future studies using host and pathogen models should enable a detailed characterization of the main steps of an infectious process; that is, persistence in the host, detection of the invading bacteria, signalling to specialized tissues, evasion of the immune response and pathogenic effects. Another promising development provided by such models concerns the effect of intestinal flora on health and disease. Commensal microbiota in *D. melanogaster* protect the host from potential ingested pathogens, and modulation of host innate immune homeostasis might modify the commensal community⁸⁶.

Studies of insect–pathogen interactions suffer from a lack of knowledge of insect pathology⁸⁷. In most cases, the physiological process(es) whose alteration is responsible for death is not known. Markers used in human pathologies comprise fever, blood analysis, urine analysis, faecal analysis and other factors. The identification of useful markers for pathogenesis together with the genetic, genomic and biochemical tools that can be used to study insects will revolutionize the understanding of bacterial infection and pathogenesis of insects.

- Boucias, D. G. & Pendland, J. C. *Principles of Insect Pathology* (Kluwer Academic Publishers, Dordrecht, 1998).
- Federici, B. A., Park, H. W., Bideshi, D. K., Wirth, M. C. & Johnson, J. J. Recombinant bacteria for mosquito control. *J. Exp. Biol.* **206**, 3877–3885 (2003).
- Aballay, A. & Ausubel, F. M. *Caenorhabditis elegans* as a host for the study of host–pathogen interactions. *Curr. Opin. Microbiol.* **5**, 97–101 (2002).
- Cosson, P. *et al.* *Pseudomonas aeruginosa* virulence analyzed in a *Dictyostelium discoideum* host system. *J. Bacteriol.* **184**, 3027–3033 (2002).
- Rahme, L. G. *et al.* Use of model plant hosts to identify *Pseudomonas aeruginosa* virulence factors. *Proc. Natl Acad. Sci. USA* **94**, 13245–13250 (1997).
- Vodovar, N., Acosta, C., Lemaitre, B. & Boccard, F. *Drosophila*: a polyvalent model to decipher host–pathogen interactions. *Trends Microbiol.* **12**, 235–242 (2004).
- Clarkson, J. M. & Charnley, A. K. New insights into the mechanisms of fungal pathogenesis in insects. *Trends Microbiol.* **4**, 197–203 (1996).
- Baumann, P. & Moran, N. A. Non-cultivable microorganisms from symbiotic associations of insects and other hosts. *Antonie Van Leeuwenhoek* **72**, 39–48 (1997).
- Gil, R., Latorre, A. & Moya, A. Bacterial endosymbionts of insects: insights from comparative genomics. *Environ. Microbiol.* **6**, 1109–1122 (2004).
- Bulla, L. A., R. A., R. & St. Julian, G. Bacteria as insect pathogens. *Annu. Rev. Microbiol.* **29**, 163–190 (1975).
- Lysenko, O. Non-sporeforming bacteria pathogenic to insects: incidence and mechanisms. *Annu. Rev. Microbiol.* **39**, 673–695 (1985).
- Kobayashi, M. *et al.* Houseflies: not simple mechanical vectors of enterohemorrhagic *Escherichia coli* O157:H7. *Am. J. Trop. Med. Hyg.* **61**, 625–629 (1999).
- Douglas, A. E. & Beard, C. B. in *Biology of the Insect Midgut* (eds Lehane, M. J. & Billingsley, P. F.) (Chapman & Hall, London, 1996).
- Kaslow, D. C. & Welburn, S. in *Biology of the Insect Midgut* (eds Lehane, M. J. & Billingsley, P. F.) (Chapman & Hall, London, 1996).
- Pai, H. H., Chen, W. C. & Peng, C. F. Cockroaches as potential vectors of nosocomial infections. *Infect. Control Hosp. Epidemiol.* **25**, 979–984 (2004).
- Gage, K. L. & Kosoy, M. Y. Natural history of plague: perspectives from more than a century of research. *Annu. Rev. Entomol.* **50**, 505–528 (2005).
- Chapman, R. F. *The Insects: Structure and Function* (Cambridge University Press, Cambridge, 1998).
- Tzou, P. *et al.* Tissue-specific inducible expression of antimicrobial peptide genes in *Drosophila* surface epithelia. *Immunity* **13**, 737–748 (2000).
- Hao, Z., Kasumba, I. & Aksoy, S. Proventriculus (cardia) plays a crucial role in immunity in tsetse fly (Diptera: Glossinidae). *Insect Biochem. Mol. Biol.* **33**, 1155–1164 (2003).
- Jarrett, C. O. *et al.* Transmission of *Yersinia pestis* from an infectious biofilm in the flea vector. *J. Infect. Dis.* **190**, 783–792 (2004).
- Ha, E. M., Oh, C. T., Bae, Y. S. & Lee, W. J. A direct role for dual oxidase in *Drosophila* gut immunity. *Science* **310**, 847–850 (2005).
- Shows that dual oxidase is indispensable for gut antimicrobial activities in adult *Drosophila*.
- Ha, E. M. *et al.* An antioxidant system required for host protection against gut infection in *Drosophila*. *Dev. Cell* **8**, 125–132 (2005).
- Shows that homeostasis in redox balance modulates host survival after ingestion of bacteria.
- Hinnebusch, B. J. *et al.* Role of *Yersinia* murine toxin in survival of *Yersinia pestis* in the midgut of the flea vector. *Science* **296**, 733–735 (2002).
- Identification of the gene that is responsible for gut persistence in fleas.
- Perry, R. D., Pendrak, M. L. & Schuetz, P. Identification and cloning of a hemin storage locus involved in the pigmentation phenotype of *Yersinia pestis*. *J. Bacteriol.* **172**, 5929–5937 (1990).
- Parsek, M. R. & Singh, P. K. Bacterial biofilms: an emerging link to disease pathogenesis. *Annu. Rev. Microbiol.* **57**, 677–701 (2003).
- Hinnebusch, B. J., Perry, R. D. & Schwan, T. G. Role of the *Yersinia pestis* hemin storage (*hms*) locus in the transmission of plague by fleas. *Science* **273**, 367–370 (1996).
- Identification of the gene responsible for proventriculus colonization and digestive tract blockage.
- Darby, C., Ananth, S. L., Tan, L. & Hinnebusch, B. J. Identification of *gmhA*, a *Yersinia pestis* gene required for flea blockage, by using a *Caenorhabditis elegans* biofilm system. *Infect. Immun.* **73**, 7236–7242 (2005).
- Flyg, C., Kenne, K. & Boman, H. G. Insect pathogenic properties of *Serratia marcescens*: phage-resistant mutants with a decreased resistance to *Cecropia* immunity and a decreased virulence to *Drosophila*. *J. Gen. Microbiol.* **120**, 173–181 (1980).
- Chugani, S. A. *et al.* QsCR, a modulator of quorum-sensing signal synthesis and virulence in *Pseudomonas aeruginosa*. *Proc. Natl Acad. Sci. USA* **98**, 2752–2757 (2001).

30. Basset, A. *et al.* The phytopathogenic bacteria *Erwinia carotovora* infects *Drosophila* and activates an immune response. *Proc. Natl Acad. Sci. USA* **97**, 3376–3381 (2000).
First report of immune-response activation after ingestion of bacteria by *Drosophila* larvae.
31. Vodovar, N. *et al.* *Drosophila* host defense after oral infection by an entomopathogenic *Pseudomonas* species. *Proc. Natl Acad. Sci. USA* **102**, 11414–11419 (2005).
Characterization of *P. entomophila* pathogenicity in *Drosophila*.
32. Blow, N. S. *et al.* *Vibrio cholerae* infection of *Drosophila melanogaster* mimics the human disease cholera. *PLoS Pathog.* **1**, e8 (2005).
33. Nehme, N. T. *et al.* A model of bacterial intestinal infections in *Drosophila melanogaster*. *PLoS Pathog.* **3**, e173 (2007).
Characterization of *S. marcescens* pathogenicity in *Drosophila*.
34. Kloepper, J. W., Brewer, J. W. & Harisson, M. D. Insect transmission of *Erwinia carotovora* var. *carotovora* and *Erwinia carotovora* var. *atroseptica* to potato plants in the field. *Am. Potato J.* **58**, 165–175 (1981).
35. Molina, J. J., Harisson, M. D. & Brewer, J. W. Transmission of *Erwinia carotovora* var. *atroseptica* by *Drosophila melanogaster* meig. I. Acquisition and transmission of the bacterium. *Am. Potato J.* **54**, 245–250 (1974).
36. Basset, A., Tzou, P., Lemaître, B. & Boccard, F. A single gene that promotes interaction of a phytopathogenic bacterium with its insect vector, *Drosophila melanogaster*. *EMBO Rep.* **4**, 205–209 (2003).
Identification of the gene that allows bacterial persistence in the *Drosophila* larval gut.
37. Acosta Muniz, C., Jaillard, D., Lemaître, B. & Boccard, F. *Erwinia carotovora* Efv antagonizes the elimination of bacteria in the gut of *Drosophila* larvae. *Cell. Microbiol.* **9**, 106–119 (2007).
38. Jackson, T. A., Boucias, D. G. & Thaler, J. O. Pathobiology of amber disease, caused by *Serratia* spp., in the New Zealand grass grub, *Costelytra zealandica*. *J. Invertebr. Pathol.* **78**, 232–243 (2001).
39. Hurst, M. R. & Jackson, T. A. Use of the green fluorescent protein to monitor the fate of *Serratia entomophila* causing amber disease in the New Zealand grass grub, *Costelytra zealandica*. *J. Microbiol. Methods* **50**, 1–8 (2002).
40. Hurst, M. R., Glare, T. R., Jackson, T. A. & Ronson, C. W. Plasmid-located pathogenicity determinants of *Serratia entomophila*, the causal agent of amber disease of grass grub, show similarity to the insecticidal toxins of *Photobacterium luminescens*. *J. Bacteriol.* **182**, 5127–5138 (2000).
Identification of determinants responsible for Amber disease.
41. Grkovic, S., Glare, T. R., Jackson, T. A. & Corbett, G. E. Genes essential for amber disease in grass grubs are located on the large plasmid found in *Serratia entomophila* and *Serratia proteamaculans*. *Appl. Environ. Microbiol.* **61**, 2218–2223 (1995).
42. Hurst, M. R., Glare, T. R. & Jackson, T. A. Cloning *Serratia entomophila* antifeding genes — a putative defective prophage active against the grass grub *Costelytra zealandica*. *J. Bacteriol.* **186**, 5116–5128 (2004).
43. Hurst, M. R., Beard, S. S., Jackson, T. A. & Jones, S. M. Isolation and characterization of the *Serratia entomophila* antifeding prophage. *FEMS Microbiol. Lett.* **270**, 42–48 (2007).
44. French-Constant, R. H., Dowling, A. & Waterfield, N. R. Insecticidal toxins from *Photobacterium* bacteria and their potential use in agriculture. *Toxicon* **49**, 436–451 (2007).
45. Onfelt Tingvall, T., Roos, E. & Engstrom, Y. The *imd* gene is required for local Cecropin expression in *Drosophila* barrier epithelia. *EMBO Rep.* **2**, 239–243 (2001).
46. Zaidman-Remy, A. *et al.* The *Drosophila* amidase PGRP-LB modulates the immune response to bacterial infection. *Immunity* **24**, 463–473 (2006).
47. Bischoff, V. *et al.* Downregulation of the *Drosophila* immune response by peptidoglycan-recognition proteins SC1 and SC2. *PLoS Pathog.* **2**, e14 (2006).
48. Ryu, J. H. *et al.* An essential complementary role of NF- κ B pathway to microbicidal oxidants in *Drosophila* gut immunity. *EMBO J.* **25**, 3693–3701 (2006).
Shows that intestinal NF- κ B- and AMP-dependent immunity is crucial to host survival against ROS-resistant bacteria.
49. Liehl, P., Blight, M., Vodovar, N., Boccard, F. & Lemaître, B. Prevalence of local immune response against oral infection in a *Drosophila/Pseudomonas* infection model. *PLoS Pathog.* **2**, e56 (2006).
Shows the importance of the bacterial metalloprotease AprA to counteract AMPs.
50. Vodovar, N. *et al.* Complete genome sequence of the entomopathogenic and metabolically versatile soil bacterium *Pseudomonas entomophila*. *Nature Biotechnol.* **24**, 673–679 (2006).
51. Leulier, F. *et al.* The *Drosophila* immune system detects bacteria through specific peptidoglycan recognition. *Nature Immunol.* **4**, 478–484 (2003).
52. Royet, J. & Dziarski, R. Peptidoglycan recognition proteins: pleiotropic sensors and effectors of antimicrobial defences. *Nature Rev. Microbiol.* **5**, 264–277 (2007).
53. Forst, S., Dowds, B., Boemare, N. & Stackebrandt, E. *Xenorhabdus* and *Photobacterium* spp.: bugs that kill bugs. *Annu. Rev. Microbiol.* **51**, 47–72 (1997).
54. Goodrich-Blair, H. & Clarke, D. J. Mutualism and pathogenesis in *Xenorhabdus* and *Photobacterium*: two roads to the same destination. *Mol. Microbiol.* **64**, 260–268 (2007).
55. Hurst, G. D., Anbutso, H., Kutsukake, M. & Fukatsu, T. Hidden from the host: *Spiroplasma* bacteria infecting *Drosophila* do not cause an immune response, but are suppressed by ectopic immune activation. *Insect Mol. Biol.* **12**, 95–97 (2003).
Host colonization by bacteria that avoid detection by the insect defences.
56. Herbert, E. E. & Goodrich-Blair, H. Friend and foe: the two faces of *Xenorhabdus nematophila*. *Nature Rev. Microbiol.* **5**, 634–646 (2007).
57. Ji, D. & Kim, Y. An entomopathogenic bacterium, *Xenorhabdus nematophila*, inhibits the expression of an antibacterial peptide, cecropin, of the beet armyworm, *Spodoptera exigua*. *J. Insect Physiol.* **50**, 489–496 (2004).
58. Apidianakis, Y. *et al.* Profiling early infection responses: *Pseudomonas aeruginosa* eludes host defenses by suppressing antimicrobial peptide gene expression. *Proc. Natl Acad. Sci. USA* **102**, 2573–2578 (2005).
59. Park, Y., Kim, Y., Putnam, S. M. & Stanley, D. W. The bacterium *Xenorhabdus nematophilus* depresses nodulation reactions to infection by inhibiting eicosanoid biosynthesis in tobacco hornworms, *Manduca sexta*. *Arch. Insect Biochem. Physiol.* **52**, 71–80 (2003).
60. Kim, Y., Ji, D., Cho, S. & Park, Y. Two groups of entomopathogenic bacteria, *Photobacterium* and *Xenorhabdus*, share an inhibitory action against phospholipase A2 to induce host immunodepression. *J. Invertebr. Pathol.* **89**, 258–264 (2005).
61. Eleftherianos, I., Millichap, P. J., French-Constant, R. H. & Reynolds, S. E. RNAi suppression of recognition protein mediated immune responses in the tobacco hornworm *Manduca sexta* causes increased susceptibility to the insect pathogen *Photobacterium*. *Dev. Comp. Immunol.* **30**, 1099–1107 (2006).
A small antibiotic molecule that is produced by *Photobacterium* spp. acts as inhibitor of phenoloxidase in *Manduca sexta*.
62. Brugirard-Ricaud, K. *et al.* Site-specific antiphagocytic function of the *Photobacterium luminescens* type III secretion system during insect colonization. *Cell. Microbiol.* **7**, 363–371 (2005).
63. Eleftherianos, I. *et al.* An antibiotic produced by an insect-pathogenic bacterium suppresses host defenses through phenoloxidase inhibition. *Proc. Natl Acad. Sci. USA* **104**, 2419–2424 (2007).
64. Haas, D. & Defago, G. Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature Rev. Microbiol.* **3**, 307–319 (2005).
65. Galan, J. E. & Collmer, A. Type III secretion machines: bacterial devices for protein delivery into host cells. *Science* **284**, 1322–1328 (1999).
66. Matsumoto, K. Role of bacterial proteases in pseudomonal and serratial keratitis. *Biol. Chem.* **385**, 1007–1016 (2004).
67. Miyoshi, S. & Shinoda, S. Microbial metalloproteases and pathogenesis. *Microbes Infect.* **2**, 91–98 (2000).
68. Travis, J., Potempa, J. & Maeda, H. Are bacterial proteinases pathogenic factors? *Trends Microbiol.* **3**, 405–407 (1995).
69. French-Constant, R. *et al.* *Photobacterium*: towards a functional genomic analysis of a symbiont and pathogen. *FEMS Microbiol. Rev.* **26**, 433–456 (2003).
70. Bowen, D. J. *et al.* Genetic and biochemical characterization of PrtA, an RTX-like metalloprotease from *Photobacterium*. *Microbiology* **149**, 1581–1591 (2003).
71. Duchaud, E. *et al.* The genome sequence of the entomopathogenic bacterium *Photobacterium luminescens*. *Nature Biotechnol.* **21**, 1307–1313 (2003).
72. Derzelle, S. *et al.* The PhoP/PhoQ two-component regulatory system of *Photobacterium luminescens* is essential for virulence in insects. *J. Bacteriol.* **186**, 1270–1279 (2004).
73. Cowles, K. N., Cowles, C. E., Richards, G. R., Martens, E. C. & Goodrich-Blair, H. The global regulator Lrp contributes to mutualism, pathogenesis and phenotypic variation in the bacterium *Xenorhabdus nematophila*. *Cell. Microbiol.* **9**, 1311–1323 (2007).
74. Fedhila, S., Nel, P. & Lereclus, D. The InhA2 metalloprotease of *Bacillus thuringiensis* strain 407 is required for pathogenicity in insects infected via the oral route. *J. Bacteriol.* **184**, 3296–3304 (2002).
Demonstrates the involvement of metalloprotease in the pathogenesis of *B. thuringiensis*.
75. Salamitou, S. *et al.* The plcR regulon is involved in the opportunistic properties of *Bacillus thuringiensis* and *Bacillus cereus* in mice and insects. *Microbiology* **146**, 2825–2832 (2000).
76. Titball, R. W., Hill, J., Lawton, D. G. & Brown, K. A. *Yersinia pestis* and plague. *Biochem. Soc. Trans.* **31**, 104–107 (2003).
77. Waterfield, N. R., Wren, B. W. & French-Constant, R. H. Invertebrates as a source of emerging human pathogens. *Nature Rev. Microbiol.* **2**, 833–841 (2004).
78. Grenier, A. M., Dupont, G., Pages, S., Condemine, G. & Rabhe, Y. The phytopathogen *Dickeya dadantii* (*Erwinia chrysanthemi* 3937) is a pathogen of the pea aphid. *Appl. Environ. Microbiol.* **72**, 1956–1965 (2006).
79. Dillon, R. J. & Dillon, V. M. The gut bacteria of insects: nonpathogenic interactions. *Annu. Rev. Entomol.* **49**, 71–92 (2004).
80. Eleftherianos, I. *et al.* Prior infection of *Manduca sexta* with non-pathogenic *Escherichia coli* elicits immunity to pathogenic *Photobacterium luminescens*: roles of immune-related proteins shown by RNA interference. *Insect Biochem. Mol. Biol.* **36**, 517–525 (2006).
81. Tzou, P., Reichhart, J. M. & Lemaître, B. Constitutive expression of a single antimicrobial peptide can restore wild-type resistance to infection in immunodeficient *Drosophila* mutants. *Proc. Natl Acad. Sci. USA* **99**, 2152–2157 (2002).
82. Dimopoulos, G. Insect immunity and its implication in mosquito-malaria interactions. *Cell. Microbiol.* **5**, 3–14 (2003).
83. Evans, J. D. *et al.* Immune pathways and defence mechanisms in honey bees *Apis mellifera*. *Insect Mol. Biol.* **15**, 645–656 (2006).
84. Zou, Z. *et al.* Comparative genomic analysis of the *Tribolium* immune system. *Genome Biol.* **8**, R177 (2007).
85. Kanost, M. R., Jiang, H. & Yu, X. Q. Innate immune responses of a lepidopteran insect, *Manduca sexta*. *Immunol. Rev.* **198**, 97–105 (2004).
86. Ryu, J. H. *et al.* Innate immune homeostasis by the homeobox gene caudal and commensal-gut mutualism in *Drosophila*. *Science* **319**, 777–782 (2008).
Describes changes in the commensal community by modification of host innate immune homeostasis and protection from ingested pathogens by the microbiota.
87. Shirasu-Hiza, M. M. & Schneider, D. S. Confronting physiology: how do infected flies die? *Cell. Microbiol.* **9**, 2775–2783 (2007).
Characterization of the colonization process of *Naucoris* by *M. ulcerans*.
88. Marsollier, L. *et al.* Protection against *Mycobacterium ulcerans* lesion development by exposure to aquatic insect saliva. *PLoS Med.* **4**, e64 (2007).
89. Marsollier, L. *et al.* Colonization of the salivary glands of *Naucoris cimicoides* by *Mycobacterium ulcerans* requires host plasmatocytes and a macrolide toxin, mycolactone. *Cell. Microbiol.* **7**, 935–943 (2005).
90. Marsollier, L. *et al.* Aquatic insects as a vector for *Mycobacterium ulcerans*. *Appl. Environ. Microbiol.* **68**, 4623–4628 (2002).
91. Marsollier, L. *et al.* Impact of *Mycobacterium ulcerans* biofilm on transmissibility to ecological niches and Buruli ulcer pathogenesis. *PLoS Pathog.* **3**, e62 (2007).

92. Brennan, C. A. & Anderson, K. V. *Drosophila*: the genetics of innate immune recognition and response. *Annu. Rev. Immunol.* **22**, 457–483 (2004).
93. Lemaitre, B. & Hoffmann, J. The host defense of *Drosophila melanogaster*. *Annu. Rev. Immunol.* **25**, 697–743 (2007).
94. Foley, E. & O'Farrell, P. H. Nitric oxide contributes to induction of innate immune responses to Gram-negative bacteria in *Drosophila*. *Genes Dev.* **17**, 115–125 (2003).
95. Dijkers, P. & O'Farrell, P. H. *Drosophila* calcineurin promotes induction of innate immune responses. *Curr. Biol.* **17**, 2087–2093 (2007).
96. Uvell, H. & Engstrom, Y. A multilayered defense against infection: combinatorial control of insect immune genes. *Trends Genet.* **23**, 342–349 (2007).
97. Kocks, C. *et al.* Eater, a transmembrane protein mediating phagocytosis of bacterial pathogens in *Drosophila*. *Cell* **123**, 335–346 (2005).
98. Cerenius, L. & Soderhall, K. The prophenoloxidase-activating system in invertebrates. *Immunol. Rev.* **198**, 116–126 (2004).
99. Gillespie, J. P., Kanost, M. R. & Trenczek, T. Biological mediators of insect immunity. *Annu. Rev. Entomol.* **42**, 611–643 (1997).
100. Hurst, G. D. & Jiggins, F. M. Male-killing bacteria in insects: mechanisms, incidence, and implications. *Emerg. Infect. Dis.* **6**, 329–336 (2000).
101. McGraw, E. A. & O'Neill, S. L. *Wolbachia pipientis*: intracellular infection and pathogenesis in *Drosophila*. *Curr. Opin. Microbiol.* **7**, 67–70 (2004).
102. Dobson, S. L. *et al.* *Wolbachia* infections are distributed throughout insect somatic and germ line tissues. *Insect Biochem. Mol. Biol.* **29**, 153–160 (1999).
103. Bourtzis, K., Pettigrew, M. M. & O'Neill, S. L. *Wolbachia* neither induces nor suppresses transcripts encoding antimicrobial peptides. *Insect Mol. Biol.* **9**, 635–639 (2000).
104. Min, K. T. & Benzer, S. *Wolbachia*, normally a symbiont of *Drosophila*, can be virulent, causing degeneration and early death. *Proc. Natl Acad. Sci. USA* **94**, 10792–10796 (1997).
105. Priest, F. G. in *Entomopathogenic Bacteria: From Laboratory to Field Application* (eds Charles, J. F., Delécluse, A. & Nielsen-LeRoux, C.) 1–22 (Kluwer Academic Publishers, Dordrecht, 2000).
106. Bravo, A., Gill, S. S. & Soberon, M. Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. *Toxicon* **49**, 423–435 (2007).
107. de Maagd, R. A., Bravo, A., Berry, C., Crickmore, N. & Schnepf, H. E. Structure, diversity, and evolution of protein toxins from spore-forming entomopathogenic bacteria. *Annu. Rev. Genet.* **37**, 409–433 (2003).
108. Schnepf, E. *et al.* *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol. Mol. Biol. Rev.* **62**, 775–806 (1998).
109. Aronson, A. I. & Shai, Y. Why *Bacillus thuringiensis* insecticidal toxins are so effective: unique features of their mode of action. *FEMS Microbiol. Lett.* **195**, 1–8 (2001).
110. Peferoen, M. Progress and prospects for field use of Bt genes in crops. *TIBTech*, 173–177 (1997).
111. Hansen, B. M. & Salamitou, S. in *Entomopathogenic Bacteria: From Laboratory to Field Application* (eds Charles, J. F., Delécluse, A. & Nielsen-LeRoux, C.) 41–64 (Kluwer Academic Publishers, Dordrecht, 2000).
112. Broderick, N. A., Raffa, K. F. & Handelsman, J. Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity. *Proc. Natl Acad. Sci. USA* **103**, 15196–15199 (2006).
- Demonstrates that commensal bacteria are required to kill gypsy moth upon *B. thuringiensis* infection.**
113. Harada, H. & Ishikawa, H. Experimental pathogenicity of *Erwinia aphidicola* to pea aphid, *Acyrtosiphon pisum*. *J. Gen. Appl. Microbiol.* **43**, 363–367 (1997).
114. Fedhila, S., Daou, N., Lereclus, D. & Nielsen-LeRoux, C. Identification of *Bacillus cereus* internalin and other candidate virulence genes specifically induced during oral infection in insects. *Mol. Microbiol.* **62**, 339–355 (2006).
115. Rolain, J. M., Franc, M., Davoust, B. & Raoult, D. Molecular detection of *Bartonella quintana*, *B. koehlerae*, *B. henselae*, *B. clarridgeiae*, *Rickettsia felis*, and *Wolbachia pipientis* in cat fleas, France. *Emerg. Infect. Dis.* **9**, 338–342 (2003).
116. Bowen, D. *et al.* Insecticidal toxins from the bacterium *Photobacterium luminescens*. *Science* **280**, 2129–2132 (1998).
- Identification of Tc-toxins.**
117. Morgan, J. A., Sergeant, M., Ellis, D., Ousley, M. & Jarrett, P. Sequence analysis of insecticidal genes from *Xenorhabdus nematophilus* PMFI296. *Appl. Environ. Microbiol.* **67**, 2062–2069 (2001).
118. Waterfield, N. R., Bowen, D. J., Fetherston, J. D., Perry, R. D. & French-Constant, R. H. The tc genes of *Photobacterium*: a growing family. *Trends Microbiol.* **9**, 185–191 (2001).
119. Charles, J. F., Silva-Filha, M.-H. & Nielsen-LeRoux, C. in *Entomopathogenic Bacteria: From Laboratory to Field Application* (eds Charles, J. F., Delécluse, A. & Nielsen-LeRoux, C.) 237–252 (Kluwer Academic Press, Dordrecht, 2000).
120. Daborn, P. J. *et al.* A single *Photobacterium* gene, makes caterpillars floppy (*mcf*), allows *Escherichia coli* to persist within and kill insects. *Proc. Natl Acad. Sci. USA* **99**, 10742–10747 (2002).
- Identification of the Mcf toxin.**

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DATABASES

Entrez Genome Project: <http://www.ncbi.nlm.nih.gov/sites/entrez?db=genomeprj>
[Bacillus cereus](http://www.ncbi.nlm.nih.gov/sites/entrez?db=genomeprj) | [Bacillus thuringiensis](http://www.ncbi.nlm.nih.gov/sites/entrez?db=genomeprj) | [Mycobacterium ulcerans](http://www.ncbi.nlm.nih.gov/sites/entrez?db=genomeprj) | [Photobacterium luminescens](http://www.ncbi.nlm.nih.gov/sites/entrez?db=genomeprj) | [Pseudomonas entomophila](http://www.ncbi.nlm.nih.gov/sites/entrez?db=genomeprj) | [Serratia marcescens](http://www.ncbi.nlm.nih.gov/sites/entrez?db=genomeprj) | [Yersinia pestis](http://www.ncbi.nlm.nih.gov/sites/entrez?db=genomeprj)

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