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Of moths, mites and microbes - The role of bacteria in the life history of two arthropod herbivores

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General introduction

General introduction

Microbes are omnipresent in the environment of macro-organisms and innumerable examples of interactions between micro and macro-organisms have been described. Microbes may colonize different organs or body cavities of macro-organisms and are then referred to as ‘symbionts’ that reside in a ‘host’. Symbiosis was originally defined as the ‘living together of unlike organisms’ by Anton de Bary (1879). Bacterial symbionts can have beneficial, neutral (commensal symbiont), harmful (parasites and pathogens) or mixed effects on the host (Dillon & Dillon, 2004; Baumann, 2005; Werren *et al.*, 2008; Himler *et al.*, 2011; Engel & Moran, 2013). The role of symbionts in hosts may also change during evolution and may be context dependent and thus differ under different circumstances (Werren *et al.*, 2008; Zug & Hammerstein, 2015). First, I will describe and discuss non-pathogenic microbe-host interactions. Thereafter, I will focus on pathogens and the effects of immune challenge on host life history.

1. Non-pathogenic associations of bacteria with arthropod hosts

Symbioses can be categorized according to the co-dependence between symbionts and their hosts. Long-term, stable associations between symbionts and hosts are more likely to lead to mutualistic symbiosis than short-term, loose associations, because host and symbionts together form a unit of selection during evolution (Zilber-Rosenberg & Rosenberg, 2008; Rosenberg *et al.*, 2009; Salem *et al.*, 2015).

Obligate endosymbionts

Obligate bacterial endosymbionts are strictly necessary for host survival, growth and/or reproduction. Obligate symbioses are evolutionarily ancient and the phylogeny of these so-called primary endosymbionts is strictly congruent with the phylogeny of their host (Munson *et al.*, 1991; Chen *et al.*, 1999; Baumann, 2005; Dale & Moran, 2006). The co-speciation of the symbiotic partners can result in specializations of the host and the bacterium (Moran & Baumann, 2000; Baumann, 2005; Dale & Moran, 2006). For instance, endosymbionts live inside specialized host cells (bacteriocytes), which can form special structures in the host, the bacteriomes and are maternally transmitted inside the eggs (Buchner, 1965; Baumann, 2005; Moran *et al.*, 2008). This tight coevolutionary living together has resulted in genomes of obligate endosymbionts that can be very small, and that are characterized by the loss of functional genes that are necessary for, e.g., recombination, replication or transcriptional regulation, while genes for the synthesis of nutrients that the host diet lacks, such as essential amino acids, are retained (Moran & Wernegreen, 2000; Baumann, 2005; Dale & Moran, 2006; Moran *et al.*, 2008).

Facultative endosymbionts

Facultative (so-called secondary) endosymbionts are not strictly necessary for survival or reproduction of their host. Secondary endosymbionts are mostly maternally transmitted inside the eggs, although horizontal transmission can also occur

(Huigens *et al.*, 2004; Caspi-Fluger *et al.*, 2012). Consequently, there is only a partial congruence between symbiont and host phylogeny (Dale & Moran, 2006; Werren *et al.*, 2008). The prevalence of facultative, maternally transmitted bacteria in a host population depends on the efficiency of vertical transmission of the bacteria, but also on host fitness (Werren, 1997; Oliver *et al.*, 2010). Many facultative endosymbionts were shown to benefit the host e.g., by protecting them against parasitoids (Oliver *et al.*, 2003), or by supporting host plant use (Tsuchida *et al.*, 2011; Su *et al.*, 2015).

Endosymbiotic reproductive parasites, such as *Wolbachia*, ‘*Candidatus Cardinium*’, *Spiroplasma* and *Rickettsia* are known to increase their prevalence in a host population by manipulating the reproduction of their host. Since these bacteria are maternally transmitted, reproductive manipulation increases the proportion of infected females in a population via mechanisms such as feminization, parthenogenesis, male-killing or cytoplasmic incompatibility (Werren, 1997; Stouthamer *et al.*, 1999; Weeks *et al.*, 2001; Werren *et al.* 2008; Duron *et al.*, 2008).

Besides reproductive manipulation, reproductive parasites may increase and strengthen their persistence in a host population by benefitting the host (Hoffmann *et al.*, 1998; Fry *et al.*, 2004; Werren *et al.*, 2008; Himler *et al.*, 2011; Zug & Hammerstein, 2015). Accordingly, reproductive parasites have recently been found to enhance host fitness and to increase host resistance to bacterial and viral pathogens (Hedges *et al.*, 2008; Teixeira *et al.*, 2008; Moreira *et al.*, 2009) or parasitoids (Xie *et al.*, 2010, 2014), act as nutritional mutualist (Hosokawa *et al.*, 2010; Nikoh *et al.*, 2014), or alter host physiology to their host’s benefit (Kaiser *et al.*, 2010).

Gut bacteria

Gut bacteria do not live intracellularly, but inhabit different regions or compartments of the gut. Associations between gut bacteria and a host range from permanent (core community) to transient (Shade & Handelsman, 2012). Gut bacteria are in some cases necessary for host survival, development or reproduction (Hosokawa *et al.*, 2007; Salem *et al.*, 2013). When gut bacteria are permanently associated with a host, they are often vertically transmitted, may reside in special compartments or pouches in the guts and provide diverse beneficial services to their hosts. If gut bacteria are vertically transmitted, transmission occurs mostly extracellularly via various mechanisms. For instance, gut bacteria may be smeared on the egg surface by a female after oviposition or, as is the case of the stinkbug *Megacopta punctatissima*, provided in capsules together with the egg mass, which ensures high transmission rates (Fukatsu & Hosokawa, 2002; Engel & Moran, 2013; Salem *et al.*, 2015). Other insects, such as the stinkbug *Riptortus pedestris*, take up bacterial symbionts each generation again from the environment (Kikuchi *et al.*, 2011). Another route of transmission for gut bacteria is coprophagy, in which bacteria are excreted together with feces and can be taken up by conspecifics (Beard *et al.*, 2002). In social insects, gut bacteria may be taken up during contact with nest mates or hive contents and may

be transferred together with food from adults to larvae (Koch & Schmid-Hempel, 2011; Martinson *et al.*, 2012; Marsh *et al.*, 2014; Powell *et al.*, 2014).

Gut bacteria may also only be transiently present in the gut if random uptake from the environment is not followed by stable colonization (Shade & Handelsman, 2012; Engel & Moran, 2013). High variability of bacterial communities between individuals of a host population or variability due to diet or developmental stage may indicate transient association of gut bacteria with a host (Shade & Handelsman, 2012). Nevertheless, the metabolic capabilities that bacteria have evolved independently of the host may suffice to enhance the fitness of an animal that takes these bacteria up into its gut, even if transiently (Mason *et al.*, 2014). For instance, some plant-associated bacteria are able to use methanol as carbon source, fix nitrogen or break down secondary plant metabolites (Sy *et al.*, 2005; Fürnkranz *et al.*, 2008; Vorholt, 2012). The latter was shown to benefit larvae of the moth *Lymantria dispar* as described below (Mason *et al.*, 2014).

1.1 Host plant utilization of herbivores in relation to bacterial symbionts

Many herbivores have colonized and feed on plants and plant parts that are poor or unbalanced in nutrients or are hard to digest (Schoonhoven *et al.*, 2005). Moreover, herbivores have to deal with physical and chemical defences that plants have evolved to reduce the damage inflicted by herbivores (Schoonhoven *et al.*, 2005; Karban & Baldwin, 2007). Symbiotic bacteria can play an important role in the ability of herbivores to use plant species or plant tissues as food and thus determine their host's ecological niche (Douglas, 2009; Feldhaar, 2011; Hansen & Moran, 2014).

Direct facilitation of host plant use

Symbiotic bacteria may directly facilitate host plant use by providing amino acids, vitamins or co-factors that are absent from the host diet or by producing enzymes that enhance the digestion of plant tissues (Buchner, 1965; Douglas, 2009; Feldhaar, 2011; Engel & Moran, 2013; Hansen & Moran, 2014). Obligate endosymbionts are well known for synthesizing nutrients for their hosts. For instance, plant sap is poor in amino acids. Hence, phloem feeders such as aphids are provided with essential amino acids by their primary endosymbionts, *Buchnera aphidicola* (Shigenobu *et al.*, 2000; van Ham *et al.*, 2003; Baumann, 2005). Nutrient provisioning is not restricted to obligate endosymbionts, although gut bacteria can fulfil this function as well. For instance, the gut symbiont of the firebug *Pyrhocoris apterus* was shown to provide its host with vitamin B, which proved essential for its ability to reproduce (Salem *et al.*, 2013, 2014). The plataspid stinkbug *Megacopta punctatissima* relies on its gut symbiont *Ishikawaella capsulata* for normal growth, development and reproduction (Fukatsu & Hosokawa, 2002; Hosokawa *et al.*, 2007). In ants, there is strong indication that gut bacteria from the order Rhizobiales may be involved in nitrogen fixation (Russell *et al.*, 2009). Even bacteria that only transiently inhabit the

gut may affect host plant use of herbivores. For instance, larval growth of the gypsy moth, *Lymantria dispar*, was enhanced by bacteria that inhabit the foliage of its host plant aspen, when phenolic glycosides, the major defence metabolites of aspen, were added to the larval (artificial) diet (Mason *et al.*, 2014). The authors further showed that these bacteria can reduce the concentration of phenolic glycosides *in vitro* (Mason *et al.*, 2014).

Indirect facilitation of host plant use

Besides the direct nutritional effects mentioned above, herbivore-associated bacteria may manipulate plant physiology to their host's benefit (Kaiser *et al.*, 2010; Chung *et al.*, 2013; Hansen & Moran, 2014; Zhu *et al.*, 2014; Su *et al.*, 2015). *Wolbachia* was shown to enhance the fitness of the lepidopteran leaf-miner *Phyllonorycter blancardella*, by preserving photosynthetically active and nutrient rich (green) islands in senescent leaves, thereby increasing host fitness (Kaiser *et al.*, 2010).

Few examples show that symbionts can also interfere with herbivore-induced plant defences. Phytohormones such as jasmonic acid (JA) or salicylic acid (SA) are central to the coordination of plant defences. The induction of phytohormone biosynthesis is specific to the type of attacker: in general, feeding by herbivorous insects induces the JA-related plant responses (Karban & Baldwin, 2007; Erb *et al.*, 2012). JA-responses were shown to cause reduced larval growth, amount of feeding and survival of herbivores and may attract their natural enemies (Howe *et al.*, 1996; Li *et al.*, 2002; Thaler *et al.*, 2002). Biotrophic phytopathogens generally induce SA-related responses in plants (Glazebrook, 2005). Importantly, JA- and SA-responses are interrelated and JA-responses can be suppressed via the induction of SA-responses and vice versa (JA-SA crosstalk) (Pieterse *et al.*, 2012; Thaler *et al.*, 2012). *Hamiltonella defensa*, the secondary symbiont of the whitefly *Bemisia tabaci*, was found to suppress JA-responses in tomato, via small non-proteinaceous molecules in the whitefly's saliva (Su *et al.*, 2015). Also, bacteria associated with the Colorado potato beetle, *Leptinotarsa decemlineata*, were shown to reduce herbivore-induced JA-responses and to increase the larval growth of the beetle (Chung *et al.*, 2013). In both examples, JA suppression did not occur in SA-deficient plants, indicating that induction of SA-related responses caused the suppression of JA-responses (Chung *et al.*, 2013)

The role of bacterial symbionts in agriculture

The ability of bacterial symbionts to determine the host plant range of herbivores can have implications for agriculture, in particular pest management. Many herbivores are destructive agricultural and forest pests. Microbes that are harboured by arthropod pests may affect their pest status by influencing the dietary range of the pest or by affecting pest management. For instance, the pest status of the stinkbug *Megacopta punctatissima* on legume crop was found to be determined by the genotype of its obligate gut symbiont *Ishikawaella capsulata* (Hosokawa *et al.*,

2007). In this study, egg hatch rate of the pest species *M. punctatissima* decreased significantly when its symbionts were replaced for symbionts of a closely related non-pest species (*M. cribraria*). On the other hand, egg hatch rate of *M. cribraria* was significantly increased after receiving symbionts of *M. punctatissima* (Hosokawa *et al.*, 2007). It was demonstrated in aphids that symbiotic bacteria are able to expand host plant range of its insect herbivore host: transfection of the facultative endosymbiont *Regiella insecticola* from the pea aphid (*Acyrtosiphon pisum*) to the vetch aphid (*Megoura crassicaula*) enabled the latter to survive and reproduce on clover (Tsuchida *et al.*, 2011).

Bacteria can also interfere with chemical pest control measures. For example, bacteria of the genus *Burkholderia* have been found to confer insecticide resistance to the bean bug, *Riptortus pedestris*, which is a pest on leguminous crops (Kikuchi *et al.*, 2012). Further, larval mortality in response to the insecticidal toxins of *Bacillus thuringiensis* was shown to be reduced upon removal of the bacterial midgut community in the gypsy moth, *Lymantria dispar*, and other Lepidoptera (Broderick *et al.*, 2006, 2009). The authors suggest that benign gut bacteria of these Lepidoptera may breach the midgut barrier after it was damaged by *B. thuringiensis* toxin and become opportunistic pathogens (Broderick *et al.*, 2006, 2009). The presence of bacterial symbionts may also affect biological control measures, e.g., *H. defensa* rendered its aphid host more resistant to parasitoid wasps (Oliver *et al.*, 2003).

2. Pathogenic effects of bacteria on insect hosts

Besides commensal and beneficial bacteria, insects may be exposed to a suite of pathogenic bacteria during their lifetime. Pathogens cause disease in organisms and pathogenicity of bacteria depends on virulence factors, such as the ability to attach to and invade host cells, the production of toxins and the secretion of proteins that modulate host cellular functions (Farthing, 2004; Galán, 2009). However, whether bacteria cause disease also strongly depends on the host or the host tissue (Ruby *et al.*, 2004).

The immune system of insects

As a first line of defence against pathogens, insects possess physical and chemical barriers, such as the cuticle or the peritrophic matrix (Dunn, 1990; Lehane, 1997; Kuraishi *et al.*, 2011; Davis & Engström, 2012). Nevertheless, pathogens might gain access to the insect hemocoel via wounds or may be ingested and be able to cross the midgut epithelium (Ashida *et al.*, 2012). As a consequence, insects have evolved an efficient innate immune system to fight pathogenic intruders. Recognition of self and non-self is the first step of immune defence (Janeway, 1992; Royet, 2004). Insects have a wide range of proteins that function as pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs) (Medzhitov & Janeway, 1997). PAMPs include surface molecules of bacteria such as lipopolysaccharides, peptidoglycans or lipoteichoic acid.

The insect immune system is a combination of cellular and humoral immune responses. Cellular immune responses include processes like phagocytosis and encapsulation that are mediated by different types of blood cells (hemocytes) (Lavine & Strand, 2002; Strand, 2008). During phagocytosis, biotic or abiotic entities are enclosed by individual hemocytes. Encapsulation response targets large invaders, such as nematodes or parasitoid larvae, that cannot be enclosed by a single cell and are therefore bound by multiple hemocytes (Strand, 2008). Phagocytized or encapsulated organisms are killed and degraded (Reeves *et al.*, 2002; Stuart & Ezekowitz, 2005; Stuart *et al.*, 2007).

Humoral defences include a broad range of antimicrobial peptides (AMPs) that are produced by the fat body or hemocytes and that are released into the hemolymph (Bulet *et al.*, 1999; Zasloff, 2002). AMPs vary in their antimicrobial spectrum. Most AMPs show activity against a wide range of pathogens, with particular efficiency against certain groups, depending on their mode of action. For example, lysozymes, which destroy bonds between peptidoglycan forming molecules (Rupley, 1967) can efficiently destroy gram-positive bacteria but are also active against gram-negative bacteria. Other AMPs have a more narrow antimicrobial spectrum. For instance, drosomycin only shows activity against filamentous fungi (Bulet *et al.*, 1999; Zhang & Zhu, 2009).

The melanin-synthesis cascade is another important part of the insect immune system. Phenoloxidase (PO) is the key enzyme in this cascade and catalyzes the reactions of phenols to toxic quinones which are polymerized to melanin (Nappi & Vass, 1993; Cerenius & Söderhäll, 2004). Intermediates of melanogenesis as well as melanin itself are cytotoxic and have been reported to be lethal for pathogens (Nappi & Vass, 1993, 1998; Nappi *et al.*, 1995; Söderhäll & Cerenius, 1998; Nappi & Christensen, 2005). Further, melanin and its intermediates have functions in the encapsulation and phagocytosis of pathogens as well as in wound healing processes (Cerenius *et al.*, 2008).

The cost of immune defence and trade-offs with other life history traits

In life history theory, the concept of trade-offs is based on the assumption that organisms have limited internal resources available that need to be partitioned among the different life history traits (Stearns, 1989; Roff & Fairbairn, 2007). Life history traits are therefore negatively linked to each other: a resource investment into one trait which increases fitness may reduce resources that are available for another fitness-relevant trait (Stearns, 1989; Roff & Fairbairn, 2007).

The immune system may prevent an organism from being colonized by pathogens. However, an immune system implicates different types of costs which may negatively affect other life history traits (Sheldon & Verhulst, 1996; Zuk & Stoehr, 2002; Schmid-Hempel, 2005). Physiological costs include costs of nutrients and energy which are necessary for the maintenance and deployment of the immune system (Schmid-Hempel, 2011). Maintenance costs are incurred to keep the immune

system at a level of readiness, even when the organism is not infected (Schmid-Hempel, 2005, 2011; Kraaijeveld & Wertheim, 2009). Deployment costs are incurred when the immune system is activated (Kraaijeveld & Wertheim, 2009; Schmid-Hempel, 2011). Immune system activation has been shown to trade off with various other life history traits such as life span (Moret & Schmid-Hempel, 2000; Krams *et al.*, 2014), growth (Soler *et al.*, 2003; Brommer, 2004) and reproduction (Gustafsson *et al.*, 1994; Sheldon & Verhulst, 1996; Adamo *et al.*, 2001; Rolff & Siva-Jothy, 2002; Hanssen *et al.*, 2005; French *et al.*, 2007).

Evolutionary costs of immunity arise through the negative genetic covariance between immunity and other fitness-related traits in uninfected organisms (Schmid-Hempel, 2005, 2011). For instance, several studies showed that highly pathogen-resistant insect strains perform worse than less resistant strains, e.g., have longer developmental time, lower larval competitive ability or lower fecundity in the absence of pathogens (Boots & Begon, 1993; Kraaijeveld & Godfray, 1997; McKean *et al.*, 2008).

Behavioural changes in the face of infection

As an alternative (or in addition) to a costly immune system (activation), organisms may have behavioural strategies that reduce the frequency of infection and minimize adverse fitness effects (Schmid-Hempel, 2011; de Roode & Lefèvre, 2012). An important behavioural adaptation to possible infections is to avoid contracting them. This behaviour can include spatial or temporal avoidance of parasites (Orr, 1992; Christe *et al.*, 1994), avoiding feeding on contaminated food (Hutchings *et al.*, 2001; Alma *et al.*, 2010) or avoiding infected conspecifics, such as infected mates (Kavaliers & Colwell, 1995; Penn & Potts, 1998; Penn *et al.*, 1998).

Behavioural adaptations to reduce pathogen loads once infection has been contracted include self-medication behaviour, in which organisms consume medicinal substances to reduce pathogen loads and increase their fitness while infected (Huffman *et al.*, 1996; Singer *et al.*, 2009; Manson *et al.*, 2010). In this context, infected females of the butterfly *Danaus plexippus* were found to engage in trans-generational medication behaviour and oviposit more on plants with high levels of toxic secondary metabolites than on plants with lower levels than uninfected females (Lefèvre *et al.*, 2010).

Another adaptive behaviour of infected animals, which does not directly aim to reduce pathogen loads but may enhance life time fecundity of infected organisms, is terminal investment. If life expectancy decreases due to age or infection, organisms may make a 'terminal investment', i.e., invest their remaining resources into current reproduction in order to maximize their fitness in the short time that remains (Williams, 1966; Clutton-Brock, 1984). Shortening of the lifespan can be caused by the pathogen that may kill the organism or by the costs of immune defence which can trade off with lifespan (Sheldon & Verhulst, 1996). A shift of investment from future to current reproduction may imply an increase in courtship activity, parental

care, oviposition rate or acceptance of less preferred host plants for oviposition (Minchella & Loverde, 1981; Part *et al.*, 1992; Adamo, 1999; Bonneaud *et al.*, 2004; Javoš & Tammaru, 2004; Creighton *et al.*, 2009).

3. Study organisms

In this thesis, I investigated possible effects of bacteria on the life histories of two generalist herbivorous arthropods. In the noctuid moth *Heliothis virescens* (Fabricius, 1777) (Lepidoptera: Noctuidae), I determined negative correlations between immunity and reproduction as well as adaptive behavioural changes in the oviposition strategy of this moth in the face of infection. Moreover, I identified bacterial communities that are associated with *H. virescens*.

In the two-spotted spider mite *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae), which is well known to harbour various (endo)symbiotic bacteria such as *Wolbachia*, ‘*Candidatus* Cardinium’ and *Spiroplasma* (Breeuwer & Jacobs, 1996; Gotoh *et al.*, 2003, 2007a; Enigl & Schausberger, 2007), I investigated effects of these bacteria on mite fitness parameters, mite transcriptome and induced plant responses of tomato leaflets after mite feeding.

Heliothis virescens

The tobacco budworm, *H. virescens* (FIGURE 1.1), is distributed throughout the American continent (Fitt, 1989). Its larvae are polyphagous and have been reported to feed on at least 37 plant species in 14 families (Barber, 1937; Stadelbacher, 1981; Fitt, 1989; Waldvogel & Gould, 1990; Sheck & Gould, 1993; Blanco *et al.*, 2007). Among the host plants of *H. virescens* are important economic crops like tobacco, cotton and chickpea (Morgan & Chamberlin, 1927; Barber, 1937; Neunzig, 1969; Blanco *et al.*, 2007), which makes this species a major agricultural pest.

Heliothis virescens females can oviposit up to 1500 eggs in their lifetime of about 30 days in the laboratory at 25 °C (Proshold *et al.*, 1982; Willers *et al.*, 1987; Fitt, 1989). Eggs are laid singly on plants throughout the night, starting at dusk (Fitt, 1989;



FIGURE 1.1 *Heliothis virescens* (a) larva, (b) pupa, and (c) mating adults. © Jan van Arkel.

General introduction

Ramaswamy, 1990). *Heliothis virescens* has five larval stages and developmental time from eggs to pupae takes about 17 days at 25 °C (Fye & McAda, 1972). Larvae pupate in the soil for 13 days at 25 °C after which the adults eclose.

Heliothis virescens was reported to have an efficient immune system (Barthel *et al.*, 2014). However, possible effects of immune response on life history traits have not been investigated in this species. Furthermore, not much is known about its microbiome, i.e., bacteria that are associated with *H. virescens*, particularly in the field.

Tetranychus urticae

The two-spotted spider mite, *T. urticae* (FIGURE 1.2) is an extremely polyphagous herbivore that occurs worldwide and has been reported to feed on over 1100 plant species, among them are economically important crops like tomato, cucumber, strawberry, bean and cotton (Bolland *et al.*, 1998; Grbic *et al.*, 2011; Migeon *et al.*, 2011) which makes this mite an important agricultural pest.

The life cycle of *T. urticae* includes egg, one larval stage, which is followed by two nymphal stages (protonymph and deutonymph) and the adult stage. The generation time of *T. urticae* takes about two weeks at 27 °C (Chain-ing *et al.*, 1976). Females have been reported to lay over 100 eggs during their lifetime (Carey & Bradley, 1982). Thus this mite can reach large population sizes in a short amount of time.

Spider mites are stylet feeders that pierce plant cells and suck out the cell content. Like other stylet feeders, *T. urticae* has been shown to induce jasmonic and salicylic acid-related plant defences during feeding (Walling, 2000; Kant *et al.*, 2004, 2008; Alba *et al.*, 2015).

Tetranychus urticae was found to harbour different endosymbionts, including *Wolbachia*, ‘*Candidatus Cardinium*’ and *Spiroplasma* (Breeuwer & Jacobs, 1996; Gotoh *et al.*, 2003, 2007a; Enigl & Schausberger, 2007). *Wolbachia* and ‘*Candida-*



FIGURE 1.2 *Tetranychus urticae* adult female. © Jan van Arkel.

tus Cardinium' were shown to cause cytoplasmic incompatibility in some populations of *T. urticae* (Breeuwer, 1997; Gotoh *et al.*, 2007a,b). In other *T. urticae* populations, *Wolbachia* did not cause cytoplasmic incompatibility (Gotoh *et al.*, 2003). Further, the effects of *Wolbachia* on mite fitness have been assessed with all possible outcomes, and *Wolbachia* had a negative effect, no effect or a positive effect on female fecundity (Breeuwer, 1997; Vala *et al.*, 2000; Perrot-Minnot *et al.*, 2002; Xie *et al.*, 2011). The presence of *Wolbachia* on mite gene expression has been investigated (Zhang *et al.*, 2015). Less is known about effects of *Spiroplasma* and '*Candidatus Cardinium*' or any combination of endosymbionts on *T. urticae*. Furthermore, the effect of endosymbionts on host-plant use of this mite has not yet been investigated.

THESIS OUTLINE

CHAPTER 2. As outlined above, bacteria can have diverse effects on their hosts. On the one hand, herbivore-associated bacteria may benefit the host, for instance by facilitating plant utilization of the host (Engel & Moran, 2013; Hansen & Moran, 2014). On the other hand, bacterial pathogens may negatively affect life history traits and thus the fitness of an organism (Grenfell & Dobson, 1995; Poulin, 2007; Schmid-Hempel, 2011). Against this background, we investigated the bacterial community that is associated with field and laboratory populations of *H. virescens* and assessed the spatial and temporal variability of this bacterial community as it depends on diet and life stage.

CHAPTER 3. The concept of 'Bateman's principle and immunity' predicts that females, who maximize their fitness by increasing longevity, invest more in immunity than males, who maximize fitness by increasing their number of matings (Rolff, 2002). Most moths, including *H. virescens*, do not correspond to the 'classical' sex roles in which females invest more into offspring than males. In this species, females and males invest substantially into offspring: females produce up to 1500 eggs whereas males produce a spermatophore that can comprise up to 5% of their bodymass (Fye & McAda, 1972; Proshold *et al.*, 1982; Blanco *et al.*, 2009). Both sexes produce a sex pheromone, but until now only males have been shown to differentiate between female sex pheromones of different quality, indicating that males are the choosy sex in this species (Vetter & Baker, 1983; Groot *et al.*, 2009, 2014). Because of the deviations in the life history of *H. virescens* from the classical Bateman case, it can be expected that differences in immunity between the sexes do not follow the predictions for 'Bateman's principle and immunity'. To assess the concept of 'Bateman's principle and immunity' in *H. virescens*, we first investigated immune system activation of males and females via immune gene expression experiments using dead cells of the entomopathogen *Serratia entomophila*. Secondly, we assessed the influence of immune activation on mating success of both sexes in mate choice experiments. Thirdly, we tested if immune defence activation

would affect the composition of the female sex pheromone as well as female calling behaviour.

CHAPTER 4. Infection may shorten the life span of an organism. One strategy for organisms to maximize fitness in the face of infection is therefore terminal investment, in which investment into future reproduction is shifted towards current reproduction (Williams, 1966; Clutton-Brock, 1984). In this chapter, we investigated whether terminal investment is part of the life history strategy of *H. virescens* and whether females of this moth adaptively change their oviposition strategy in response to an immune challenge with dead cells of the entomopathogen *S. entomophila*. Specifically, we assessed whether *H. virescens* females would increase their egg output in response to an immune challenge, and whether oviposition site choice of *H. virescens* females would be influenced by an immune challenge.

CHAPTER 5. Endosymbionts, such as *Wolbachia*, ‘*Candidatus Cardinium*’ and *Spiroplasma*, are well-known reproductive manipulators which may increase the proportion of infected females to increase their prevalence in a host population (Werren, 1997; Duron *et al.*, 2008; Werren *et al.*, 2008; Engelstädter & Hurst, 2009). However, several studies indicate that these bacteria may also positively affect host fitness (Teixeira *et al.*, 2008; Kaiser *et al.*, 2010; Xie *et al.*, 2010; Zug & Hammerstein, 2015). We studied the role of bacterial (endo)symbionts in two strains of the two-spotted spider mite *T. urticae*, one strain is a plant defence inducer and the other strain a plant defence suppressor. We investigated the bacterial community associated with these two strains and tested if the presence of (endo)symbionts was correlated with performance and gene expression profiles of the mites. Further, we assessed if the symbionts were associated with induction or suppression of plant responses in tomato (*Solanum lycopersicum*).

REFERENCES

- Adamo, S.A. 1999. Evidence for adaptive changes in egg laying in crickets exposed to bacteria and parasites. *Anim Behav* **57**: 117-124.
- Adamo, S.A., Jensen, M. & Younger, M. 2001. Changes in lifetime immunocompetence in male and female *Gryllus texensis* (formerly *G. integer*): trade-offs between immunity and reproduction. *Anim Behav* **62**: 417-425.
- Alba, J.M., Schimmel, B.C.J., Glas, J.J., Ataide, L.M.S., Pappas, M.L., Villarroel, C.A., Schuurink, R.C., Sabelis, M.W. & Kant, M.R. 2015. Spider mites suppress tomato defenses downstream of jasmonate and salicylate independently of hormonal crosstalk. *New Phytol* **205**: 828-840.
- Alma, C.R., Gillespie, D.R., Roitberg, B.D. & Goettel, M.S. 2010. Threat of infection and threat-avoidance behavior in the predator *Dicyphus hesperus* feeding on whitefly nymphs infected with an entomopathogen. *J Insect Behav* **23**: 90-99.
- Ashida, H., Ogawa, M., Kim, M., Mimuro, H. & Sasakawa, C. 2012. Bacteria and host interactions in the gut epithelial barrier. *Nat Chem Biol* **8**: 36-45.
- Barber, G.W. 1937. Seasonal availability of food plants of two species of *Heliothis* in eastern Georgia. *J Econ Entomol* **30**: 150-158.

- Barthel, A., Kopka, I., Vogel, H., Zipfel, P., Heckel, D.G. & Groot, A.T. 2014. Immune defence strategies of generalist and specialist insect herbivores. *Proc R Soc B Biol Sci* **281**: 20140897.
- Baumann, P. 2005. Biology of bacteriocyte-associated endosymbionts of plant sap-sucking insects. *Annu Rev Microbiol* **59**: 155-189.
- Beard, C.B., Cordon-Rosales, C. & Durvasula, R.V. 2002. Bacterial symbionts of the triatominae and their potential use in control of Chagas disease transmission. *Annu Rev Entomol* **47**: 123-141.
- Blanco, C.A., Terán-Vargas, A.P., López Jr, J.D., Kauffman, J.V. & Wei, X. 2007. Densities of *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae) in three plant hosts. *Fla Entomol* **90**: 742-750.
- Blanco, C.A., Guadalupe Rojas, M., Groot, A.T., Morales-Ramos, J. & Abel, C.A. 2009. Size and chemical composition of *Heliothis virescens* (Lepidoptera: Noctuidae) spermatophores. *Ann Entomol Soc Am* **102**: 629-637.
- Bolland, H.R., Gutierrez, J. & Flechtman, C.H. 1998. *World catalogue of the spider mite family (Acari: Tetranychidae)*. Brill, Leiden, The Netherlands.
- Bonneaud, C., Mazuc, J., Chastel, O., Wester Dahl, H. & Sorci, G. 2004. Terminal investment induced by immune challenge and fitness traits associated with major histocompatibility complex in the house sparrow. *Evolution* **58**: 2823-2830.
- Boots, M. & Begon, M. 1993. Trade-offs with resistance to a granulosis virus in the Indian meal moth, examined by a laboratory evolution experiment. *Funct Ecol*: 528-534.
- Breeuwer, J.A.J. & Jacobs, G. 1996. *Wolbachia*: intracellular manipulators of mite reproduction. *Exp Appl Acarol* **20**: 421-434.
- Breeuwer, J.A. 1997. *Wolbachia* and cytoplasmic incompatibility in the spider mites *Tetranychus urticae* and *T. turkestanii*. *Heredity* **79**: 41-47.
- Broderick, N.A., Raffa, K.F. & Handelsman, J. 2006. Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity. *Proc Natl Acad Sci USA* **103**: 15196-15199.
- Broderick, N.A., Robinson, C.J., McMahon, M.D., Holt, J., Handelsman, J. & Raffa, K.F. 2009. Contributions of gut bacteria to *Bacillus thuringiensis*-induced mortality vary across a range of Lepidoptera. *BMC Biol* **7**: 11.
- Brommer, J.E. 2004. Immunocompetence and its costs during development: an experimental study in blue tit nestlings. *Proc R Soc B Biol Sci* **271**: S110-S113.
- Buchner, P. 1965. *Endosymbiosis of animals with plant microorganisms*. Interscience, New York, USA.
- Bulet, P., Hetru, C., Dimarcq, J.-L. & Hoffmann, D. 1999. Antimicrobial peptides in insects; structure and function. *Dev Comp Immunol* **23**: 329-344.
- Carey, J. & Bradley, J. 1982. Developmental rates, vital schedules, sex ratios and life tables for *Tetranychus urticae*, *T. turkestanii* and *T. pacificus* (Acarina: Tetranychidae) on cotton. *Acarologia* **23**: 333-345.
- Caspi-Fluger, A., Inbar, M., Mozes-Daube, N., Katzir, N., Portnoy, V., Belausov, E., Hunter, M.S. & Zchori-Fein, E. 2012. Horizontal transmission of the insect symbiont *Rickettsia* is plant-mediated. *Proc R Soc B Biol Sci* **279**: 1791-1796.
- Cerenius, L. & Söderhäll, K. 2004. The prophenoloxidase-activating system in invertebrates. *Immunol Rev* **198**: 116-126.
- Cerenius, L., Lee, B.L. & Söderhäll, K. 2008. The proPO-system: pros and cons for its role in invertebrate immunity. *Trends Immunol* **29**: 263-271.
- Chain-ing, T.S., Poe, S.L. & Cromroy, H.L. 1976. Biology, life table, and intrinsic rate of increase of *Tetranychus urticae*. *Ann Entomol Soc Am* **69**: 362-364.
- Chen, X., Li, S. & Aksoy, S. 1999. Concordant evolution of a symbiont with its host insect species: molecular phylogeny of genus *Glossina* and its bacteriome-associated endosymbiont, *Wigglesworthia glossinidia*. *J Mol Evol* **48**: 49-58.
- Christe, P., Oppliger, A. & Richner, H. 1994. Ectoparasite affects choice and use of roost sites in the great tit, *Parus major*. *Anim Behav* **47**: 895-898.

General introduction

- Chung, S.H., Rosa, C., Scully, E.D., Peiffer, M., Tooker, J.F., Hoover, K., Luthe, D.S. & Felton, G.W. 2013. Herbivore exploits orally secreted bacteria to suppress plant defenses. *Proc Natl Acad Sci USA* **110**: 15728-15733.
- Clutton-Brock, T.H. 1984. Reproductive effort and terminal investment in iteroparous animals. *Am Nat*: 212-229.
- Creighton, J.C., Heflin, N.D. & Belk, M.C. 2009. Cost of reproduction, resource quality, and terminal investment in a burying beetle. *Am Nat* **174**: 673-684.
- Dale, C. & Moran, N.A. 2006. Molecular interactions between bacterial symbionts and their hosts. *Cell* **126**: 453-465.
- Davis, M.M. & Engström, Y. 2012. Immune response in the barrier epithelia: lessons from the fruit fly *Drosophila melanogaster*. *J Innate Immun* **4**: 273-283.
- Dillon, R. & Dillon, V. 2004. The gut bacteria of insects: nonpathogenic interactions. *Annu Rev Entomol* **49**: 71-92.
- Douglas, A. 2009. The microbial dimension in insect nutritional ecology. *Funct Ecol* **23**: 38-47.
- Dunn, P.E. 1990. Humoral immunity in insects. *BioScience*: 738-744.
- Duron, O., Bouchon, D., Boutin, S., Bellamy, L., Zhou, L., Engelstädter, J. & Hurst, G.D. 2008. The diversity of reproductive parasites among arthropods: *Wolbachia* do not walk alone. *BMC Biol* **6**: 27.
- Engel, P. & Moran, N.A. 2013. The gut microbiota of insects—diversity in structure and function. *FEMS Microbiol Rev* **37**: 699-735.
- Engelstädter, J. & Hurst, G.D. 2009. The ecology and evolution of microbes that manipulate host reproduction. *Annu Rev Ecol Evol Syst* **40**: 127-149.
- Enigl, M. & Schausberger, P. 2007. Incidence of the endosymbionts *Wolbachia*, *Cardinium* and *Spiroplasma* in phytoseiid mites and associated prey. *Exp Appl Acarol* **42**: 75-85.
- Erb, M., Meldau, S. & Howe, G.A. 2012. Role of phytohormones in insect-specific plant reactions. *Trends Plant Sci* **17**: 250-259.
- Farthing, M.J. 2004. Bugs and the gut: an unstable marriage. *Best Pract Res Clin Gastroenterol* **18**: 233-239.
- Feldhaar, H. 2011. Bacterial symbionts as mediators of ecologically important traits of insect hosts. *Ecol Entomol* **36**: 533-543.
- Fitt, G.P. 1989. The ecology of *Heliothis* species in relation to agroecosystems. *Annu Rev Entomol* **34**: 17-53.
- French, S.S., DeNardo, D.F. & Moore, M.C. 2007. Trade-offs between the reproductive and immune systems: facultative responses to resources or obligate responses to reproduction? *Am Nat* **170**: 79-89.
- Fry, A., Palmer, M. & Rand, D. 2004. Variable fitness effects of *Wolbachia* infection in *Drosophila melanogaster*. *Heredity* **93**: 379-389.
- Fukatsu, T. & Hosokawa, T. 2002. Capsule-transmitted gut symbiotic bacterium of the Japanese common plataspid stinkbug, *Megacopta punctatissima*. *Appl Environ Microbiol* **68**: 389-396.
- Fürnkranz, M., Wanek, W., Richter, A., Abell, G., Rasche, F. & Sessitsch, A. 2008. Nitrogen fixation by phyllosphere bacteria associated with higher plants and their colonizing epiphytes of a tropical lowland rainforest of Costa Rica. *ISME J* **2**: 561-570.
- Fye, R.E. & McAda, W. 1972. *Laboratory studies on the development, longevity, and fecundity of six lepidopterous pests of cotton in Arizona*. Agricultural Research Service, US Dept. of Agriculture.
- Galán, J.E. 2009. Common themes in the design and function of bacterial effectors. *Cell Host Microbe* **5**: 571-579.
- Glazebrook, J. 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu Rev Phytopathol* **43**: 205-227.

- Gotoh, T., Noda, H. & Hong, X. 2003. *Wolbachia* distribution and cytoplasmic incompatibility based on a survey of 42 spider mite species (Acari: Tetranychidae) in Japan. *Heredity* **91**: 208-216.
- Gotoh, T., Noda, H. & Ito, S. 2007a. *Cardinium* symbionts cause cytoplasmic incompatibility in spider mites. *Heredity* **98**: 13-20.
- Gotoh, T., Sugasawa, J., Noda, H. & Kitashima, Y. 2007b. *Wolbachia*-induced cytoplasmic incompatibility in Japanese populations of *Tetranychus urticae* (Acari: Tetranychidae). *Experimental and Applied Acarology* **42**: 1-16.
- Grbic, M., Van Leeuwen, T., Clark, R.M., Rombauts, S., Rouze, P., Grbic, V., Osborne, E.J., Dermauw, W., Thi Ngoc, P.C., Ortego, F., Hernandez-Crespo, P., Diaz, I., Martinez, M., Navajas, M., Sucena, E., Magalhaes, S., Nagy, L., Pace, R.M., Djuranovic, S., Smagghe, G., Iga, M., Christiaens, O., Veenstra, J.A., Ewer, J., Villalobos, R.M., Hutter, J.L., Hudson, S.D., Velez, M., Yi, S.V., Zeng, J., Pires-daSilva, A., Roch, F., Cazaux, M., Navarro, M., Zhurov, V., Acevedo, G., Bjelica, A., Fawcett, J.A., Bonnet, E., Martens, C., Baele, G., Wissler, L., Sanchez-Rodriguez, A., Tirry, L., Blais, C., Demeestere, K., Henz, S.R., Gregory, T.R., Mathieu, J., Verdon, L., Farinelli, L., Schmutz, J., Lindquist, E., Feyerisen, R. & Van de Peer, Y. 2011. The genome of *Tetranychus urticae* reveals herbivorous pest adaptations. *Nature* **479**: 487-492.
- Grenfell, B.T. & Dobson, A.P. 1995. *Ecology of infectious diseases in natural populations*. Cambridge University Press, Cambridge, UK.
- Groot, A.T., Inglis, O., Bowdridge, S., Santangelo, R.G., Blanco, C., Lopez Jr, J.D., Vargas, A.T., Gould, F. & Schal, C. 2009. Geographic and temporal variation in moth chemical communication. *Evolution* **63**: 1987-2003.
- Groot, A.T., Schöfl, G., Inglis, O., Donnerhacke, S., Classen, A., Schmalz, A., Santangelo, R.G., Emerson, J., Gould, F. & Schal, C. 2014. Within-population variability in a moth sex pheromone blend: genetic basis and behavioural consequences. *Proc R Soc B Biol Sci* **281**: 20133054.
- Gustafsson, L., Nordling, D., Andersson, M., Sheldon, B. & Qvarnstrom, A. 1994. Infectious diseases, reproductive effort and the cost of reproduction in birds. *Philos Trans R Soc B Biol Sci* **346**: 323-331.
- Hansen, A.K. & Moran, N.A. 2014. The impact of microbial symbionts on host plant utilization by herbivorous insects. *Mol Ecol* **23**: 1473-1496.
- Hanssen, S.A., Hasselquist, D., Folstad, I. & Erikstad, K.E. 2005. Cost of reproduction in a long-lived bird: incubation effort reduces immune function and future reproduction. *Proc R Soc B Biol Sci* **272**: 1039-1046.
- Hedges, L.M., Brownlie, J.C., O'Neill, S.L. & Johnson, K.N. 2008. *Wolbachia* and virus protection in insects. *Science* **322**: 702-702.
- Himler, A.G., Adachi-Hagimori, T., Bergen, J.E., Kozuch, A., Kelly, S.E., Tabashnik, B.E., Chiel, E., Duckworth, V.E., Dennehy, T.J. & Zchori-Fein, E. 2011. Rapid spread of a bacterial symbiont in an invasive whitefly is driven by fitness benefits and female bias. *Science* **332**: 254-256.
- Hoffmann, A.A., Hercus, M. & Dagher, H. 1998. Population dynamics of the *Wolbachia* infection causing cytoplasmic incompatibility in *Drosophila melanogaster*. *Genetics* **148**: 221-231.
- Hosokawa, T., Kikuchi, Y., Shimada, M. & Fukatsu, T. 2007. Obligate symbiont involved in pest status of host insect. *Proc R Soc B Biol Sci* **274**: 1979-1984.
- Hosokawa, T., Koga, R., Kikuchi, Y., Meng, X.-Y. & Fukatsu, T. 2010. *Wolbachia* as a bacteriocyte-associated nutritional mutualist. *Proc Natl Acad Sci USA* **107**: 769-774.
- Howe, G.A., Lightner, J. & Ryan, C. 1996. An octadecanoid pathway mutant (JL5) of tomato is compromised in signaling for defense against insect attack. *Plant Cell* **8**: 2067-2077.
- Huffman, M.A., Page, J.E., Sukhdeo, M.V., Gotoh, S., Kalunde, M.S., Chandrasiri, T. & Towers, G.N. 1996. Leaf-swallowing by chimpanzees: a behavioral adaptation for the control of strongyle nematode infections. *Int J Primatol* **17**: 475-503.

General introduction

- Huigens, M., De Almeida, R., Boons, P., Luck, R. & Stouthamer, R. 2004. Natural interspecific and intraspecific horizontal transfer of parthenogenesis-inducing *Wolbachia* in *Trichogramma* wasps. *Proc R Soc B Biol Sci* **271**: 509-515.
- Hutchings, M.R., Kyriazakis, I. & Gordon, I.J. 2001. Herbivore physiological state affects foraging trade-off decisions between nutrient intake and parasite avoidance. *Ecology* **82**: 1138-1150.
- Janeway, C.A. 1992. The immune system evolved to discriminate infectious nonself from noninfectious self. *Immunol Today* **13**: 11-16.
- Javoiš, J. & Tammaru, T. 2004. Reproductive decisions are sensitive to cues of life expectancy: the case of a moth. *Anim Behav* **68**: 249-255.
- Kaiser, W., Huguet, E., Casas, J., Commin, C. & Giron, D. 2010. Plant green-island phenotype induced by leaf-miners is mediated by bacterial symbionts. *Proc R Soc B Biol Sci*: rspb20100214.
- Kant, M.R., Ament, K., Sabelis, M.W., Haring, M.A. & Schuurink, R.C. 2004. Differential timing of spider mite-induced direct and indirect defenses in tomato plants. *Plant Physiol* **135**: 483-495.
- Kant, M.R., Sabelis, M.W., Haring, M.A. & Schuurink, R.C. 2008. Intraspecific variation in a generalist herbivore accounts for differential induction and impact of host plant defences. *Proc R Soc B Biol Sci* **275**: 443-452.
- Karban, R. & Baldwin, I.T. 2007. *Induced responses to herbivory*. University of Chicago Press.
- Kavaliers, M. & Colwell, D.D. 1995. Discrimination by female mice between the odours of parasitized and non-parasitized males. *Proc R Soc B Biol Sci* **261**: 31-35.
- Kikuchi, Y., Hosokawa, T. & Fukatsu, T. 2011. Specific developmental window for establishment of an insect-microbe gut symbiosis. *Appl Environ Microbiol* **77**: 4075-4081.
- Kikuchi, Y., Hayatsu, M., Hosokawa, T., Nagayama, A., Tago, K. & Fukatsu, T. 2012. Symbiont-mediated insecticide resistance. *Proc Natl Acad Sci USA* **109**: 8618-8622.
- Koch, H. & Schmid-Hempel, P. 2011. Socially transmitted gut microbiota protect bumble bees against an intestinal parasite. *Proc Natl Acad Sci USA* **108**: 19288-19292.
- Kraaijeveld, A. & Godfray, H. 1997. Trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. *Nature* **389**: 278-280.
- Kraaijeveld, A.R. & Wertheim, B. 2009. Costs and genomic aspects of *Drosophila* immunity to parasites and pathogens. In: *Insect Infection and Immunity* (J. Rolff & C. Reynolds, eds), pp. 187-205. Oxford University Press, Oxford.
- Krams, I., Kecko, S., Kangassalo, K., Moore, F.R., Jankevics, E., Inashkina, I., Krama, T., Lietuviētis, V., Meija, L. & Rantala, M.J. 2014. Effects of food quality on trade-offs among growth, immunity and survival in the greater wax moth *Galleria mellonella*. *Insect Sci* **22**: 431-439.
- Kuraishi, T., Binggeli, O., Oputa, O., Buchon, N. & Lemaître, B. 2011. Genetic evidence for a protective role of the peritrophic matrix against intestinal bacterial infection in *Drosophila melanogaster*. *Proc Natl Acad Sci USA* **108**: 15966-15971.
- Lavine, M.D. & Strand, M.R. 2002. Insect hemocytes and their role in immunity. *Insect Biochem Mol Biol* **32**: 1295-1309.
- Lefèvre, T., Oliver, L., Hunter, M.D. & De Roode, J.C. 2010. Evidence for trans-generational medication in nature. *Ecol Lett* **13**: 1485-1493.
- Lehane, M. 1997. Peritrophic matrix structure and function. *Annu Rev Entomol* **42**: 525-550.
- Li, C., Williams, M.M., Loh, Y.-T., Lee, G.I. & Howe, G.A. 2002. Resistance of cultivated tomato to cell content-feeding herbivores is regulated by the octadecanoid-signaling pathway. *Plant Physiol* **130**: 494-503.
- Manson, J.S., Otterstatter, M.C. & Thomson, J.D. 2010. Consumption of a nectar alkaloid reduces pathogen load in bumble bees. *Oecol* **162**: 81-89.

- Marsh, S.E., Poulsen, M., Pinto-Tomás, A. & Currie, C.R. 2014. Interaction between workers during a short time window is required for bacterial symbiont transmission in *Acromyrmex* leaf-cutting ants. *PLoS ONE* **9**: e103269.
- Martinson, V.G., Moy, J. & Moran, N.A. 2012. Establishment of characteristic gut bacteria during development of the honeybee worker. *Appl Environ Microbiol* **78**: 2830-2840.
- Mason, C.J., Couture, J.J. & Raffa, K.F. 2014. Plant-associated bacteria degrade defense chemicals and reduce their adverse effects on an insect defoliator. *Oecol* **175**: 901-910.
- McKean, K.A., Yourth, C.P., Lazzaro, B.P. & Clark, A.G. 2008. The evolutionary costs of immunological maintenance and deployment. *BMC Evol Biol* **8**: 76.
- Medzhitov, R. & Janeway, C.A. 1997. Innate immunity: the virtues of a nonclonal system of recognition. *Cell* **91**: 295-298.
- Migeon, A., Nouguié, E. & Dorkeld, F. 2011. Spider Mites Web: a comprehensive database for the Tetranychidae. In: *Trends in Acarology* (M.W. Sabelis & J. Bruin, eds), pp. 557-560. Springer, The Netherlands.
- Minchella, D.J. & Loverde, P.T. 1981. A cost of increased early reproductive effort in the snail *Biomphalaria glabrata*. *Am Nat* **118**: 876-881.
- Moran, N.A. & Baumann, P. 2000. Bacterial endosymbionts in animals. *Curr Opin Microbiol* **3**: 270-275.
- Moran, N.A. & Wernegreen, J.J. 2000. Lifestyle evolution in symbiotic bacteria: insights from genomics. *Trends Ecol Evol* **15**: 321-326.
- Moran, N.A., McCutcheon, J.P. & Nakabachi, A. 2008. Genomics and evolution of heritable bacterial symbionts. *Annu Rev Genet* **42**: 165-190.
- Moreira, L.A., Iturbe-Ormaetxe, I., Jeffery, J.A., Lu, G., Pyke, A.T., Hedges, L.M., Rocha, B.C., Hall-Mendelin, S., Day, A. & Riegler, M. 2009. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and *Plasmodium*. *Cell* **139**: 1268-1278.
- Moret, Y. & Schmid-Hempel, P. 2000. Survival for immunity: the price of immune system activation for bumblebee workers. *Science* **290**: 1166-1168.
- Morgan, A. & Chamberlin, F.S. 1927. tobacco budworm and its control in the Georgia and Florida tobacco-growing region. *USDA Farmer's Bull* **1531**: 1-91.
- Munson, M., Baumann, P., Clark, M., Baumann, L., Moran, N., Voegtlin, D. & Campbell, B. 1991. Evidence for the establishment of aphid-eubacterium endosymbiosis in an ancestor of four aphid families. *J Bacteriol* **173**: 6321-6324.
- Nappi, A. & Vass, E. 1993. Melanogenesis and the generation of cytotoxic molecules during insect cellular immune reactions. *Pigm Cell Res* **6**: 117-126.
- Nappi, A. & Vass, E. 1998. Hydrogen peroxide production in immune-reactive *Drosophila melanogaster*. *J Parasitol* **84**: 1150-1157.
- Nappi, A. & Christensen, B. 2005. Melanogenesis and associated cytotoxic reactions: applications to insect innate immunity. *Insect Biochem Mol Biol* **35**: 443-459.
- Nappi, A.J., Vass, E., Frey, F. & Carton, Y. 1995. Superoxide anion generation in *Drosophila* during melanotic encapsulation of parasites. *Eur J Cell Biol* **68**: 450-456.
- Neunzig, H. 1969. Biology of the tobacco budworm and the corn earworm in North Carolina. *North Carolina Agr. Exp. Sta. Tech. Bull.* **196**: 1-76.
- Nikoh, N., Hosokawa, T., Moriyama, M., Oshima, K., Hattori, M. & Fukatsu, T. 2014. Evolutionary origin of insect-*Wolbachia* nutritional mutualism. *Proc Natl Acad Sci USA* **111**: 10257-10262.
- Oliver, K.M., Russell, J.A., Moran, N.A. & Hunter, M.S. 2003. Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proc Natl Acad Sci USA* **100**: 1803-1807.
- Oliver, K.M., Degnan, P.H., Burke, G.R. & Moran, N.A. 2010. Facultative symbionts in aphids and the horizontal transfer of ecologically important traits. *Annu Rev Entomol* **55**: 247-266.

General introduction

- Orr, M.R. 1992. Parasitic flies (Diptera: Phoridae) influence foraging rhythms and caste division of labor in the leaf-cutter ant, *Atta cephalotes* (Hymenoptera: Formicidae). *Behav Ecol Sociobiol* **30**: 395-402.
- Part, T., Gustafsson, L. & Moreno, J. 1992. 'Terminal investment' and a sexual conflict in the collared flycatcher (*Ficedula albicollis*). *Am Nat* **140**: 868-882.
- Penn, D. & Potts, W.K. 1998. Chemical signals and parasite-mediated sexual selection. *Trends Ecol Evol* **13**: 391-396.
- Penn, D., Schneider, G., White, K., Slev, P. & Potts, W. 1998. Influenza infection neutralizes the attractiveness of male odour to female mice (*Mus musculus*). *Ethol* **104**: 685-694.
- Perrot-Minnot, M.J., Cheval, B., Migeon, A. & Navajas, M. 2002. Contrasting effects of *Wolbachia* on cytoplasmic incompatibility and fecundity in the haplodiploid mite *Tetranychus urticae*. *J Evol Biol* **15**: 808-817.
- Pieterse, C.M., Van der Does, D., Zamioudis, C., Leon-Reyes, A. & Van Wees, S.C. 2012. Hormonal modulation of plant immunity. *Annu Rev Cell Dev Biol* **28**: 489-521.
- Poulin, R. 2007. *Evolutionary ecology of parasites*. Princeton University Press, New Jersey.
- Powell, J.E., Martinson, V.G., Urban-Mead, K. & Moran, N.A. 2014. Routes of acquisition of the gut microbiota of the honey bee *Apis mellifera*. *Appl Environ Microbiol* **80**: 7378-7387.
- Proshold, F., Karpenko, C. & Graham, C. 1982. Egg production and oviposition in the tobacco budworm: effect of age at mating. *Ann Entomol Soc Am* **75**: 51-55.
- Ramaswamy, S.B. 1990. Periodicity of oviposition, feeding, and calling by mated female *Heliothis virescens* in a field cage. *J Insect Behav* **3**: 417-427.
- Reeves, E.P., Lu, H., Jacobs, H.L., Messina, C.G., Bolsover, S., Gabella, G., Potma, E.O., Warley, A., Roes, J. & Segal, A.W. 2002. Killing activity of neutrophils is mediated through activation of proteases by K⁺ flux. *Nature* **416**: 291-297.
- Roff, D. & Fairbairn, D. 2007. The evolution of trade-offs: where are we? *J Evol Biol* **20**: 433-447.
- Rolff, J. 2002. Bateman's principle and immunity. *Proc R Soc B Biol Sci* **269**: 867-872.
- Rolff, J. & Siva-Jothy, M.T. 2002. Copulation corrupts immunity: a mechanism for a cost of mating in insects. *Proc Natl Acad Sci USA* **99**: 9916-9918.
- Rosenberg, E., Sharon, G. & Zilber-Rosenberg, I. 2009. The hologenome theory of evolution contains Lamarckian aspects within a Darwinian framework. *Environ Microbiol* **11**: 2959-2962.
- Royet, J. 2004. Infectious non-self recognition in invertebrates: lessons from *Drosophila* and other insect models. *Mol Immunol* **41**: 1063-1075.
- Ruby, E., Henderson, B. & McFall-Ngai, M. 2004. We get by with a little help from our (little) friends. *Science* **303**: 1305-1307.
- Rupley, J. 1967. The binding and cleavage by lysozyme of N-acetylglucosamine oligosaccharides. *Proc R Soc B Biol Sci* **167**: 416-428.
- Russell, J.A., Moreau, C.S., Goldman-Huertas, B., Fujiwara, M., Lohman, D.J. & Pierce, N.E. 2009. Bacterial gut symbionts are tightly linked with the evolution of herbivory in ants. *Proc Natl Acad Sci USA* **106**: 21236-21241.
- Salem, H., Kreutzer, E., Sudakaran, S. & Kaltenpoth, M. 2013. Actinobacteria as essential symbionts in firebugs and cotton stainers (Hemiptera, Pyrrhocoridae). *Environ Microbiol* **15**: 1956-1968.
- Salem, H., Bauer, E., Strauss, A.S., Vogel, H., Marz, M. & Kaltenpoth, M. 2014. Vitamin supplementation by gut symbionts ensures metabolic homeostasis in an insect host. *Proc R Soc B Biol Sci* **281**: 20141838.
- Salem, H., Florez, L., Gerardo, N. & Kaltenpoth, M. 2015. An out-of-body experience: the extracellular dimension for the transmission of mutualistic bacteria in insects. *Proc R Soc B Biol Sci* **282**: 20142957.
- Schmid-Hempel, P. 2005. Evolutionary ecology of insect immune defenses. *Annu Rev Entomol* **50**: 529-551.

- Schmid-Hempel, P. 2011. *Evolutionary parasitology: the integrated study of infections, immunology, ecology, and genetics*. Oxford University Press, New York.
- Schoonhoven, L.M., van Loon, J.J.A. & Dicke, M. 2005. *Insect-Plant Biology, 2nd edn*. Oxford University Press, Oxford.
- Shade, A. & Handelsman, J. 2012. Beyond the Venn diagram: the hunt for a core microbiome. *Environ Microbiol* **14**: 4-12.
- Sheck, A. & Gould, F. 1993. The genetic basis of host range in *Heliothis virescens*: larval survival and growth. *Entomol Exp Appl* **69**: 157-172.
- Sheldon, B.C. & Verhulst, S. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends Ecol Evol* **11**: 317-321.
- Shigenobu, S., Watanabe, H., Hattori, M., Sakaki, Y. & Ishikawa, H. 2000. Genome sequence of the endocellular bacterial symbiont of aphids *Buchnera* sp. APS. *Nature* **407**: 81-86.
- Singer, M.S., Mace, K.C. & Bernays, E.A. 2009. Self-medication as adaptive plasticity: increased ingestion of plant toxins by parasitized caterpillars. *PLoS ONE* **4**: e4796-e4796.
- Söderhäll, K. & Cerenius, L. 1998. Role of the prophenoloxidase-activating system in invertebrate immunity. *Curr Opin Immunol* **10**: 23-28.
- Soler, J.J., de Neve, L., Pérez-Contreras, T., Soler, M. & Sorci, G. 2003. Trade-off between immunocompetence and growth in magpies: an experimental study. *Proc R Soc B Biol Sci* **270**: 241-248.
- Stadelbacher, E. 1981. Role of early-season wild and naturalized host plants in the buildup of the F1 generation of *Heliothis zea* and *H. virescens* in the Delta of Mississippi. *Environ Entomol* **10**: 766-770.
- Stearns, S.C. 1989. Trade-offs in life-history evolution. *Funct Ecol* **3**: 259-268.
- Stouthamer, R., Breeuwer, J.A. & Hurst, G.D. 1999. *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. *Annu Rev Microbiol* **53**: 71-102.
- Strand, M.R. 2008. The insect cellular immune response. *Insect Sci* **15**: 1-14.
- Stuart, L., Boulais, J., Charriere, G., Hennessy, E., Brunet, S., Jutras, I., Goyette, G., Rondeau, C., Letarte, S. & Huang, H. 2007. A systems biology analysis of the *Drosophila* phagosome. *Nature* **445**: 95-101.
- Stuart, L.M. & Ezekowitz, R.A.B. 2005. Phagocytosis: elegant complexity. *Immunity* **22**: 539-550.
- Su, Q., Oliver, K.M., Xie, W., Wu, Q., Wang, S. & Zhang, Y. 2015. The whitefly-associated facultative symbiont *Hamiltonella defensa* suppresses induced plant defences in tomato. *Funct Ecol* **29**: 1007-1018.
- Sy, A., Timmers, A.C., Knief, C. & Vorholt, J.A. 2005. Methylothetic metabolism is advantageous for *Methylobacterium extorquens* during colonization of *Medicago truncatula* under competitive conditions. *Appl Environ Microbiol* **71**: 7245-7252.
- Teixeira, L., Ferreira, Á. & Ashburner, M. 2008. The bacterial symbiont *Wolbachia* induces resistance to RNA viral infections in *Drosophila melanogaster*. *PLoS Biol*: e1000002
- Thaler, J.S., Farag, M.A., Paré, P.W. & Dicke, M. 2002. Jasmonate-deficient plants have reduced direct and indirect defences against herbivores. *Ecol Lett* **5**: 764-774.
- Thaler, J.S., Humphrey, P.T. & Whiteman, N.K. 2012. Evolution of jasmonate and salicylate signal crosstalk. *Trends Plant Sci* **17**: 260-270.
- Tsuchida, T., Koga, R., Matsumoto, S. & Fukatsu, T. 2011. Interspecific symbiont transfection confers a novel ecological trait to the recipient insect. *Biol Lett* **7**: 245-248.
- Vala, F., Breeuwer, J.A. & Sabelis, M.W. 2000. *Wolbachia*-induced 'hybrid breakdown' in the two-spotted spider mite *Tetranychus urticae* Koch. *Proc R Soc B Biol Sci* **267**: 1931-1937.
- van Ham, R.C., Kamerbeek, J., Palacios, C., Rausell, C., Abascal, F., Bastolla, U., Fernández, J.M., Jiménez, L., Postigo, M. & Silva, F.J. 2003. Reductive genome evolution in *Buchnera aphidicola*. *Proc Natl Acad Sci USA* **100**: 581-586.

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- Vetter, R.S. & Baker, T.C. 1983. Behavioral responses of male *Heliothis virescens* in a sustained-flight tunnel to combinations of seven compounds identified from female sex pheromone glands. *J Chem Ecol* **9**: 747-759.
- Vorholt, J.A. 2012. Microbial life in the phyllosphere. *Nat Rev Microbiol* **10**: 828-840.
- Waldvogel, M. & Gould, F. 1990. Variation in oviposition preference of *Heliothis virescens* in relation to macroevolutionary patterns of Heliothine host range. *Evolution* **44**: 1326-1337.
- Walling, L.L. 2000. The myriad plant responses to herbivores. *J Plant Growth Regul* **19**: 195-216.
- Weeks, A.R., Marec, F. & Breeuwer, J.A. 2001. A mite species that consists entirely of haploid females. *Science* **292**: 2479-2482.
- Werren, J.H. 1997. Biology of *Wolbachia*. *Annu Rev Entomol* **42**: 587-609.
- Werren, J.H., Baldo, L. & Clark, M.E. 2008. *Wolbachia*: master manipulators of invertebrate biology. *Nat Rev Microbiol* **6**: 741-751.
- Willers, J., Schneider, J. & Ramaswamy, S. 1987. Fecundity, longevity and caloric patterns in female *Heliothis virescens*: changes with age due to flight and supplemental carbohydrate. *J Insect Physiol* **33**: 803-808.
- Williams, G.C. 1966. Natural selection, the costs of reproduction, and a refinement of Lack's principle. *Am Nat* **100**: 687-690.
- Xie, J., Vilchez, I. & Mateos, M. 2010. *Spiroplasma* bacteria enhance survival of *Drosophila hydei* attacked by the parasitic wasp *Leptopilina heterotoma*. *PLoS ONE* **5**: e12149.
- Xie, J., Butler, S., Sanchez, G. & Mateos, M. 2014. Male killing *Spiroplasma* protects *Drosophila melanogaster* against two parasitoid wasps. *Heredity* **112**: 399-408.
- Xie, R.-R., Chen, X.-L. & Hong, X.-Y. 2011. Variable fitness and reproductive effects of *Wolbachia* infection in populations of the two-spotted spider mite *Tetranychus urticae* Koch in China. *Appl Entomol Zool* **46**: 95-102.
- Zasloff, M. 2002. Antimicrobial peptides of multicellular organisms. *Nature* **415**: 389-395.
- Zhang, Y.K., Ding, X.L., Rong, X. & Hong, X.Y. 2015. How do hosts react to endosymbionts? A new insight into the molecular mechanisms underlying the *Wolbachia*-host association. *Insect Mol Biol* **24**: 1-12.
- Zhang, Z.T. & Zhu, S.Y. 2009. Drosomycin, an essential component of antifungal defence in *Drosophila*. *Insect Mol Biol* **18**: 549-556.
- Zhu, F., Poelman, E.H. & Dicke, M. 2014. Insect herbivore-associated organisms affect plant responses to herbivory. *New Phytol* **204**: 315-321.
- Zilber-Rosenberg, I. & Rosenberg, E. 2008. Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiol Rev* **32**: 723-735.
- Zug, R. & Hammerstein, P. 2015. Bad guys turned nice? A critical assessment of *Wolbachia* mutualisms in arthropod hosts. *Biol Rev* **90**: 89-111.
- Zuk, M. & Stoehr, A.M. 2002. Immune defense and host life history. *Am Nat* **160**: S9-S22.