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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; Javošič & 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 (Rolf, 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*).

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