Mites and endosymbionts –

towards improved biological control

Thèse de doctorat

présentée par

Renate Zindel

Université de Neuchâtel, Suisse, 16.12.2012

Cover photo: *Hypoaspis miles (Stratiolaelaps scimitus)*

UNIVERSITÉ DE
NEUCHÂTEL

FACULTE DES SCIENCES
Secrétariat-Décanat de la faculté
Rue Emile-Argand 11
CH-2000 Neuchâtel

IMPRIMATUR POUR LA THESE

Mites and endosymbionts – towards improved biological control

Renate ZINDEL

UNIVERSITE DE NEUCHATEL

FACULTE DES SCIENCES

La Faculté des sciences de l'Université de Neuchâtel autorise l'impression de la présente thèse

sur le rapport des membres du jury :

Prof. Ted Turlings, Université de Neuchâtel, directeur de thèse
Dr Alexandre Aebi (co-directeur de thèse), Université de Neuchâtel
Prof. Pilar Junier (Université de Neuchâtel)
Prof. Christoph Vorburger (ETH Zürich, EAWAG, Dübendorf)

Le doyen

Prof. Peter Kropf

Neuchâtel, le 18 décembre 2012

Téléphone : +41 32 718 21 00 E-mail : secretariat.sciences@unine.ch www.unine.ch/sciences

Index

Foreword

The work presented here is the result of my PhD dissertation conducted at the Swiss federal research station Agroscope Reckenholz-Tänikon and at the University of Neuchâtel.

It is structured in the following way: The **first chapter** is an overview of the current state of the research on mites and their associated bacteria, in the context of biological control. Based on this review, I conclude that mites and their endosymbionts (ES) have been studied, but the focus has clearly been on their presence and distribution andonparticular species such as the spider mite *Tetranychus urticae*. In contrast, many other mite families and the further characterization of the effects ES can have on their hosts, have received less attention. We would like to publish this chapter, adapted and changed, as a review.

The **second chapter** summarizes the different ways microorganisms associated with arthropods can have an influence on biological pest- and disease vector control, through the many ways they can be involved in their host's biology. The review, which was published in *the Journal of Applied Ecology* in 2011, provides practical recommendations on the handling of ES in biological control agents and pests.

In the **third chapter**an extended range of mites was screened for known endosymbionts. Using a PCR-based approach, we screened 21 phytophagous, predatory or parasitic mite species for *Wolbachia* spp*., Cardinium* spp*., Rickettsia* spp*., Flavobacterium* spp*., Arsenophonus* spp. and *Spiroplasma* spp.. Results are discussed in the context of their importance for biological control with and of mites. This chapter will be submitted to *Biological Control*.

The **fourth chapter** is dedicated to the mite *Rhizoglyphus robini* and its microbiome. The aim of this study was to shed light on the mite's microbiome in order to better understand the pest's biology. The plant pest *R. robini* had previously been observed to feed mainly on fungus infected plants and actively chose this substrate. We demonstrated chitinolytic activity of mite homogenate and linked it

to associated bacteria. The mite's microbiome was described through a metagenomic analysis. The bacterial involvement in the ability to digest chitin was then demonstrated in a series of bio-assays. These results will enable us to better understand the biology of this pest mite. This chapter we will re-submit to the *Faseb-Journal*.

The **fifth chapter** is a continuation of the fourth chapter. We isolated a chitinolytic bacterium associated with *Rhizoglyphus robini* and identified it by 16S sequencing as *Serratia marcescens*. We aim at publishing this chapter in *Symbiosis*.

Summary

The microbiome associated with arthropods is very diverse and may significantly influence the biology of biological biocontrol agents and arthropod pests. Some endosymbiont bacteria may even be usedto achieve pest or disease vector biological control.

The subclass Acari (mites and ticks) is very diverse in form, habitat and behaviour. Mites can be plant pests, parasites of domestic animals and humans or predators of major crop-pests.

The aim of this first chapter was to collect and present an overview of the present literature and knowledge on the prevalence and distribution of maternally-inherited ES (*Arsenophonus* spp*., Cardinium* spp*., Flavobacteria* spp*., Rickettsia* spp*., Spiroplasma* spp*.* and *Wolbachia* spp.) in mite species (chapter 1). We completed this knowledge by adding the results of our own screening (chapter 3), including mite species of three different lifestyles (herbivorous, predatory and parasitic). We found that mites and their ES have been studied, but the focus has clearly been on presence and distribution of insect ES in spider mites, whereas many other mite families as well as the further characterization of effects of ES on their hosts have received less attention. As a next step we describe the different ways microorganisms associated with arthropods can have an influence on biological pest- and disease vector control, through the many ways they can be involved in their hosts biology and we then provide practical recommendations on the handling of ES in biological control agents and pests (chapter 2).

The bulb mite *Rhizoglyhus robini* is below-ground pest mostly on Liliacean crop plants, which has been observed mainly on plants already infested with a fungus. As not many animals carry the enzymatic machinery to digest fungal carbohydrates, we tested the hypothesis that associated bacteria are involved in the chitin digetion. Preliminary DGGE assessment with general bacterial primers, revealed a high diversity. Investigating the bacterial community associated with this mite in more detail by 454 metagenomic analysis, we found several genera which contain chitin-degrading species. Bioassays confirmed the mite's preference for - and high fitness on - a fungal food source. Finally we could make the link between the fungal food source of the mite and the bacterial

chitinolytic ability by demonstrating the digestion of chitin by mite homogenate and isolating bacteria from the chitin-free zones (chapter 4). We molecularly identified these isolated clones as *Serratia marcescens* (chapter 5), a bacterium well known for its chitinolytic machinery.

The biology of *Rhizoglyphus robini* can be understood only if the associated bacteria are also considered. The mites' status has to be reassessed now, as it was previously described as a primary pest, feeding on the bulbs and tubers of plants. If the mite attacks plants preferentially after previous plant- infection with a fungus, this could be exploited in biological control. The fact that the mite might need the bacteria to digest the fungus also opens up new possibilities for control of the mite. We believe that the bacterial community in most pest and beneficial mites will deliver valuable information, which can be used in the development of new control strategies or to explain and potentially solve problems in existing control programs (chapter 2). In chapter two we explain in detail why we recommend including an investigation of the associated microbiome in standard assessments of pests and control agents.

Zusammenfassung

Gliederfüsser (Arthropoden) beherbergen sehr diverse Bakteriengesellschaften in ihrem Innern. Sie können durch ihre Wirte grosse Auswirkungen auf die biologische Schädlingsbekämpfung haben. Vor allem dann nämlich, wenn sie ihren Wirt auf eine Weise beeinflussen, dass dieser entweder in seiner Funktion als Nützling beeinträchtigt wird oder aber, wenn sie einem Schädling zur erhöhten Resistenz gegen seine natürlichen Feinde verhelfen. Manchmal werden bakterielle Endosymbionten (ES) sogar gezielt zur Bekämpfung von Schädlingen eingesetzt.

Die Arthropoden-Untereinheit Acari (Milben und Zecken) ist sehr vielfältig in Form, Verhalten und Lebensraum. Es gibt Milben, die als Schädlinge auf Pflanzen leben, solche, die parasitisch auf Haustieren oder sogar Menschen leben und dann gibt es Milben-Nützlinge, die zur Eindämmung von Spinnmilben (Raubmilben) oder von Pflanzenschädlingen (Eriophyidae) eingesetzt werden. Milben-Symbionten wurden bisher wenig untersucht, trotz der Tatsache, dass alle Erkenntnisse aus diesem Forschungszweig für die biologischen Schädlingsbekämpfung von grossem Nutzen sein können.

Das erste Ziel der vorliegenden Arbeit bestand darin, alle vorhandenen Studien (und ihre Resultate) zum Thema zu sammeln und eine gute Übersicht über ihre Resultate zu erstellen. Wir haben alle Literatur zur Häufigkeit und Verbreitung mütterlich-vererbter Endosymbionten (*Arsenophonus* spp., *Cardinium* spp., *Flavobacteria* spp., *Rickettsia* spp., *Spiroplasma* spp. und *Wolbachia* spp.) in Milben, sowie alles, was über die Auswirkungen der ES auf ihre Wirte bekannt ist zusammengetragen (Kapitel 1 dieser Arbeit) und sie durch unsere eigenen Resultate (Kapitel 3) ergänzt. Zusammenfassend kann gesagt werden, dass es mehr Studien zu Milben-ES gibt als erwartet, dass die Forschung sich allerdings mehrheitlich darauf beschränkt, die An- bzw. Abwesenheit dieser ES in einem bestimmten Wirt festzustellen.Weitaus am besten untersucht sind die Spinnmilben und die ihnen zugehörigen Bakterien, während andere Milbenfamilien bisher wenig wissenschaftliche Aufmerksamkeit erhalten haben.

In einem nächsten Schritt haben wir an Beispielen aufgezeigt auf welche unterschiedlichen Weisen biologische Schädlingsbekämpfungsprogramme von ES beeinflusst werden können (Kapitel 2). Das

geht von unausgewogenen Geschlechterverhältnissen in den Zuchten bis zur Inkompatibilität von eingeführten Nützlingen mit den ansässigen Populationen. Auch in der Kontrolle der Vektoren von Krankheitserregern können ES-Wirkungen auf die Wirte Folgen haben. Schlussendlich empfehlen wir die Präsenz von ES in Nützlingen und auch in den Schädlingen zu untersuchen, vor allem wenn Probleme wie Resistenzen oder Zuchtschwierigkeiten auftreten (Kapitel 2).

Unsere Theorie wird praktisch untermauert mit der Untersuchung der Wurzelmilbe (*Rhizoglyphus robini*). Die Wurzelmilbe ist ein Pflanzenschädling, der vor allem an den unterirdischen Knollen von Liliengewächsen frisst. Es wurde beobachtet, dass *R. robini* meistens auf Pflanzen gefunden wird, die zusätzlichauch von einem Pilz befallen sind. Nicht vielen Tieren jedoch sind Kohlenstoffe aus Pilzen als Nahrung zugänglich, da sie nicht die richtigen Enzyme besitzen für den Verdau von Chitin. Wir haben uns nun gefragt, ob die Bakterien im Innern der Milbe bei der Verdauung eine Rolle spielen könnten. Eine Voruntersuchung (Denaturing gradient gel electrophoresis (DGGE) mit breitamplifizierenden Bakterienprimern) hatte gezeigt, dass *R. robini* eine grosse Bakterienvielfalt beherbergt. In einer mehr detaillierten Untersuchung durch 454 Sequenzierung der nächsten Generation konnten wir dann mehrere Bakteriengattungen finden, in denen Arten mit chitinolytischer Aktivität vorkommen. Mithilfe von Bio-Assays konnten wir die Vorliebe für Pilz-Nahrung und hohe Fortpflanzungsrate der Milben bestätigen. Den aktiven Chitinverdau des Milbenhomogenates wiesen wir nach, indem wir jeweils 1µl davon auf eine Chitin-beschichtete Platte gaben. Schon nach 12 Stunden bildeten sich klare Zonen, sogenannte „haloes“ (Heiligenscheine) um den Tropfen (Kapitel 4). Wir konnten Bakterien aus den klaren Zonen isolieren, kultivieren und molekular (durch Sequenzierung eines grossen 16S-Fragmentes) der Bakterienart *Serratia marcescens* zuweisen (Kapitel 5).

Man kann die Biologie von *Rhizoglyphus robini* nur verstehen, wenn man die dazugehörigen Bakterien in die Betrachtung miteinbezieht. Der Pflanzenschädlingsstatus dieser Milbe muss nun überarbeitet werden, da sie wahrscheinlich vor allem den Pilz frisst oder zumindest für eine Pflanze eine viel grössere Gefahr darstellt, wenn diese schon von einem Pilz befallen ist. Die am Beispiel der

Wurzelmilbe gewonnen Erkenntnisse sind wichtig für die weitere Forschung in der biologischen Schädlingsbekämpfung, denn sie eröffnen viele neue Möglichkeiten. Die Bakterien von Milben in der biologischen Schädlingsbekämpfung sollten untersucht werden, da sie einen grossen Einfluss auf den Erfolg der Programme haben können. Allerdings sollte der Aufwand im Verhältnis dazu stehen, welchen Schaden mögliche ES anrichten könnten oder welchen Nutzen man sich verspricht.

Résumé

Le microbiome vivant en association avec certains arthropodes est très divers et peut significativement influencer la biologie de nombreux agents de lutte biologique ou de ravageurs de culture. Certains endosymbiontes bactériens peuvent même être utilisés contre des populations de insectes ravageurs de ou de vecteurs de maladies dans un programme de lutte biologique.

La espèces de la sous-classe des Acari (acariens et tiques) sont très diverses en forme, habitats et comportements. Il y a des acariens ravageurs, des parasites d'animaux domestiques ou de l'homme et même des acariens utiles, prédateurs pouvant s'attaquer à d'autres ravageurs de culture.

Le but du premier chapitre est de faire la synthèse de la littérature concernant les symbiontes d'acariens et en particulier sur l'incidence et la prévalence d'endosymbiontes maternellement transmis comme *Arsenophonus* spp., *Cardinium* spp., *Flavobacteria* spp., *Rickettsia* spp., *Spiroplasma* spp. et *Wolbachia* spp. chez les acariens (Chapitre 1). Bien que de nombreux articles aient été publié sur ce sujet, la plupart des études portent sur la présence d'endosymbiontes chez les tétranynques alors que de nombreuses espèces d'acariens ou des études sur les effets de ces bactéries sur leurs hôtes ont reçu moins d'attention. Ces données ont ensuite été complétées par notre propre étude ou des acariens phytophages, prédateurs et parasites ont été analysés pour la présence d'endosymbiontes (Chapitre 3). L'étape suivante de cette thèse a été de décrire les différents mécanismes par lesquelles les endosymbiontes peuvent influencer le succès de programme de contrôle biologique de ravageurs de culture ou de vecteur de maladies. Nous nous sommes également penchés sur l'apport de ces connaissances afin de mieux appréhender ou comprendre la biologie de ces arthropodes. Dans le chapitre deux, nous faisons des recommandations pratiques sur les façons de prendre en compte les endosymbiontes dans le cadre de la lutte biologique (Chapitre 2).

L'acarien phytophage *Rhizoglyhus robini* s'attaque principalement aux racines de plantes cultivées de la famille des Liliacées. Bien que la plupart des animaux ne possèdent pas les enzymes nécessaires à la digestion d'hydrates de carbones d'origine fongique, cette espèce note une préférence alimentaire

pour les plantes infectées par des champignons sur lesquelles elle est principalement trouvée.Par une approche multidisciplinaire, nous avons testé l'hypothèse que le bactériome était nécessaire à l'acarien pour digerer la chitine. Une analyse génétique préliminaire réalisée avec des sondes moléculaires générales a révélé une la présence d'une riche communauté de bactéries vivant avec cet acarien. Une analyse plus détaillée de cette communauté bactérienne par une approche métagénomique (454) a révélé la présence de plusieurs genres de bactéries connue pour être capables de dégrader la chitine. Des expériences en laboratoire ont confirmé la préférence des acariens pour une source de nourriture contenant des champignons et un taux de reproduction plus élevé sur ce type de régime alimentaire. Nous avons donc pu faire le lien entre la présence des bactéries et leur activité chitinolytique en démontrant l'activité de digestion d'homogénats d'acariens ainsi que de bactéries isolées de ces derniers (4). Nous avons enfin identifié la bactérie isolée comme *Serratia marcescens* (chapitre 5), une bactérie possédant une machinerie efficace pour dégrader la chitine des champignons.

Les détails de la biologie de l'acarien *Rhizoglyphus robini* ne peuvent donc être comprise qu'en tenant en compte de la biologie des bactéries qui lui sont associées. Cette étude complète nous a amené à reconsidérer le statut de peste de l'acarien. En effet *R. robini* était considéré comme une peste primaire, se nourrissant directement sur les racines des plantes alors que maintenant il est considéré comme une peste secondaire s'attaquant préférentiellement aux plantes préalablement infectées par un champignon. Le fait que l'acarien dépende de la présence de bactéries pour pouvoir digérer les champignons ouvre la voie pour de nouvelles stratégies de lutte. Nous sommes persuadés qu'une meilleure connaissance des communautés bactériennes vivant en association avec la plupart des acariens phytophages et des acariens bénéficiaires amèneront des informations cruciales pour le développement de nouvelles stratégies de lutte ou pour mieux comprendre les problèmes dans des programmes de lutte biologique existants. Dans le chapitre deux, nous expliquons en détail pourquoi nous recommandons de se pencher sur les interactions entre les arthropodes utilisés en lutte biologique et leur microbiome (Chapitre 2).

Glossary

This glossary was created for the context of this thesis. Definitions might only be applicable for this specific context or contain information only relevant for this field.

Biological control (BC): Biological control is a method of controlling pests (including insects, mites, weeds and plant diseases) using other living organisms. It relies on predation, parasitism, herbivory, or other natural mechanisms. There are three basic types of biological pest control strategies: classical biological control, augmentation and conservation. For the context of this thesis: There are mite species, which are pests and other mite species that can be used as control agents in biological control.

Cytoplasmic incompatibility (CI): One of the reproductive manipulations known from *Wolbachia* strains to ensure their spread in a host population. Infected males and uninfected females are unable to produce viable offspring.

Disease vector: The carrier of a pathogen, while self not a target, infecting more individuals with the disease. Example: Malaria and the TseTse fly. Biological control aiming to control a pathogen often targets the vector.

Endosymbiont: An endosymbiont is a symbiont living on the inside of its host. In this thesis the term is used for bacterial symbionts of arthropod hosts such as for example *Wolbachia* spp., *Cardinium* spp. and *Flavobacterium* spp.. In the research field of bacterial endosymbionts the bacteria are often described by naming their host rather than defining the species. *Wolbachia pipientis* for example will hence often simply be referred to as "*Wolbachia,* endosymbiont of *Culex pipiens*". Most of the endosymbiotic bacteria cannot be cultured outside of their host and are therefore hard to describe other than with molecular tools. This makes it hard to describe and identify species.

Feminization: One of the reproductive manipulations known from *Wolbachia* strains to ensure their spread in a host population. Infected genetic males turn into phenotypic females.

Holobiont: Organisms can be seen as single species' or as species complexes, also called holobionts, where many species contribute to the biology of a core organism and many genomes to the phenotype.

Male Killing: Infected males are killed by the endosymbiont, supposedly because they represent a dead end and can serve as food for their newly hatched sisters. Causes female biased sex ratios in host populations. Male killing is found in lady beetles and butterflies.

Maternal inheritance: Usually information or material passed on from mother to offspring within or on the outside of the egg, but not contained in the nucleus (for example cytoplasmic components like mitochondria).

Mutualism: A type of interaction between two organisms, where both benefit in some way. The classical example is the pollination of plants by insects which are collecting the nectar in the flowers.

Parthenogenesis induction (PI): One of the reproductive manipulations known from *Wolbachia* strains to ensure their spread in a host population. Infected females can reproduce through unfertilized eggs.

Pathogen: A biological agent causing disease or illness in its host. It can be bacteria, virus, fungi, nematodes or other microbes.

Symbiont (facultative, obligatory): An organism living in symbiosis with another. There are obligatory (primary) and facultative (secondary) symbionts.
The definition of symbiosis is controversial among scientists. For this thesis we apply the term "symbiosis" to any types of persistent biological interactions (the symbiosis can be neutral, beneficial or detrimental for both parties involved).

Chapter 1

Arthropod symbioses: a neglected parameter in pest- and disease- control programmes

This article was published in the *Journal of Applied Ecology* in 2011.

Journal of Applied Ecology

British Ecological Society

Journal of Applied Ecology doi: 10.1111/j.1365-2664.2011.01984.x

REVIEW

Arthropod symbioses: a neglected parameter in pest- and disease-control programmes

Renate Zindel[1]*, Yuval Gottlieb[2] and Alexandre Aebi[1]

[1]*Agroscope Reckenholz-Tänikon, Research Station ART, Reckenholzstrasse 191, 8046 Zürich, Switzerland; and*
[2]*Koret School of Veterinary Medicine, the Hebrew University of Jerusalem, Rehovot 76100, Israel*

Summary

1. Arthropods are important players in biological control as pests, control agents and transmitters of invertebrate diseases. Arthropods are frequently infected with one or several micro-organisms, serving as micro-ecosystems in which multiple interactions can take place. These micro-organisms include disease agents and symbiotic micro-organisms. The latter are usually vertically transmitted and can have a broad spectrum of effects on their hosts, ranging from reproductive manipulations to protection against natural enemies. These interactions may directly or indirectly alter the biology of many arthropods in agriculturally, medically and ecologically relevant ecosystems.

2. Symbiotic micro-organism-induced reproductive manipulations such as cytoplasmic incompatibility and parthenogenesis induction can substantially affect the rearing of biological control agents. Many insects, and recently also mites and nematodes, have been found to be infected, displaying a wide range of effects. We discuss examples of arthropod-micro-organism interactions and effects, which could have consequences for the practical application of arthropods in biological control.

3. Symbiotic micro-organisms can also be involved in host protection against natural enemies such as parasitoids, pathogenic bacteria, fungi and viruses.

4. Symbiotic bacteria can influence the vectorial capacity of disease-vectoring arthropods and may be very helpful in decreasing the transmission of disease agents.

5. *Synthesis and applications*. The effect of micro-organisms on the outcome of biological control programmes is usually not considered in risk assessments and failure analyses. This review emphasizes the importance of endosymbiotic micro-organisms in comprehensive biological control programmes and provides recommendations on how to recognize, avoid or benefit from these influential tenants.

Key-words: arthropod biology, biological control, crop pest, disease vector, endosymbiont, reproduction manipulation, risk assessment, *Wolbachia*

Introduction

Arthropods are responsible for severe economic and ecological damage world-wide. In the United States, pest insects destroy approximately 13% of crop production, accounting for a loss of $33 billion annually (Pimentel *et al*. 2003). Emerging or re-emerging pest species, such as the Argentine ant *Linepithema humile*, can also potentially affect whole ecosystems (e.g. Jenkins, Aber & Canham 1999; Gomez & Oliveras 2003; Fowler 2004). In addition, arthropods function as disease vectors, and as ecto- and endoparasites of humans and animals, creating a world-wide health risk [World Health Organization, WHO (http://www.who.int) and World Organization for Animal Health, OIE (http://www.oie.int/eng/en_index.htm)]. A very prominent example are the hard ticks (Acari: Ixodidae), which can vector several human disease agents (Beugnet & Marie 2009) such as the bacterium *Borrelia burgdorferi*, causative agent of lyme disease and the viral agent of tick-borne encephalitis (Nazzi *et al*. 2010; Randolph 2010).

Arthropods are also the most frequently used organisms in augmentative biological control (BC; in numbers released – J. Klapwijk, pers. comm.), and most of them are hosts to one or several endosymbiotic bacteria (Zchori-Fein & Perlman 2004; Weinert *et al*. 2007; Duron *et al*. 2008; Hilgenboecker

*Correspondence author. E-mail: renate.zindel@art.admin.ch

et al. 2008). The two most studied maternally inherited bacterial endosymbionts (ESs) are *Wolbachia* and *Cardinium*, which infect 66% and 6%–7% of insect species, respectively (Hurst & Jiggins 2000; Kittayapong *et al.* 2003; Zchori-Fein & Perlman 2004). For example, recent studies on large samples of ladybirds (Coccinellidae) showed that 52% of the species were infected with either *Wolbachia*, *Rickettsia* or *Spiroplasma* (Weinert *et al.* 2007). Given that arthropods represent a large part of our planet's biomass, maternally inherited ESs are probably the most common bacteria living in association with living organisms on Earth. Endosymbiotic bacteria can be divided into obligatory (primary) and facultative (secondary) symbionts. Obligatory symbionts are involved in and sometimes solely responsible for vital functions of their host (Baumann 2005). Thus, their hosts would not survive without them. One of the most studied examples is *Buchnera aphidicola*, the primary symbiont of the pea aphid *Acyrthosiphon pisum*, providing its host with essential amino acids (Douglas 1998). Facultative ESs, on the other hand, are not essential for host survival and their presence can be neutral, beneficial or detrimental to the host (Oliver *et al.* 2003; Perotti *et al.* 2006). Facultative ESs can be involved in their hosts' feeding (Gunduz & Douglas 2009; Hosokawa *et al.* 2010), reproductive (O'Neill, Hoffmann & Werren 1997; Werren, Baldo & Clark 2008) or defence strategies (Haine 2007; Brownlie & Johnson 2009). There is growing evidence that the interactions between facultative ESs and their hosts vary with environmental stressors such as the presence or absence of a natural enemy; in extreme cases, ESs being beneficial in one situation and costly in another (Haine 2007).

To improve their own fitness, endosymbiotic bacteria have to guarantee their maintenance in a host population. Many facultative ESs are maternally inherited. As vertical transmission rates (from mother to offspring) are usually $< 100\%$, they would eventually be lost from the population in the absence of some measure of horizontal transmission (Lipsitch *et al.* 1995; Lively *et al.* 2005). To ensure its persistence in a host population, an ES can reduce the fitness of non-infected female hosts by manipulating their reproductive strategies (O'Neill, Hoffmann & Werren 1997). Such manipulations include cytoplasmic incompatibility (CI) between infected males and uninfected (or differently infected) females, selective male-killing in broods, feminization of genetic males or parthenogenesis induction.

Protecting their host against natural enemies is another strategy that allows ESs to spread in their host's population. The ES can provide the infected host with a selection advantage or increased fitness (Lively *et al.* 2005; Jones, White & Boots 2007), although surprisingly little evidence for this has been found in the field. Interestingly, some ESs have been shown to protect their hosts from predators, macroparasites (Olsen, Reynolds & Hoffmann 2001; Harcombe & Hoffmann 2004; Chiel *et al.* 2009a), bacteria and viruses (Davidson *et al.* 2001; Lopanik, Lindquist & Targett 2004; Brownlie & Johnson 2009) and to be involved in pesticide-resistance mechanisms (Kontsedalov *et al.* 2008). Such ES-mediated resistance to pathogens or chemicals can give an organism a substantial advantage over nonresistant conspecifics in an environment where biotic or abiotic antagonists are present. The mechanisms underlying the various types of resistance are often unknown even if, in some cases, the ES produces toxic compounds that affect its host's enemies (Gil-Turnes, Hay & Fenical 1989; Gil-Turnes & Fenical 1992; Kellner 2002; Oliver *et al.* 2009). In other cases, ES-induced behavioural changes in the host (Haine, Boucansaud & Rigaud 2005; Rigaud & Haine 2005), such as deterrence effects that protect the prey from its predator (Davidson *et al.* 2001; Lopanik, Lindquist & Targett 2004), have been suggested or observed.

Endosymbionts can infect any beneficial arthropod species and may dramatically affect the outcome of a BC programme. By manipulating their host's biology, they have the potential to dramatically affect all phases of BC, from the rearing of a biological control agent (BCA) to its establishment in the field. In this review, we will explain how ESs can influence their arthropod hosts, give examples of known interactions among micro-organisms and their pest or disease-vectoring hosts, explain how ESs can play a role in the context of BC systems, and give practical advice on how to seek and recognize potential ES-mediated effects. Finally, we discuss how ES–host interactions can be profitably integrated into pest- and disease-control programmes.

Direct interactions among microbes and arthropods

The outcome of a BC programme can be positively or negatively influenced by many unpredictable biotic factors, ESs being one of them. Some bacteria are known to affect their host's reproduction strategies in a wide variety of ways. They can induce parthenogenic reproduction and thereby improve their own transmission to the next generation, as for example *Wolbachia* in a phytophagous pest mite of the genus *Bryobia* (Weeks & Breeuwer 2001). Another strategy is to disable crosses between infected males and uninfected females by causing CI, such as *Cardinium* in *Encarsia pergandiella* (Perlman, Kelly & Hunter 2008). Unidirectional or bidirectional CI may also occur between host populations carrying different strains of the bacterium (Bordenstein, O'Hara & Werren 2001). In another wasp, *Encarsia hispida*, *Cardinium* can turn genetic males into females (feminization; Giorgini *et al.* 2009). In arthropod hosts that lay their eggs in batches, freshly hatched siblings are often the first food source for young larvae. Vertically transmitted ES of the ladybeetle *Adalia bipunctata* can kill infected male embryos, an appreciated and crucial first meal for their sisters, providing them with a competitive advantage over larvae hatched from an uninfected brood (Schulenburg *et al.* 2002, among others). Remarkably, different species of *Wolbachia*, *Cardinium* and other endosymbiotic bacteria can be responsible for different reproductive manipulations in different hosts.

How do parthenogenesis-inducing ESs affect BC? These ESs are only known from haplo-diploid organisms (Floate, Kyei-Poku & Coghlin 2006). The sex determination of Hymenoptera and Thysanoptera, as well as some Acarida, Hemiptera and Coleoptera (Normark 2003), is determined by the numbers of chromosome sets, i.e. males are haploid and develop from

unfertilized eggs, whereas females are diploid and the eggs are usually fertilized. Parasitoid wasps, beetle larvae and predatory mites can be very important BCAs. Parthenogenic reproduction may considerably increase population growth and facilitate the rearing of beneficial organisms by making the presence of males unnecessary and reducing reproductive costs. Parthenogenic reproduction induced by ESs will influence the sex ratio towards females, thereby considerably increasing the success of the programme if sexes differ in efficiency and effectiveness as BCAs. In many cases, only females act as BCAs by laying their eggs in the pest, e.g. in parasitoid wasps, females lay their eggs in or on their hosts and hatched larvae kill the host by consuming it. In this case, males are reared solely to fertilize females. In the case of parthenogenic parasitoids, theoretically, twice as many pest hosts can be parasitized by a similar BCA population size. Sexually reproducing BCAs may hybridize with native species in the field, affecting their genetic integrity and thereby having a dramatic nontarget effect on the environment (Yara 2004; Hopper, Brich & Wajnberg 2006). By using parthenogenic BCAs, BC practitioners can avoid this risk. However, there are also disadvantages with parthenogenetically reproducing populations. In some species, the reproductive rate can be higher in sexual lines compared to ES-induced parthenogenic lines, due, for example, to the high mortality of *Wolbachia*-infected offspring (Lamb & Willey 1979), or delays in development time (Corley & Moore 1999; Matsuura & Kobayashi 2007). Stouthamer (1993) compared the efficiency of parasitic wasp (*Trichogramma)* females of sexual and asexual lines in controlling pest moth populations and found that their relative efficiency depends on host density. At a high density of pest hosts, sexual females produce more offspring and parasitize more, whereas at low density, the asexual female wasps perform better. Ongoing discussions about the evolution and maintenance of sexual versus asexual reproduction highlight many theoretical advantages and disadvantages of both reproduction modes (Stouthamer 1993; Hurst & Peck 1996, among others).

To date, *Wolbachia* (Tagami, Miura & Stouthamer 2001; Weeks & Breeuwer 2001), *Cardinium* (Gotoh, Noda & Ito 2007) and *Rickettsia* (Hagimori *et al*. 2006) infection have been found to induce parthenogenesis. When rearing haplo-diploid species on a commercial scale, we recommend paying attention to potential parthenogenesis-inducing ES infections.

How do CI-inducing ESs affect BC? CI is another reproductive manipulation which can have severe consequences for BC programmes. CI suppresses the development of offspring from crosses between infected males and uninfected females. There have been attempts to use CI-inducing ESs directly in BC, to deplete uninfected pest populations by releasing an excess of males carrying CI-inducing ESs. This technique is analogous to the sterile insect technique, considered to be one of the only strategies that can successfully eradicate a detrimental insect population (Krafsur 1998). Zabalou *et al*. (2004, 2009) demonstrated a rapid decrease in laboratory Mediterranean fly *Ceratitis capitata* populations inundated with males artificially infected with a CI strain isolated from a closely related species, *Rhagoletis cerasi*. This approach could be taken much further in the future. Given that the host's genes, located on mitochondrial DNA, will spread into a population in the same way (because mitochondria and ESs are both vertically transmitted), the use of CI-inducing ESs has been suggested for the introduction of a gene impeding malaria transmission into an *Anopheles* population (Curtis & Sinkins 1998). Brelsfoard, St Clair & Dobson (2009) discuss the use of CI-inducing ESs in combination with a low dose of radiation for the control of lymphatic filiariasis-transmitting mosquitoes. The repeated release of incompatible males only could deplete the mosquito population. It is crucial not to release any females infected with the CI ES into the population to avoid a spread of infection that would lead to population replacement (uninfected to infected). To prevent accidental release of females, low-dose irradiation of BCAs was suggested to sterilize any females present in the material to be freed. CI-inducing ESs may also cause indirect negative effects in an augmentative BC programme. If the aim is to artificially increase a pre-existing population of beneficial arthropods, the presence of CI-inducing ESs in the mass-reared and released individuals may not allow them to produce any offspring in the field. If the released and native populations are both infected but with different strains, reproductive isolation might be near complete, although in most cases CI is not 100% efficient, leaving a few uninfected offspring of infected males to reach adulthood.

Endosymbionts can be crucial for host egg production. In the braconid wasp genus *Asobara*, the symbiont *Wolbachia* does not only manipulate reproduction – in some strains it is indispensable for oogenesis. The exact mechanism is not clear: without the symbiont, either females fail to produce oocytes at all or the offspring generated by the oocytes do not develop properly (Dedeine, Bouletreau & Vavre 2005). Although this example is fairly unique in current research, its occurrence should still be considered when using Hymenopteran BCAs.

Symbionts can protect their hosts from abiotic stress, thus increasing the survival of pests and vectors in the environment. A striking example of a direct interaction between a symbiont and its host comes from the tick *Ixodes scapularis*, in which the bacterium *Anaplasma phagocytophillum*, the causative agent of human granulocytic anaplasmosis, induces the expression of antifreeze glycoprotein which helps the host survive in cold temperatures (Neelakanta *et al*. 2010). In this case, the symbiont is also a horizontally transmitted mammalian pathogen, protecting its vector in stressful environments and enhancing its vectorial capacity. In the pea aphid *Acyrthosiphon pisum*, the symbiont *Serratia symbiotica* enables its host to survive under heat shock by providing a rapid supply of essential metabolites to the aphid or to the essential primary symbiont, through its own lysis (Burke, Fiehn & Moran 2009). In contrast, a symbiotic *Rickettsia* in the whitefly *Bemisia tabaci* was shown to reduce the whitefly's resistance to specific pesticides, an important component of the environment (Kontsedalov *et al*. 2008). This *Rickettisa* was shown to be transferred to the immature stages of the whitefly parasitoids *Eretmocerus mundus* and *Encarsia pergandiella*; however, its effects are not known and it does not persist later in adulthood (Chiel *et al*. 2009b; Fig. 1). Infection of the parasitoids during larval

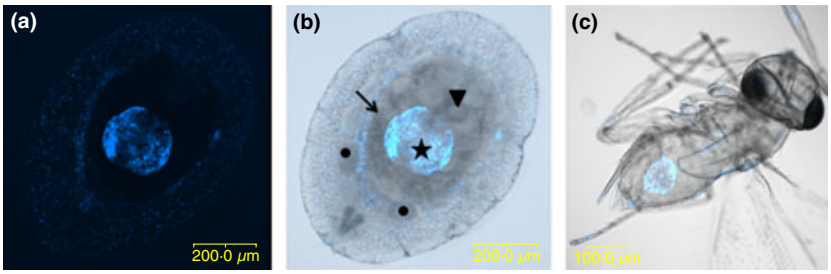


Fig. 1. Demonstration of the multitrophic interaction: Pest-BCA-ES using fluorescent *in situ* hybridization (FISH) of *Rickettsia* endosymbiont (blue) in the whitefly *Bemisia tabaci* and in its parasitoid *Eretmocerus emiratus*. (a) *B. tabaci* larva parasitized with *E. emiratus* larvae, fluorescent channel showing *Rickettsia* only, (b) Same as (a), fluorescent and bright field channel, arrow – parasitoid larvae, arrowhead – *E. emiratus* egg-shell, star – *E. emiratus* gut filled with *Rickettsia*, dots – *B. tabaci* bacteriomes (c) *E. emiratus* adult showing *Rickettsia* in its abdomen, fluorescent and bright field channels. For more details please see Chiel *et al.* 2009b.

development may nevertheless affect them later as adults either by modifying their commensal bacterial community or directly by inflicting a cost of infection carried throughout the life of the parasitoid. Developmental media were demonstrated to affect the mating behaviour of *Drosophila* by influencing their commensal bacteria (Sharon *et al.* 2010). Therefore, feeding media and multitrophic interactions need to be further investigated and taken into consideration when mass rearing pests and their BCA.

Microbial interactions within the arthropod host

Microbes can interact to protect their host against its natural enemies. In the case of the pea aphid and its ES, the presence of the ES alters the host's interaction with *Aphidius ervi*, protecting it from the parasitoid (Oliver, Moran & Hunter 2005). *Aphidius ervi* is used world-wide to control aphid populations in legumes and ornamentals. Pea aphid clones, however, vary greatly in their resistance to *A. ervi*, which oviposits in an adult aphid with the developing larva slowly killing it from the inside. Oliver *et al.* (2003) demonstrated that the development of resistant populations is owing to differences in secondary symbiont infections. Both *Hamiltonella defensa* and *Regiella insecticola* conferred resistance to *A. ervi* by decreasing its chances of completing development within the aphid host. Recent studies have shown that the bacteriophage associated with *Hamiltonella defensa* is responsible for the resistance, encoding a toxin capable of killing the parasitoid larvae (Oliver *et al.* 2009). As a bacterial strain can lose its associated phage, the levels of protection and resulting fitness of the aphids, and thus the success of a BC programme using *A. ervi*, may vary greatly in the field.

Nematodes are macroparasites of many arthropods. Females of the North American *Drosophila neotestacea* become completely sterile when infected with the nematode *Howardula aoronymphibium*. Recently, however, the protective effect of *Spiroplasma*, one of two symbionts found in this fly, has been described. While *Spiroplasma* does not change the fecundity of unparasitized flies, it can rescue most of the eggs in females infected with *H. aoronymphibium* (Jaenike *et al.* 2010). Because of the selective advantage provided by the ES to its host in the presence of the nematode, rapid spread of *Spiroplasma* infection can be observed across continents.

Having beneficial and pathogenic bacteria as roommates may lead to conflicts of interest. The ES depends on the host for its own reproduction, while the pathogen uses it as a resource and may harm or kill it. It is thus advantageous for the ES to protect the host from the pathogen. Microbial pathogens are typically protozoa (including microsporidia), fungi, bacteria or viruses, and sometimes even occupy the same location as the symbiont within the host.

Beauveria bassiana is a fungal pathogen infecting a diverse range of insect hosts (Riedel & Steenberg 1998). It lives in the soil, often infecting species that are in close contact with the ground for part of their life cycle. It is known to be an important natural mortality factor in insect populations and it has been suggested or used as a BCA of pests of many insect orders (Quesada-Moraga *et al.* 2006; Akello *et al.* 2008; Espinel *et al.* 2008; Pardey 2009). In a laboratory study, females of *Drosophila melanogaster* with identical genetic backgrounds but different *Wolbachia* infection status (W+ or W−) showed differences in their resistance to *B. bassiana* (Panteleev *et al.* 2007). Overall, the proportion of surviving females 7 days after infection with *B. bassiana* was three times greater in the infected (W+) female group than in the non-infected (W−) female group. Infected (W+) females also exhibited behavioural changes, such as variation in oviposition substrate preference. Moreover, infected (W+) males exhibited greater reproduction success than non-infected (W−) males (Panteleev *et al.* 2007). *Beauveria bassiana* is considered a potential control agent of the Asian ladybird *Harmonia axyridis* as it causes a significant reduction in the fertility of infected individuals. Roy *et al.* (2008) found that native ladybird populations are much more susceptible to *B. bassiana* than populations collected in Britain, which is part of the invasive range. Different genetic backgrounds could explain this pattern (Roy *et al.* 2008), but the potential influence of ESs infecting *H. axyridis* (Aebi & Zindel 2010) remains an open question that warrants evaluation.

Endosymbionts can provide their hosts with antibiotic components. The European beewolf *Philanthus triangulum* engages in a close association with a *Streptomyces* species that protects

the offspring in the brood cells from fungal pathogens. The bacteria reside in the antennae of female digger wasps and are added to brood cells prior to oviposition. The hatched larva spins a cocoon, into which it integrates the bacteria. As the brood cells are also ideal habitats for pathogens, the bacteria provide the beewolves with a selective advantage (Kaltenpoth *et al*. 2006). Recently, antibiotic substances have been found in the brain of cockroaches which are now being considered for the treatment of methicillin-resistent *Staphylococcus aureus* (MRSA) (http://www.smartplanet.com). To the best of our knowledge, there are no records of antibiotic metabolites produced by insects or other animals that support the hypothesis of symbiotically living micro-organisms in the brains of these cockroaches.

Arthopods are often harmed by viruses, and *Wolbachia* has been found to alter arthropod resistance to viruses. *Drosophila melanogaster* has been used as a model organism to study resistance to viruses in arthropods. Two research groups (Hedges *et al*. 2008; Teixeira, Ferreira & Ashburner 2008) independently demonstrated that *Wolbachia* infection increases its host's resistance to *Drosophila* C Virus (DCV), a single-stranded RNA virus, but also to three other RNA viruses: Cricket Paralysis Virus (Hedges *et al*. 2008), Nora Virus and Flock House (FH) Virus (Teixeira, Ferreira & Ashburner 2008). The mechanisms involved in the ES-mediated protection against viruses are not fully understood. In the case of DCV and Nora virus, W+ flies contained up to 10^4-fold less viruses. To date, no *Wolbachia*-induced resistance to a DNA virus has been reported (Teixeira, Ferreira & Ashburner 2008). Interactions between ESs and viruses are quite likely to be common (see below), as ESs and viruses are often found together in the intracellular space. Any found resistance to a virus can theoretically be attributed to an ES, although only ES-mediated resistance to RNA viruses has been reported to date.

Interaction between arthropod symbiont and vectored pathogen

The ability of an arthropod host to serve as a vector for pathogenic agents can be described in two ways: 'vector competence' or 'vectorial capacity' (Reisen 2002). The former term refers to the ability of the vector to support pathogen infection, replication and/or development (depending on the pathogen group) and transmission (nearly always by bite). The latter term includes vector competence as a factor, but is a field-derived estimate obtained through vector biting rates and survival, which can greatly influence disease transmission. Thus, vectorial capacity is the more comprehensive and relevant factor in describing the ability of a potential vector to transmit pathogens. ESs may thus have an influence on vectorial capacity by two means: interactions between the vector and its symbionts, and interactions between the symbionts (enduring microbes) and the pathogens (transient microbes) within the vector. Here, we will elaborate on microbial interactions and their potential to reduce vectorial capacity by directly influencing the survival of the disease agent within its vector.

Vertically transmitted symbionts are usually considered beneficial to their host and are thus expected to have an advantage over nonbeneficial, potentially harmful, transient microbes such as vectored pathogenic agents. For example, the causative agent of Rocky Mountain spotted fever, *Rickettsia rickettsii*, is transmitted by the tick *Dermacentor andersoni* (Burgdorfer, Hayes & Mavros 1981) and is also pathogenic to the tick itself (Niebylski, Peacock & Schwan 1999). Competitive displacement of *R. rickettsii* by the nonvectored, symbiotic *Rickettsia peackokii* (Niebylski *et al*. 1997) not only protects the tick from the harmful effects of *R. ricketsii*, it also prevents its transmission. In contrast, ESs can increase the vectorial capacity of their hosts by contributing to the transmission of the pathogen. For example, in the B biotype of the whitefly *Bemisia tabaci*, plant viruses have been shown to positively (Jiu *et al*. 2007) or negatively (Rubinstein & Czosnek 1997) affect vector fitness. Recently, Gottlieb *et al*. (2010) showed that the efficacy of Tomato Yellow Leaf Curl Virus (TYLCV) transmission by *B. tabaci* depends on the presence of the whitefly symbiont *Hamiltonella*. The interaction between a specific *Hamiltonella* GroEL and the virus coat protein protects the virus from proteolysis in the haemolymph, enhancing its chances of infecting the whitefly salivary gland and increasing its probability of being transmitted to the next plant. Symbionts can be viewed as part of their host's immune system, a statement which has recently gained some experimental support (see examples in section Microbial interactions within the arthropod host). The defence mechanisms explained above could, in the near future, be used for the control of vector-borne diseases. Tracking the quantity of three symbionts infecting tsetse flies showed active and dynamic colonization of the host which was dependent on host or environmental factors (Rio *et al*. 2006). Challenging the host with a transient microbe, *Trypanosoma brucei rhodesiense*, the agent of African trypanosomiasis (sleeping sickness), only affected the density of a facultative symbiont, *Wolbachia*, but had no significant effect on the density of the obligatory symbionts (Rio *et al*. 2006). Although the response to trypanosome infection was shown to be cellular (Hao *et al*. 2001; Boulanger *et al*. 2002; Hao, Kasumba & Aksoy 2003), the contribution of symbionts to the immune response cannot be ruled out.

Correlations between trypanosome infections and the presence of *Wolbachia* have also been described in the bug *Rhodnius pallescens* (Espino *et al*. 2009), a vector of *Trypanosoma cruzi*, the agent of American trypanosomiasis, or Chagas disease (Calzada *et al*. 2006), and *Trypanosoma rangeli*, a non-human pathogen (Guhl & Vallejo 2003). In that work, all field-collected triatomines were infected with *Wolbachia*, and the prevalence of trypanosome infection was between 25% and 56% for single infection (only one trypanosome species), whereas it was only 12% for double infection with the two trypanosome species. Thus, *Wolbachia* infection may reduce the number of double trypanosome infections via a competitive mechanism among all microbes, or by affecting the immune system of its host to prevent trypanosome development. The first hypothesis may be supported by the fact that *Wolbachia* has been found in the gonads, gut, salivary glands and faeces,

indicating the likelihood of its interaction with trypanosomes as both organisms share common locations in their host (Espino *et al.* 2009). The latter hypothesis may be supported by the recent work of Kambris *et al.* (2009) showing a reduction in the filarial nematode *Brugia pahangi*'s development in *Aedes aegypti* after artificial infection with the strain wMelPop of *Wolbachia* because of upregulation of immune system genes. These hypotheses, however, require further study. Symbiont manipulation (paratransgenesis) was used by Durvasula *et al.* (1997) to interfere with trypanosome transmission. The obligate symbiont of another triatomine, *Rhodnius prolixus*, was engineered to produce antimicrobial peptide against *T. cruzi*, and introduction of the engineered bacterium *Rhodococcus rhudnii* prevented *T. cruzi* establishment in several individuals in the laboratory. The ability to infect *R. prolixus* with modified symbionts via stercoraria (transmission through the faeces), the symbionts' natural mode of transmission, has great applicative potential. The paratransgenesis model was also tested for controlling Pierce's disease in grapevines caused by *Xylella fastidiosa*, a bacterium transmitted by the glassy-winged sharpshooter, *Homalodisca vitripennis*. The sharpshooter symbiont, *Alcaligenes xylosoxidans denitrificans*, was genetically modified to express single-chain antibodies that were specific to *X. fastidiosa* (Ramirez, Perring & Miller 2007), thus preventing persistence of the plant pathogen in the vector and reducing the vectorial capacity.

Gut microbes are expected to be the first barrier against transient microbes. Moreover, these microbiota are believed to actively inhibit pathogen transmission (summarized in Azambuja, Garcia & Ratcliffe 2005). Studies on the gut microbiota of malaria vectors have shown that the host immune genes modulate the symbiotic bacteria's gut community after intake of a blood meal (Meister *et al.* 2009). Natural responses of the microbiota in the mosquito gut may mediate antimicrobial immune responses against *Plasmodium* (Dong, Manfredini & Dimopoulos 2009). The interaction between *Aedes* mosquito microbiota, its defence response and infections with *Plasmodium* could then be targeted for specific control of malaria.

Analyses of the microbiota of other dipteran vectors of important veterinary and human diseases could reveal more natural interactions or potential candidates for manipulation. The sand fly *Phlebotomus argentipes*, vector of Kala-Azar, harbours specific bacteria (*Bacillus megaterium* and *Brevibacterium linens*) that are suitable for a paratransgenesis approach to controlling lishmaniasis. These bacteria can cause persistent infections and can be cultured (Hillesland *et al.* 2008). Comparisons between biting midges (*Culicoides*) vectoring bluetongue virus and nonvectoring midges revealed significant differences in microbial community composition (Campbell *et al.* 2004). These findings suggest that the microbial community may naturally determine vectorial capacity. This is also supported by the differing bacterial species richness of the flea vector *Ctenocephalides felis* when infected or not infected with *Rickettsia felis* (Pornwiroon *et al.* 2007).

To date, there is no conclusive evidence for a natural role for *Wolbachia* in direct protection against transient microbes, but a study has shown that it can be a target for manipulation, specifically when introduced into a novel host. In a pioneering work, McMeniman *et al.* (2009) injected a life-shortening *Wolbachia* strain into the *Dengue* virus vector, *Aedes aegypti*, and directly reduced its vectorial capacity by influencing its survival and biting ability. Other works (e.g. Dobson, Marsland & Rattanadechakul 2009; Espino *et al.* 2009) showing natural *Wolbachia* infection in important vector species may open the way for direct manipulation of the symbiont or its environment to prevent transmission.

Conclusions and implications

Several biotic and abiotic factors affecting the BCA or the target crop-pest species can influence the success of a BC programme. One of the most variable and commonly underestimated factors is endosymbiotically living organisms such as bacteria, fungi or viruses. In addition to drastically influencing the dynamics and structure of BCA or pest/vector populations, ESs can (i) be involved in the evolution of resistance against natural enemies commonly used in BC programmes, (ii) induce resistance to pesticides and (iii) influence the vectorial capacity of some disease vectors. Evidence for ES-mediated protection strategies against very different enemies or chemicals is accumulating, even if it is still not clear how often ESs are actually responsible for an observed effect. A determination of the presence and influence of ESs in arthropods involved in BC programmes should be included in the risk assessment protocol prior to the BCA's release. Although ESs fall into the definition of contaminants that could potentially affect the efficacy of BCAs, defining their associated risk is an arduous task. In risk-assessment procedures, risk is usually defined as 'hazard x probability', hazard being any identifiable adverse effect that has a probability or likelihood of occurring. In the case of ESs infecting BCAs, both hazards and probabilities remain poorly described in BCAs, and it is virtually impossible to calculate the probability of a hazard occurring as most of the above-mentioned examples have been described in only a limited number of biological systems. Although there are currently no quality-control standards for contaminants associated with BCAs, we strongly encourage BC practitioners to perform a survey of potential ESs infecting the BCA, possibly threatening the success of a BC programme (Goettel & Inglis 2006). However, the effort invested in the detection of potentially adverse ESs should be in direct proportion to the risk they pose to the BCA or to the outcome of a BC programme.

Different methods of investigating the presence of ESs or other associated bacteria are available. Whole bacterial communities associated with a given species can be described by denaturing gradient gel electrophoresis (DGGE) (http://www.eeescience.utoledo.edu/faculty/sigler/research/protocols/dgge/dgge.pdf) or clone library analysis on bacterial 16S rDNA gene product, followed by a sequencing procedure. If the presence of bacteria is suspected (sex-ratio bias, unexplained resistance to natural enemies, unexplained rearing crashes, incompatibility between strains), polymerase chain reaction (PCR) with specific primers can, in some cases, help

confirm its presence (Enigl & Schausberger 2007; Weinert *et al.* 2007; Duron *et al.* 2008). An online, open-access catalogue of widely used BCAs (EPPO Standards on Safe Use of Biological Control – PM 6/3 – Version 2010), known bacterial associations and their potential effects is available on the following webpage: http://www.symbiontsincontrol.ch. The aim of this catalogue is to guide and inform BC practitioners on ES–arthropod interactions.

Acknowledgements

This review was supported by the COST-SER project C08·0109 to AA, part of the COST Action FA0701 and the Research Grant Award No. IS-4185-09F to YG from BARD, the United States – Israel Binational Agricultural Research and Development Fund. We thank the Prof. Hunter group from the University of Arizona for letting us use their wasp culture to create the figures. We thank Steven Bacon, the editors and two anonymous reviewers for helpful comments.

References

Aebi, A. & Zindel, R. (2010) What can endosymbionts tell about the *Harmonia axyridis* invasion? *Study Group 'Benefits and Risks of Exotic Biological Control Agents' at Engelberg (Switzerland), 6–10 September 2009* (eds D. Babendreier, M. Kenis, A. Aebi & H. Roy), pp. 5–6. IOBC, Darmstadt (Germany).

Akello, J., Dubois, T., Coyne, D. & Kyamanywa, S. (2008) Effect of endophytic *Beauveria bassiana* on populations of the banana weevil, *Cosmopolites sordidus*, and their damage in tissue-cultured banana plants. *Entomologia Experimentalis Et Applicata*, **129**, 157–165.

Azambuja, P., Garcia, E.S. & Ratcliffe, N.A. (2005) Gut microbiota and parasite transmission by insect vectors. *Trends in Parasitology*, **21**, 568–572.

Baumann, P. (2005) Biology of bacteriocyte-associated endosymbionts of plant sap-sucking insects. *Annual Review of Microbiology*, **59**, 155–189.

Beugnet, F. & Marie, J.L. (2009) Emerging arthropod-borne diseases of companion animals in Europe. *Veterinary Parasitology*, **163**, 298–305.

Bordenstein, S.R., O'Hara, F.P. & Werren, J.H. (2001) *Wolbachia*-induced incompatibility precedes other hybrid incompatibilities in *Nasonia*. *Nature*, **409**, 707–710.

Boulanger, N., Brun, R., Ehret-Sabatier, L., Kunz, C. & Bulet, P. (2002) Immunopeptides in the defense reactions of *Glossina morsitans* to bacterial and *Trypanosoma brucei brucei* infections. *Insect Biochemistry and Molecular Biology*, **32**, 369–375.

Brelsfoard, C.L., St Clair, W. & Dobson, S.L. (2009) Integration of irradiation with cytoplasmic incompatibility to facilitate a lymphatic filariasis vector elimination approach. *Parasites and Vectors*, **2**, ????–????.

Brownlie, J.C. & Johnson, K.N. (2009) Symbiont-mediated protection in insect hosts. *Trends in Microbiology*, **17**, 348–354.

Burgdorfer, W., Hayes, S.F. & Mavros, A.J. (1981) Nonpathogenic Rickettsiae in *Dermacentor andersoni*: a limiting factor for the distribution of *Rickettsia rickettsii*. *Rickettsiae and Rickettsial Diseases* (eds W. Burgdorfer & R.L. Anacker), pp. 585–594. Academic Press, New York.

Burke, G., Fiehn, O. & Moran, N. (2009) Effects of facultative symbionts and heat stress on the metabolome of pea aphids. *The ISME Journal*, **4**, 242–252.

Calzada, J.E., Pineda, V., Montalvo, E., Alvarez, D., Santamaria, A.M., Samudio, F., Bayard, V., Caceres, L. & Saldana, A. (2006) Human trypanosome infection and the presence of intradomicile *Rhodnius pallescens* in the Western Border of the Panama Canal, Panama. *American Journal of Tropical Medicine and Hygiene*, **74**, 762–765.

Campbell, C.L., Mummey, D.L., Schmidtmann, E.T. & Wilson, W.C. (2004) Culture-independent analysis of midgut microbiota in the arbovirus vector *Culicoides sonorensis* (Diptera: Ceratopogonidae). *Journal of Medical Entomology*, **41**, 340–348.

Chiel, E., Inbar, M., Mozes-Daube, N., White, J.A., Hunter, M.S. & Zchori-Fein, E. (2009a) Assessments of Fitness Effects by the Facultative Symbiont *Rickettsia* in the Sweetpotato Whitefly (Hemiptera: Aleyrodidae). *Annals of the Entomological Society of America*, **102**, 413–418.

Chiel, E., Zchori-Fein, E., Inbar, M., Gottlieb, Y., Adachi-Hagimori, T., Kelly, S.E., Asplen, M.K. & Hunter, M.S. (2009b) Almost there: transmission routes of bacterial symbionts between trophic levels. *PLoS One*, **4**, e4767.

Corley, L. & Moore, A. (1999) Fitness of alternative modes of reproduction: developmental constraints and the evolutionary maintenance of sex. *Proceedings of the Royal Society B-Biological Sciences*, **266**, 471–476.

Curtis, C.F. & Sinkins, S.P. (1998) *Wolbachia* as a possible means of driving genes into populations. *Parasitology*, **116**, S111–S115.

Davidson, S.K., Allen, S.W., Lim, G.E., Anderson, C.M. & Haygood, M.G. (2001) Evidence for the biosynthesis of bryostatins by the bacterial symbiont '*Candidatus Endobugula sertula*' of the bryozoan *Bugula neritina*. *Applied and Environmental Microbiology*, **67**, 4531–4537.

Dedeine, F., Bouletreau, M. & Vavre, F. (2005) *Wolbachia* requirement for oogenesis: occurrence within the genus *Asobara* (Hymenoptera, Braconidae) and evidence for intraspecific variation in *A. tabida*. *Heredity*, **95**, 394–400.

Dobson, S.L., Marsland, E.J. & Rattanadechakul, W. (2009) *Wolbachia*-Induced Cytoplasmic Incompatibility in Single- and Superinfected *Aedes albopictus* (Diptera: Culicidae). *Journal of Medical Entomology*, **38**, 382–387.

Dong, Y., Manfredini, F. & Dimopoulos, G. (2009) Implication of the mosquito midgut microbiota in the defense against malaria parasites. *PLoS Pathogens*, **5**, e1000423.

Douglas, A.E. (1998) Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria *Buchnera*. *Annual Review of Entomology*, **43**, 17–37.

Duron, O., Bouchon, D., Boutin, S., Bellamy, L., Zhou, L.Q., Engelstadter, J. & Hurst, G.D. (2008) The diversity of reproductive parasites among arthropods: *Wolbachia* do not walk alone. *BMC Biology*, **6**, 27.

Durvasula, R.V., Gumbs, A., Panackal, A., Kruglov, O., Aksoy, S., Merrifield, R.B., Richards, F.F. & Beard, C.B. (1997) Prevention of insect-borne disease: an approach using transgenic symbiotic bacteria. *Proceedings of the National Academy of Sciences of the U S A*, **94**, 3274–3278.

Enigl, M. & Schausberger, P. (2007) Incidence of the endosymbionts *Wolbachia, Cardinium* and *Spiroplasma* in phytoseiid mites and associated prey. *Experimental and Applied Acarology*, **42**, 75–85.

Espinel, C., Torres, L., Grijalba, E., Villamizar, L. & Cotes, A.M. (2008) Preformulations for control of the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) under laboratory conditions. *Revista Colombiana De Entomologia*, **34**, 22–27.

Espino, C.I., Gomez, T., Gonzalez, G., do Santos, M.F., Solano, J., Sousa, O., Moreno, N., Windsor, D., Ying, A., Vilchez, S. & Osuna, A. (2009) Detection of *Wolbachia* bacteria in multiple organs and feces of the triatomine insect *Rhodnius pallescens* (Hemiptera, Reduviidae). *Applied and Environmental Microbiology*, **75**, 547–550.

Floate, K.D., Kyei-Poku, G.K. & Coghlin, P.C. (2006) Overview and relevance of *Wolbachia* bacteria in biocontrol research. *Biocontrol Science and Technology*, **16**, 767–788.

Fowler, S.V. (2004) Biological control of an exotic scale, *Orthezia insignis* Browne (Homoptera: Ortheziidae), saves the endemic gumwood tree, *Commidendrum robustum* (Roxb.) DC. (Asteraceae) on the island of St. Helena. *Biological Control*, **29**, 367–374.

Gil-Turnes, M.S. & Fenical, W. (1992) Embryos of *Homarus americanus* are protected by epibiotic bacteria. *Biological Bulletin*, **182**, 105–108.

Gil-Turnes, M.S., Hay, M.E. & Fenical, W. (1989) Symbiotic Marine-Bacteria Chemically Defend Crustacean Embryos from a Pathogenic Fungus. *Science*, **246**, 116–118.

Giorgini, M., Monti, M.M., Caprio, E., Stouthamer, R. & Hunter, M.S. (2009) Feminization and the collapse of haplodiploidy in an asexual parasitoid wasp harboring the bacterial symbiont *Cardinium*. *Heredity*, **102**, 365–371.

Goettel, M.S. & Inglis, G. (2006) Methods for assessments of contaminants of invertebrate biological control agents and associated risks. *Environmental Impact of Invertebrates for Biological Control of Arthropods: Methods and Risk Assessment* (eds F. Bigler, D. Babendreier & U. Kuhlmann), pp. 145–165. CABi publishing, Wallingford, UK.

Gomez, C. & Oliveras, J. (2003) Can the Argentine ant (*Linepithema humile* Mayr) replace native ants in myrmecochory? *Acta Oecologica-International Journal of Ecology*, **24**, 47–53.

Gotoh, T., Noda, H. & Ito, S. (2007) *Cardinium* symbionts cause cytoplasmic incompatibility in spider mites. *Heredity*, **98**, 13–20.

Gottlieb, Y., Zchori-Fein, E., Mozes-Daube, N., Kontsedalov, S., Skaljac, M., Brumin, M., Sobol, I., Czosnek, H., Vavre, F., Fleury, F. & Ghanim, M. (2010) The Transmission Efficiency of Tomato Yellow Leaf Curl Virus by the Whitefly *Bemisia tabaci* Is Correlated with the Presence of a Specific Symbiotic Bacterium Species. *Journal of Virology*, **84**, 9310–9317.

Guhl, F. & Vallejo, G.A. (2003) *Trypanosoma* (Herpetosoma) *rangeli* Tejera, 1920 – An updated review. *Memorias Do Instituto Oswaldo Cruz*, **98**, 435–442.

Gunduz, E.A. & Douglas, A.E. (2009) Symbiotic bacteria enable insect to use a nutritionally inadequate diet. *Proceedings of the Royal Society B-Biological Sciences*, **276**, 987–991.

Hagimori, T., Abe, Y., Date, S. & Miura, K. (2006) The first finding of a *Rickettsia* bacterium associated with parthenogenesis induction among insects. *Current Microbiology*, **52**, 97–101.

Haine, E.R. (2007) Symbiont-mediated protection. *Proceedings of the Royal Society B-Biological Sciences*, **275**, 353–361.

Haine, E.R., Boucansaud, K. & Rigaud, T. (2005) Conflict between parasites with different transmission strategies infecting an amphipod host. *Proceedings of the Royal Society B-Biological Sciences*, **272**, 2505–2510.

Hao, Z., Kasumba, I. & Aksoy, S. (2003) *Proventriculus* (*cardia*) plays a crucial role in immunity in tsetse fly (Diptera: Glossinidiae). *Insect Biochemistry and Molecular Biology*, **33**, 1155–1164.

Hao, Z., Kasumba, I., Lehane, M.J., Gibson, W.C., Kwon, J. & Aksoy, S. (2001) Tsetse immune responses and trypanosome transmission: implications for the development of tsetse-based strategies to reduce trypanosomiasis. *Proceedings of the National Academy of Sciences of the U S A*, **98**, 12648–12653.

Harcombe, W. & Hoffmann, A.A. (2004) *Wolbachia* effects in *Drosophila melanogaster*: in search of fitness benefits. *Journal of Invertebrate Pathology*, **87**, 45–50.

Hedges, L.M., Brownlie, J.C., O'Neill, S.L. & Johnson, K.N. (2008) *Wolbachia* and Virus Protection in Insects. *Science*, **322**, 702.

Hilgenboecker, K., Hammerstein, P., Schlattmann, P., Telschow, A. & Werren, J.H. (2008) How many species are infected with *Wolbachia*? – a statistical analysis of current data. *FEMS Microbiology Letters*, **281**, 215–220.

Hillesland, H., Read, A., Subhadra, B., Hurwitz, I., McKelvey, R., Ghosh, K., Das, P. & Durvasula, R. (2008) Identification of aerobic gut bacteria from the kala azar vector, *Phlebotomus argentipes*: a platform for potential paratransgenic manipulation of sand flies. *American Journal of Tropical Medicine and Hygiene*, **79**, 881–886.

Hopper, K., Brich, S. & Wajnberg, E. (2006) Risks of interbreeding between species used in biological control and native species, and methods for evaluating their occurrence and impact. *Environmental Impact of Invertebrates for Biological Control of Arthropods: Methods and Risk Assessment* (eds F. Bigler, D. Babendreier & U. Kuhlmann), pp. 78–97. CABi publishing, Wallingford, UK.

Hosokawa, T., Koga, R., Kikuchi, Y., Meng, X.Y. & Fukatsu, T. (2010) *Wolbachia* as a bacteriocyte-associated nutritional mutualist. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 769–774.

Hurst, G.D.D. & Jiggins, F.M. (2000) Male-killing bacteria in insects: mechanisms, incidence, and implications. *Emerging Infectious Diseases*, **6**, 329–336.

Hurst, L.D. & Peck, J.R. (1996) Recent advances in understanding of the evolution and maintenance of sex. *Trends in Ecology and Evolution*, **11**, A46–A52.

Jaenike, J., Unckless, R., Cockburn, S.N., Boelio, L.M. & Perlman, S.J. (2010) Adaptation via symbiosis: recent spread of a Drosophila defensive symbiont. *Science*, **329**, 212–215.

Jenkins, J.C., Aber, J.D. & Canham, C.D. (1999) Hemlock woolly adelgid impacts on community structure and N cycling rates in eastern hemlock forests. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere*, **29**, 630–645.

Jiu, M., Zhou, X.-P., Tong, L., Xu, J., Yang, X., Wan, F.-H. & Liu, S.-S. (2007) Vector-Virus Mutualism Accelerates Population Increase of an Invasive Whitefly. *PLoS One*, **2**, e182.

Jones, E.O., White, A. & Boots, M. (2007) Interference and the persistence of vertically transmitted parasites. *Journal of Theoretical Biology*, **246**, 10–17.

Kaltenpoth, M., Goettler, W., Dale, C., Stubblefield, J.W., Herzner, G., Roeser-Mueller, K. & Strohm, E. (2006) *Candidatus Streptomyces philanthi*, an endosymbiotic streptomycete in the antennae of *Philanthus* digger wasps. *International Journal of Systematic and Evolutionary Microbiology*, **56**, 1403–1411.

Kambris, Z., Cook, P.E., Phuc, H.K. & Sinkins, S.P. (2009) Immune Activation by Life-Shortening *Wolbachia* and Reduced Filarial Competence in Mosquitoes. *Science*, **326**, 134–136.

Kellner, R.L.L. (2002) Molecular identification of an endosymbiotic bacterium associated with pederin biosynthesis in *Paederus sabaeus* (Coleoptera: Staphylinidae). *Insect Biochemistry and Molecular Biology*, **32**, 389–395.

Kittayapong, P., Jamnongluk, W., Thipaksorn, A., Milne, J.R. & Sindhusake, C. (2003) *Wolbachia* infection complexity among insects in the tropical rice-field community. *Molecular Ecology*, **12**, 1049–1060.

Kontsedalov, S., Zchori-Fein, E., Chiel, E., Gottlieb, Y., Inbar, M. & Ghanim, M. (2008) The presence of *Rickettsia* is associated with increased susceptibility of *Bemisia tabaci* (Homoptera: Aleyrodidae) to insecticides. *Pest Management Science*, **64**, 789–792.

Krafsur, E.S. (1998) Sterile insect technique for suppressing and eradicating insect populations: 55 years and counting. *Journal of Agricultural Entomology*, **15**, 303–317.

Lamb, R. & Willey, R. (1979) Are parthenogenetic and related bisexual insects equal in fertility? *Evolution*, **33**, 774–775.

Lipsitch, M., Nowak, M.A., Ebert, D. & May, R.M. (1995) The population dynamics of vertically and horizontally transmitted parasites. *Proceedings of the Royal Society B-Biological Sciences*, **260**, 321–327.

Lively, C.M., Clay, K., Wade, M.J. & Fuqua, C. (2005) Competitive co-existence of vertically and horizontally transmitted parasites. *Evolutionary Ecology Research*, **7**, 1183–1190.

Lopanik, N., Lindquist, N. & Targett, N. (2004) Potent cytotoxins produced by a microbial symbiont protect host larvae from predation. *Oecologia*, **139**, 131–139.

Matsuura, K. & Kobayashi, N. (2007) Size, hatching rate, and hatching period of sexually and asexually produced eggs in the facultatively parthenogenetic termite *Reticulitermes speratus* (Isoptera: Rhinotermitidae). *Applied Entomology and Zoology*, **42**, 241–246.

McMeniman, C.J., Lane, R.V., Cass, B.N., Fong, A.W., Sidhu, M., Wang, Y.F. & O'Neill, S.L. (2009) Stable introduction of a life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti*. *Science*, **323**, 141–144.

Meister, S., Agianian, B., Turlure, F., Relogio, A., Morlais, I., Kafatos, F.C. & Christophides, G.K. (2009) *Anopheles gambiae* PGRPLC-mediated defense against bacteria modulates infections with malaria parasites. *PLoS Pathogens*, **5**, e1000542.

Nazzi, F., Martinelli, E., Del Fabbro, S., Bernardinelli, I., Milani, N., Iob, A., Pischiutti, P., Campello, C. & D'Agaro, P. (2010) Ticks and Lyme borreliosis in an alpine area in northeast Italy. *Medical and Veterinary Entomology*, **24**, 220–226.

Neelakanta, G., Sultana, H., Fish, D., Anderson, J.F. & Fikrig, E. (2010) *Anaplasma phagocytophilum* induces *Ixodes scapularis* ticks to express an antifreeze glycoprotein gene that enhances their survival in the cold. *The Journal of Clinical Investigation*, **120**, 3179–3190.

Niebylski, M.L., Peacock, M.G. & Schwan, T.G. (1999) Lethal effect of *Rickettsia rickettsii* on its tick vector (*Dermacentor andersoni*). *Applied and Environmental Microbiology*, **65**, 773–778.

Niebylski, M.L., Peacock, M.G., Fischer, E.R., Porcella, S.F. & Schwan, T.G. (1997) Characterization of an endosymbiont infecting wood ticks, *Dermacentor andersoni*, as a member of the genus *Francisella*. *Applied and Environmental Microbiology*, **63**, 3933–3940.

Normark, B.B. (2003) The evolution of alternative genetic systems in insects. *Annual Review of Entomology*, **48**, 397–423.

Oliver, K.M., Moran, N.A. & Hunter, M.S. (2005) Variation in resistance to parasitism in aphids is due to symbionts not host genotype. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 12795–12800.

Oliver, K.M., Russell, J.A., Moran, N.A. & Hunter, M.S. (2003) Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 1803–1807.

Oliver, K.M., Degnan, P.H., Hunter, M.S. & Moran, N.A. (2009) Bacteriophages Encode Factors Required for Protection in a Symbiotic Mutualism. *Science*, **325**, 992–994.

Olsen, K., Reynolds, K.T. & Hoffmann, A.A. (2001) A field cage test of the effects of the endosymbiont *Wolbachia* on *Drosophila melanogaster*. *Heredity*, **86**, 731–737.

O'Neill, S.L., Hoffmann, A.A. & Werren, J.H. (1997) *Influential Passengers*. Oxford University Press Inc., New York, USA.

Panteleev, D.Y., Goryacheva I.I., Andrianov, B.V., Reznik, N.L., Lazebny, O.E. & Kulikov, A.M. (2007) The endosymbiotic bacterium *Wolbachia* enhances the nonspecific resistance to insect pathogens and alters behavior of *Drosophila melanogaster*. *Russian Journal of Genetics*, **43**, 1066–1069.

Pardey, A.E.B. (2009) Evaluation of chemical and biological insecticides to control *Frankliniella occidentalis* (Thysanoptera: Thripidae) in asparagus crops. *Revista Colombiana De Entomologia*, **35**, 12–17.

Perlman, S.J., Kelly, S.E. & Hunter, M.S. (2008) Population biology of cytoplasmic incompatibility: maintenance and spread of *Cardinium* symbionts in a parasitic wasp. *Genetics*, **178**, 1003–1011.

Perotti, M.A., Clarke, H.K., Turner, B.D. & Braig, H.R. (2006) *Rickettsia* as obligate and mycetomic bacteria. *FASEB Journal*, **20**, 2372–2383.

Pimentel, D., Lach, L., Zuniga, R. & Morrison, D. (2003) Environmental and Economic Costs of Alien Arthropods and Other Organisms in the United States. *Invasive Arthropods in Agriculture – Problems and Solutions* (eds G.J. Hallmann & C.P. Schwalbe), pp. 107–117. Science Publishers Inc., Enfield (NH), USA.

Pornwiroon, W., Kearney, M.T., Husseneder, C., Foil, L.D. & Macaluso, K.R. (2007) Comparative microbiota of *Rickettsia felis*-uninfected and -infected colonized cat fleas, *Ctenocephalides felis*. *The ISME Journal*, **1**, 394–402.

Quesada-Moraga, E., Maranhao, E.A.A., Valverde-Garcia, P. & Santiago-Alvarez, C. (2006) Selection of *Beauveria bassiana* isolates for control of the whiteflies *Bemisia tabaci* and *Trialeurodes vaporariorum* on the basis of their virulence, thermal requirements, and toxicogenic activity. *Biological Control*, **36**, 274–287.

Ramirez, J.L., Perring, T.M. & Miller, T.A. (2007) Fate of a Genetically Modified Bacterium in Foregut of Glassy-Winged Sharpshooter (Hemiptera: Cicadellidae). *Journal of Economic Entomology*, **101**, 1519–1525.

Randolph, S.E. (2010) Human activities predominate in determining changing incidence of tick-borne encephalitis in Europe. *Eurosurveillance*, **15**, 24–31.

Reisen, W.K. (2002) Epidemiology of vector-borne diseases. *Medical and Veterinary Entomology* (eds G. Mullen & L. Durden), pp. 15–27. Elsevier, NY.

Riedel, W. & Steenberg, T. (1998) Adult polyphagous coleopterans overwintering in cereal boundaries: winter mortality and susceptibility to the entomopathogenic fungus *Beauveria bassiana*. *BioControl*, **43**, 175–188.

Rigaud, T. & Haine, E.R. (2005) Conflict between co-occurring parasites as a confounding factor in manipulation studies? *Behavioural Processes*, **68**, 259–262.

Rio, R.V., Wu, Y.N., Filardo, G. & Aksoy, S. (2006) Dynamics of multiple symbiont density regulation during host development: tsetse fly and its microbial flora. *Proceedings of the Royal Society B-Biological Sciences*, **273**, 805–814.

Roy, H.E., Brown, P.M.J., Rothery, P., Ware, R.L. & Majerus, M.E.N. (2008) Interactions between the fungal pathogen *Beauveria bassiana* and three species of coccinellid: *Harmonia axyridis*, *Coccinella septempunctata* and *Adalia bipunctata*. *BioControl*, **53**, 265–276.

Rubinstein, G. & Czosnek, H. (1997) Long-term association of tomato yellow leaf curl virus with its whitefly vector *Bemisia tabaci*: effect on the insect transmission capacity, longevity and fecundity. *Journal of General Virology*, **78**, 2683–2689.

Schulenburg, J., Hurst, G.D.D., Tetzlaff, D., Booth, G.E., Zakharov, I.A. & Majerus, M.E.N. (2002) History of infection with different male-killing bacteria in the two-spot ladybird beetle *Adalia bipunctata* revealed through mitochondrial DNA sequence analysis. *Genetics*, **160**, 1075–1086.

Sharon, G., Segal, D., Ringo, J., Hefetz, A., Zilber-Rosenberg, I. & Rosenberg, E. (2010) Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. *PNAS*, **107**, 20051–20056.

Stouthamer, R. (1993) The use of sexual versus asexual wasps in biological control. *Entomophaga*, **38**, 3–6.

Tagami, Y., Miura, K. & Stouthamer, R. (2001) How does infection with parthenogenesis-inducing *Wolbachia* reduce the fitness of *Trichogramma*? *Journal of Invertebrate Pathology*, **78**, 267–271.

Teixeira, L., Ferreira, A. & Ashburner, M. (2008) The Bacterial Symbiont *Wolbachia* Induces Resistance to RNA Viral Infections in *Drosophila melanogaster*. *PLoS Biology*, **6**, 2753–2763.

Weeks, A.R. & Breeuwer, J.A.J. (2001) *Wolbachia*-induced parthenogenesis in a genus of phytophagous mites. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **268**, 2245–2251.

Weinert, L.A., Tinsley, M.C., Temperley, M. & Jiggins, F.M. (2007) Are we underestimating the diversity and incidence of insect bacterial symbionts? A case study in ladybird beetles. *Biology Letters*, **3**, 678–681.

Werren, J.H., Baldo, L. & Clark, M.E. (2008) *Wolbachia*: master manipulators of invertebrate biology. *Nature Reviews Microbiology*, **6**, 741–751.

Yara, K. (2004) Relationship between the introduced and indigenous parasitoids *Torymus sinensis* and *T. beneficus* (Hymenoptera: Torymidae) as inferred from mt-DNA (COI) sequences. *Applied Entomology and Zoology*, **39**, 427–433.

Zabalou, S., Riegler, M., Theodorakopoulou, M., Stauffer, C., Savakis, C. & Bourtzis, K. (2004) *Wolbachia*-induced cytoplasmic incompatibility as a means for insect pest population control. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 15042–15045.

Zabalou, S., Apostolaki, A., Livadaras, I., Franz, G., Robinson, A.S., Savakis, C. & Bourtzis, K. (2009) Incompatible insect technique: incompatible males from a *Ceratitis capitata* genetic sexing strain. *Entomologia Experimentalis Et Applicata*, **132**, 232–240.

Zchori-Fein, E. & Perlman, S.J. (2004) Distribution of the bacterial symbiont *Cardinium* in arthropods. *Molecular Ecology*, **13**, 2009–2016.

Received 1 October 2010; accepted 17 February 2011
Handling Editor: Joan Cossentine

Chapter 2

Introduction and background

We would like to publish this chapter, adapted and changed, as a review.

Introduction and background

Multi-genome organism and associated bacteria

Arthropods may engage in obligate or facultative interactions with microbes. Close associations between host and microbes are described in the literature (O'Neill, Hoffmann & Werren 1997; Baumann 2005). On one hand, the endosymbiont may ensure its maintenance in the host through maternal transmission, and these associations can be obligate for one or both partners and last for the entire life span of the host. These associations are sometimes called symbioses, a term originally referring to "the intimate living together of dissimilar organisms" (O'Neill, Hoffmann & Werren 1997). On the other hand, we observe a large array of bacteria which seem to be loosely associated with the arthropod they live in. Such microorganisms are possibly taken up from the environment during early development or a specific life-stage of the host or transmitted externally between generations (Jones, Knight & Martin 2010). However, even those symbionts can be beneficial to the host or engage in a mutualistic interaction.

Terms used to classify these diverse symbioses are obligatory (primary) and facultative (secondary), describing the intensity of the interaction; mutualistic (beneficial), parasitic or commensal, referring to the "motivation" of the partners to engage in such a relationship. Finally, the extent of vertical or horizontal transmission is an indication of the fidelity of the association through generations and the microorganism's way to maintain itself in a host population. However, symbiont-host associations are very complex, as their nature can change over time, with different phenotypes expressed in changing environments (O'Neill, Hoffmann & Werren 1997).

Maternally-inherited endosymbiotic bacteria (endosymbionts, ES) are very widespread among arthropod species. The two most studied endosymbionts, *Wolbachia* spp. and *Cardinium ssp.* infect 40 and 20% respectively of insect species according to the newest calculations (Kittayapong *et al.* 2003; Zchori-Fein & Perlman 2004; Hilgenboecker *et al.* 2008; Zug & Hammerstein 2012). Infections occur vertically via the egg cytoplasm from infected mother to their offspring. Therefore infection

can spread in the host population if infected females have a reproductive advantage over non-infected females. Four strategies evolved among these ES to achieve this goal; i.e. parthenogenesis induction (PI), cytoplasmic incompatibility (CI), feminization (F) and male-killing (MK) (O'Neill, Hoffmann & Werren 1997). Interestingly, ES were also shown to significantly alter the overall fitness of their host. Aphids for example harbour several endosymbiotical bacteria of different status and with different strategies to sustain their populations in the host: *Wolbachia* can increase its aphid host's survival (Foster *et al.* 2005) and fecundity (Fry, Palmer & Rand 2004). *Hamiltonella defensa* (more precisely its bacteriophage) and *Serratia symbiotica* increase the resistance against parasitoid attack from the genus *Aphidius* (Oliver *et al.* 2003; Ferrari *et al.* 2004; Oliver *et al.* 2009). Interestingly, *Serratia symbiotica* increases its host's heat-tolerance (Montllor, Maxmen & Purcell 2002) and can compensate for the absence of the primary aphid's symbiont *Buchnera* sp. (Koga, Tsuchida & Fukatsu 2003). Finally *Regiella insecticola* was shown to affect aphid's host-plant range, susceptibility to environmental factors, resistance to fungi, dispersal and mating of its host (Chiel *et al.* 2007). Alternatively, detrimental effects related to *Wolbachia* spp. such as increased mortality or reduced life-span were reported (Min & Benzer 1997; Reynolds, Thomson & Hoffmann 2003; Cook, McMeniman & O'Neill 2008).

The same ES species can be associated with very different host species and has individual effects on them. Different strains of *Wolbachia* spp. can, in different hosts, induce all four described reproductive manipulations (PI, CI F and MK), shorten lifespan, and change susceptibility to natural enemies and pathogens (Fytrou *et al.* 2006; Panteleev *et al.* 2007; Hedges *et al.* 2008; Teixeira, Ferreira & Ashburner 2008). Cases have been reported where several ES species (or strains of one) were found in one host population (Duron, Raymond & Weill 2011; Schuler *et al.* 2011; Graham *et al.* 2012; Nakamura *et al.* 2012) or even a single host individual, each inducing their separate phenotype in the host (Graham *et al.* 2012). There have been successful artificial transmissions of ES to a new host, resulting in the same phenotype (McMeniman *et al.* 2009).

In the last few years a number of comprehensive surveys of arthropods' bacterial communities have been published (Jones, Knight & Martin 2010; Chandler *et al.* 2011; Hulcr *et al.* 2012). Rather than focusing on faithful symbiosis throughout generations, these provide a snapshot image of the bacteria present in a given arthropod host, at a particular moment. This potentially includes gut bacteria, which were ingested with the food. Part of the described community might then be transient (not directly transmitted to the offspring). Nevertheless these bacteria may have a function in their host's biology as a part of the multi-genome-organism.

Due to the inability to culture many of arthropod associated bacteria outside the host's body it has, until recently, been difficult to study them. Within the last 20 years, symbiosis research has had gained many tools and has used them widely, which is reflected in a wide palette of published results (see for examples references of this chapter). Metagenomical approaches made possible by next generation sequencing, have provided a new angle to look at microorganismal communities associated with arthropods. The discussions are now directed towards more comprehensive systems, involving many partners as opposed to the earlier focus on bilateral interactions. Examples of discussions on these multi-lateral, looser systems are provided on honeybees (Cox-Foster *et al.* 2007) and ticks (Andreotti *et al.* 2011; Lalzar *et al.* 2012) among others besides our own study on the microbiome of *Rhizoglyphus robini* (chapter 4 of this thesis).

Arthropod- bacteria association and biological control

Endosymbiotical bacteria in biological control can be discussed from two completely different points of view (Floate, Kyei-Poku & Coghlin 2006): There are on one hand symbiont – based control strategies, where the bacteria are directly used to control a pest (McMeniman *et al.* 2009; Zabalou *et al.* 2009 among others). On the other hand, there is the fact that arthropods are very well represented among control agents and pests and therefore very likely to be affected by endosymbionts, which can dramatically affect (positively or negatively) their biology. These infections could affect the success of the biocontrol program and should be considered to optimize

the performance of the control agent or the susceptibility of the pest to the natural enemy used (Zindel, Gottlieb & Aebi 2011; chapter 2, this thesis).

ES-based control strategies have several advantages over other techniques: a) The method does not involve any pesticides, which pose a threat to the environment, b) no non-target effects are expected as the agents are selected to be host specific and c) they possess their own spreading strategy, being adapted to drive themselves into a host population after being introduced via a few infected individuals (Riegler *et al.* 2005) .

Several biological systems have been evaluated for the use of symbiont-based biological control of pest - or disease vector species (Ahantarig & Kittayapong 2011), (Brelsfoard, St Clair & Dobson 2009). There are two main approaches 1) the incompatible insect technique (IIT), based on the incompatibility of differentially infected hosts (Brelsfoard, St Clair & Dobson 2009; Zabalou *et al.* 2009) and the 2) the introduction of a gene or phenotype into a population using a genetically modified endosymbiont (Wang *et al.* 2012). Examples for both approaches are given in the paragraphs below. A third approach, specific to this one system, is presented in a third paragraph.

As an example of the first approach in pest control I would like to mention the transfection of the med fly with a CI-inducing *Wolbachia* spp. strain from the cherry fly *Rhagoletis cerasi* (Zabalou *et al.* 2004). They achieved a stable infection and the rates of suppression in laboratory populations reached 99%. A combination of Cytoplasmic incompatibility (Incompatible insect technique IIT) and radiation is being tested to eradicate the vector of lymphatic filariasis, caused by the nematode *Wuchereria bancroftii*. Male *Aedes polynensis* with a different infection status (quantitative or qualitative) than the field population are repeatedly mass-released into a population. By inundating the population with males carrying an ES causing cytoplasmic incompatibility, most mating in the field will be unfertile. However, the release of a single female carrying the same ES as the released male threatens the entire program as its ES may propagate in the entire population and alleviate the cytoplasmic incompatibility. Thus a combined approach is suggested. The mosquito male carrying an

ES are exposed to radiation for sterilization,before being released (Brelsfoard, Sechan & Dobson 2008; Brelsfoard, St Clair & Dobson 2009; Brelsfoard & Dobson 2011).

Another technique, called paratransgenesis, consists of genetically manipulating the symbiont carried by a pathogen or a pest. The symbiont is engineered to produce a toxin will eliminate the pathogen before it can be transmitted by its vector (Ben Beard, Cordon-Rosales & Durvasula 2002). Symbiont manipulation was tentatively used to disable Trypanosome transmission by the triatomines *Rhodnius prolixus* and *Triatoma infestans (Durvasula et al. 2008)*. The paratransgenesis model was also tested for controlling *Xylella fastidiosa*, the causative agent of Pierce's disease in grapevines, vectored by the glassy-winged sharpshooter *Homalodisca vitripennis* (Ramirez, Perring & Miller 2008) by modifying its symbiont *Alcaligenes xylosoxidans denitrificans*.

A third approach, and possibly the most promising ES-involving vector control approach for field application, might be the introduction of life-shortening *Wolbachia* WmelPop-CLA into the Dengue-virus vector *Aedes aegypti* (McMeniman *et al.* 2009; Walker *et al.* 2011). Indeed, the vectorial capability is age-dependent. Short lived vector individuals can statistically transmit less pathogens to another host than long-lived vectors. However, no estimates of the control rate could be given. Another advantage of the *Wolbachia* strain used in this study is that it also induces complete CI and hence should be able to drive itself into the population.

The research on unintended ES-effects in biological control is emerging and many interesting studies have recently been published (Mochiah *et al.* 2002; Machtelinckx *et al.* 2009; Vasquez *et al.* 2011; Graham *et al.* 2012; Machtelinckx *et al.* 2012). Having written a comprehensive review article on endosymbionts in biocontrol (chapter 2), I will here only discuss the topic with regard to a recent example.

The case study of the African armyworm (Graham *et al.* 2012)

After a few studies have shown the capacity of *Wolbachia* to protect its host from viruses (Hedges *et al.* 2008; Teixeira, Ferreira & Ashburner 2008; Turley *et al.* 2009; Walker *et al.* 2011), Graham *et al.*

(2012) show an example of the contrary: *Wolbachia* spp. can increase the African armyworm Moth's (*Spodoptera exempta*) susceptibility to the Spex-NPVirus.

Spodoptera exempta is one of the most devastating migratory pests in sub-Saharan Africa, affecting staple cereals such as maize, wheat and sorghum among others. During the larval stage it is susceptible to an endemic baculovirus, the *S. exempta* nucleo polyhedrovirus, SpexNPV, which is currently being tested as a biopesticide. Graham *et al.* (2012) found that *S. exempta* populations can be infected with three different W strains, one of them being a male-killer, the others with unknown effects on their host. All three strains have been shown to increase their host's susceptibility to the virus between 6 and 14 times. The authors suggest to a) take into account predictable spatio-temporal fluctuations of *Wolbachia* in the application management of the biopesticide and b) to investigate the possibility of manipulating *Wolbachia* levels in the wild in order to increase negative effects of the virus. The first seems to be a novel way of involving *Wolbachia* in biological control.

Mites – economically important species

The arthropod-subclass Acari (mites and ticks) is very diverse in form, habitat and behaviour. Many of them are of some importance to agriculture, as pests or beneficials. Some mite species are parasites of domestic animals (livestock, poultry, companion animals, honey bees) and humans. A minority of mite species can cause allergic reactions or serious clinical diseases. Apart from direct effects on the vertebrate host, mites may serve as vectors or intermediate hosts for pathogens.

Pest and beneficial mites are of major importance in agricultural environments all over the world. Spidermites can cause damages and loss in several crop plants. Predatory mites such as for example *Hypoaspis miles* or *Phytoseiulus persimilis* prey upon severe pest species and are produced in large quantities of biological control companies and sold worldwide. Predatory mites are among the most important bio control products.

Plant pests

There are four main mite families of crop-pests: the Tetranychidae, the Tenuipalpidae, the Tarsonemidae and the Eryiophidae. Some members of the Acaridae also cause damage to crop plants.

The spider mites and false spider mites (Tetranychidae and Tenuipalpidae) are the most important family comprising more than 1200 known species which are capable to damage a wide range of agricultural crops such as fruit trees, berries, vegetables, field crops and ornamentals (Helle & Sabelis 1985; Gerson 2001). If not controlled, spider mite outbreaks can lead to partial or complete yield losses. The false spider mites in the genus *Brevipalpus* are also known to act as plant pathogen vectors (Childers, Rodrigues & Welbourn 2003).

In the family Tarsonemidae we find two important pest species, the rice mite (*Steneotarsonemus spinki*) and the broad mite (*Polyphagotarsonemus latus*). The broad mite is a pest on fruits and berries while the rice mite, also called panicle rice mite, causes damage by feeding on reproductive tissues of the rice plant as well by transmitting pathogens such as *Fusarium sp*. and *Spiroplasma citri*.

The third mite family of particular interest in agriculture is the family of Eriophyidae, consisting of many plant parasitic gall formers. Many of them (*E. vitis* among others) can cause substantial damage to a wide range of crop species such as coconut trees, wine and plum among many others (Galvao *et al.* 2012; Miller *et al.* 2012). However, some species of this family can be used as biological agents to control weeds and invasive plant species. For example *Aceria malherbae* is being used against field bindweed, *A. chondrillae* against skeletonweed (Milan *et al.* 2006; Skoracka *et al.* 2010; Rodriguez-Navarro *et al.* 2011; Stoeva *et al.* 2012).

Parasites

Among the ectoparasitic mites of vertebrates in temperate climates, several stationary mite species causing mange and scab in livestock and temporary species such as the poultry red mite *Dermanyssus gallinae,* are of major economic importance. Scab mite species are usually controlled

via veterinary treatment of their host. In contrast, *D. gallinae* has to be controlled in the environment of its host, which imposes the application of non-chemotherapeutical control strategies, e.g. biocontrol. Honey bees are parasitized by several mite species: *Varroa destructor* feeds on the hemolymph of honey bee larvae and adults. Besides feeding on the bees, the mite transmits several viruses affecting bee colonies' viability(Chen *et al.* 2004; Gisder, Aumeier & Genersch 2009). Two *Tropilaelaps* spp., which feed on honey bee larvae, are expected to emerge in Switzerland in the future. Besides the ectoparasites, there are also endoparasitical mites in honey bees such as the honey bee tracheal mite *Acarapis woodi*. *Leptus sp*. are also insect parasites, rather large and considered for biological control of agricultural and invasive insect pests (DiBlasi *et al.* 2011). *Sarcoptes scabiei*, (order: Sarcoptiformes) is the causative agent of scabies in humans, mammals in general and other large animals. The itching rashes are caused by females when they burry themselves into the skin of their host for oviposition.

Mite species impacting on human health

The European house dust mite (*Dermatophagoides pteronyssinus*) and the American house dust mite (*Dermatophagoides farinae*) are two different species, but are not necessarily confined to Europe or North America. It is some of their digestive enzymes which persist in their feces and trigger allergic reactions. Ticks are impacting on human health mainly by vectoring bacterial and viral pathogens such as *Rickettsia rickettsii*, the causative agent of Rocky mountain spotted fever, and the tick-borne encephalitis virus TBEV.

Beneficial mites

Beneficial mites are the main natural enemies of several pest mites (e.g. spider mites, stored food mites, bulb mites) or other important pests (e.g. thrips, whiteflies, aphids, mushroom insects, slugs and ants) (Gerson 2001) or used for biological weed control (e.g. *Aceria malherbae* against bindweed) (Rodriguez-Navarro, Torres-Martinez & Olivares-Orozco 2004). Several beneficial mites

are of global importance and commercially used as biological control agents. In recent years about 50 companies were selling 28 species in 7 families (Gerson, Smiley & Ochoa 2003).

Mites and endosymbionts

There is more research on mites and associated bacteria being done than expected and our literature research provides a general overview of the research being conducted in this field.The best studied among mites in this context are the spidermites *Tetranychus sp*., *Panonychus sp*., the false spider mite *Brevipalpus phoenicis*, *Bryobia sp*., the predatory mites *Metaseiulus occidentalis*, and the poultry red mite *Dermanyssus gallinae*. I will review the existing studies and use these species as case studies to highlight the potential and importance of this research field.

Spider mites /plant- and other pest mites

Spider mites and their ES have been extensively studied in Japan (Gotoh, Noda & Hong 2003) and Europe (Breeuwer & Jacobs 1996; Nakamura *et al.* 2009). The distribution of infections of the two best known ES, *Wolbachia* spp. and *Cardinium* spp., has been investigated widely, however, the research has often been abandoned after the detection of an ES or in some cases the exploration of Cytoplasmic Incompatibility (CI), neglecting other potential ES and ES-effects on the spider mite hosts. The best studied species is by far *Tetranychus urticae* (appendix 1), probably due to its worldwide distribution and pest status.

The following ES-effects have been found in spidermites: Cases of *Wolbachia*- and *Cardinium*-induced CI have been shown in several spider mite species such as *Bryobia sarothami*, *Eutetranychus suginamensis* and *T. urticae* (Vala *et al.* 2002; Gotoh, Noda & Ito 2007; Gotoh *et al.* 2007; Ros & Breeuwer 2009). Unspecified reproductive manipulation was shown for 3 *Brevipalpus sp*. by Groot and Breeuwer (2006). Vala and colleagues (2004) discovered behavioural changes (in oviposition and mating) due to *Wolbachia* infection in *T. urticae*. For *T. urticae* an extended microbial community

description was performed and apart from *Wolbachia*, a *Rickettsia* and a *Caulobacter* sequence were molecularly described (Hoy & Jeyaprakash 2005).

Predatory mites

The few studies performed on predatory mites' endosymbionts have not revealed many infections. Breeuwer and Jacobs (Breeuwer & Jacobs 1996) and Enigl and Schausberger (2007) have screened a number of predatory mite species for *Wolbachia* spp., *Cardinium* spp. and *Spiroplasma* spp. respectively and Hoy and Jeyprakash (2005) have investigated the bacterial community associated with *Metaseiulus occidentalis* by PCR (16S rRNA gene) methods (see appendix 2 for their results among others).

Parasitic mites

Parasitic mites and blood feeding ticks and their associated bacterial communities have been researched mainly with the aim to determine their microbial vectoring potential (for example Moro *et al.* 2011) as they are often in contact with several host- individuals or even species, during their lifespan. Apart from the ticks, only *Dermanyssus gallinae* was examined for the common arthropod symbionts (De Luna *et al.* 2009; Moro *et al.* 2009a; Moro *et al.* 2009b; Moro *et al.* 2011) (appendix 3). Jaenike *et al.* (Jaenike *et al.* 2007) have been able to demonstrate the transmission of *Spiroplasma* between *Drosophila melanogaster* individuals by the ectoparasitic mite *Macrocheles subbadius*. *Leptus sp.* have also been discussed in the context of pathogen vectoring in general (Welbourn & Jennings 1991). DiBlasi and colleagues (DiBlasi *et al.* 2011) report closely related *Spiroplasma* strains in *Leptus* mites and their *Agathemera* walking stick hosts, hinting at horizontal transmission.
The bacterial community of poultry red mite, *D.gallinae*, has been extensively studied (De Luna *et al.* 2009; Moro *et al.* 2009b; Moro *et al.* 2011). Besides specific PCR-analysis for the common arthropod-ES C, S and W (see table 3 for results) in several populations, they performed 16S rRNA -TTGE, assessing unknown associated bacteria and found a *Schineria* spp.-ES and a *Rickettsiella* spp.-ES,

although no experiment to confirm intracellular location or maternal transmission was performed (De Luna *et al.* 2009).

Orientia tsutsugamushi, is a less known mite ES, which is also a human pathogen. It causes scrub typhus in East Asia, (Sonthayanon *et al.* 2010). Not much information can be found on its effects on the trombiculid host mites (*Leptotrobidium* spp*.,* chiggers).

Also for ticks, the research has mainly been aimed at identifying vectored pathogens. Bacterial associates of ticks are besides the common arthropod symbionts *Wolbachia*, *Rickettsia* and *Spiroplasma* species: *Arsenophonus nasoniae*, *Coxiella*-like bacteria, *Midichloria mitochondrii* (Sassera *et al.* 2006 among others; Heise, Elshahed & Little 2010; Andreotti *et al.* 2011), *Ehrlichia* and *Francisella sp*. (Scoles 2004).There is only little literature available on the effects or functions these bacteria may have on their hosts. According to a recent review, no "classical" primary or secondary endosymbiont has been reported for ticks (Taylor *et al.* 2012) (appendix 4). However, several associated bacteria possess primary or secondary symbiont characteristica: *Ehrlichia* and *Midichloria,* were recently described to be intracellular (Benson *et al.* 2004; Sassera *et al.* 2006) and a reduction in numbers of the *Coxiella* symbiont in *A. americanum* decreased the fitness of the host (Zhong, Jasinskas & Barbour 2007). *Midichloria mitochondrii* occupies a unique niche within several tick host species- the mitochondriae -, and can infect 100% of individuals in a population in its main host *Ixodes ricinus* (Sassera *et al.* 2006; Epis *et al.* 2008). In some cases, ticks harbor endosymbiont and pathogen species, which are very closely related, such as for example the genera *Francisella*, *Rickettsia* and *Spiroplasma*, which contain tick-endosymbionts as well as tick-borne pathogens (Tully *et al.* 1995; Macaluso *et al.* 2002; Scoles 2004; Bastian *et al.* 2007).

Newer tick studies make use of the new approaches such as metagenomics by next generation sequencing and present a wider view on the microbiome of ticks (Andreotti *et al.* 2011; Lalzar *et al.* 2012). A table with comprehensive tick-bacteria research results can be found in the appendices (appendix 4).

Mites, endosymbionts and biocontrol

In the context of biocontrol the potential use of mite-ES interactions can be separated in 1) symbiont-based control strategies and 2) symbionts as an, often neglected, parameter in pest-and/or control agent biology. I believe that the first point has not been addressed in any published research. The potential ES-based approaches will largely be the same as in insects (see respective paragraph above). Their potential use has been briefly brought up in the context of sheep scab mite (*Psoroptesovis* sp.*)* control (Hall 2011) and for ticks in general (Ghosha, Azhahianambia & Yadavb 2007).

Conclusions

In most presentations on mite-ES I heard at ES-conferences, the speakers related to the mites as a understudied side-topic. While writing this chapter I realized that there is more research being performed on mites and their ES than I expected. I think it is a mistake to treat the mites as a side-topic of the insects rather than their own important group of organisms, especially in the context of endosymbionts.

For both, mites and insects, and their associated bacterial communities the recent progress in molecular methods presents many new possibilities. For over a century the field of microbiology has relied on the ability to culture microorganisms from very different habitats in the laboratory, unwillingly selecting among the diversity present in the environment. In soil habitats an estimated 99% of active microorganisms are not detectible by conventional culture-dependant methods. Studying communities containing organisms with possible intracellular lifestyle the percentage of inculturable microorganisms might be even higher. A combination of old and new molecular methods and culture dependent methods as well as traditional microscopy and histological staining has triggered exciting research in this area.

Many mite-bacteria associations are detected, but there is no further information available on the relationship between the host and the bacterium. Very little ES-effects on mite hosts or functions in

the symbiosis have been identified (tables 1-4) compared to many well-investigated insect-symbioses (Oliver *et al.* 2003; Oliver, Moran & Hunter 2005; Oliver, Moran & Hunter 2006; Oliver *et al.* 2009; Caspi-Fluger *et al.* 2011; Caspi-Fluger *et al.* 2012). This could be related to the low number of research projects on mite ES or to inconclusive, often unpublished, results. Another reason could be the focus on the investigation of bacteria known to be symbionts of insects, rather than approaches directly and unprejudistly investigating bacteria associated with mites. However, even here, new metagenomic surveys promise a considerable change in approaches and findings.

In biological control, mites are already as important as insects (or nearly). The fact that no ES-involving control strategies of – and with mites have been suggested so far, is surprising. This chapter is meant to provide a summary of existing knowledge as a starting point for all research directed towards ES-involving biological control and potentially even people working with mites in biological control. As a long-time goal in mite-ES research the endosymbiont-knowledge should be available and as a result easily testable, whenever problems might appear with the mites, in rearings or in the field.

References

Ahantarig, A. & Kittayapong, P. (2011) Endosymbiotic *Wolbachia* bacteria as biological control tools of disease vectors and pests. *Journal of Applied Entomology,* **135,** 479-486.

Andreotti, R., de Leon, A.A.P., Dowd, S.E., Guerrero, F.D., Bendele, K.G. & Scoles, G.A. (2011) Assessment of bacterial diversity in the cattle tick *Rhipicephalus* (Boophilus) microplus through tag-encoded pyrosequencing. *BMC Microbiology,* **11**.

Bastian, F.O., Sanders, D.E., Forbes, W.A., Hagius, S.D., Walker, J.V., Henk, W.G. & Enright, F.M. (2007) *Spiroplasma* spp. isolated from rabbit ticks or TSE-affected brains induce spongiform encephalopathy in ruminants. *FASEB Journal,* **21,** 1235-1242.

Baumann, P. (2005) Biology of bacteriocyte-associated endosymbionts of plant sap-sucking insects. *Annual Review of Microbiology,* **59,** 155-189.

Ben Beard, C., Cordon-Rosales, C. & Durvasula, R.V. (2002) Bacterial symbionts of the triatominae and their potential use in control of Chagas disease transmission. *Annual Review of Entomology,* **47,** 123-141.

Benson, M.J., Gawronski, J.D., Eveleigh, D.E. & Benson, D.R. (2004) Intracellular symbionts and other bacteria associated with deer ticks (*Ixodes scapularis*) from Nantucket and Wellfleet, Cape Cod, Massachusetts. *Applied and Environmental Microbiology,* **70,** 616-620.

Breeuwer, J.A.J. & Jacobs, G. (1996) *Wolbachia*: Intracellular manipulators of mite reproduction. *Experimental & Applied Acarology,* **20,** 421-434.

Brelsfoard, C.L. & Dobson, S.L. (2011) *Wolbachia* effects on host fitness and the influence of male aging on cytoplasmic incompatibility in *Aedes polynesiensis* (Diptera: Culicidae). *Journal of Medical Entomology,* **48,** 1008-1015.

Brelsfoard, C.L., Sechan, Y. & Dobson, S.L. (2008) Interspecific hybridization yields strategy for south pacific filariasis vector elimination. *Plos Neglected Tropical Diseases,* **2**.

Brelsfoard, C.L., St Clair, W. & Dobson, S.L. (2009) Integration of irradiation with cytoplasmic incompatibility to facilitate a lymphatic filariasis vector elimination approach. *Parasites & Vectors,* **2**.

Brinton, L.P. & Burgdorfer, W. (1976) Cellular and subcellular organization of 277F-Agent, a *Spiroplasma* from rabbit tick *Haemaphysalis leporispalustris* (Acari: Ixodidae). *International Journal of Systematic Bacteriology,* **26,** 554-560.

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. *Proceedings of the Royal Society B-Biological Sciences,* **279,** 1791-1796.

Caspi-Fluger, A., Inbar, M., Mozes-Daube, N., Mouton, L., Hunter, M.S. & Zchori-Fein, E. (2011) *Rickettsia* 'In' and 'Out': Two different localization patterns of a bacterial symbiont in the same insect species. *Plos One,* **6**.

Chandler, J.A., Lang, J.M., Bhatnagar, S., Eisen, J.A. & Kopp, A. (2011) Bacterial communities of diverse *Drosophila* Species: Ecological context of a host-microbe model system. *Plos Genetics,* **7**.

Chen, Y.P., Pettis, J.S., Evans, J.D., Kramer, M. & Feldlaufer, M.F. (2004) Transmission of Kashmir bee virus by the ectoparasitic mite *Varroa destructor*. *Apidologie,* **35,** 441-448.

Chiel, E., Gottlieb, Y., Zchori-Fein, E., Mozes-Daube, N., Katzir, N., Inbar, M. & Ghanim, M. (2007) Biotype-dependent secondary symbiont communities in sympatric populations of *Bemisia tabaci*. *Bulletin of Entomological Research,* **97,** 407-413.

Childers, C.C., Rodrigues, J.C.V. & Welbourn, W.C. (2003) Host plants of *Brevipalpus californicus*, *B. obovatus*, and *B. phoenicis* (Acari: Tenuipalpidae) and their potential involvement in the spread of viral diseases vectored by these mites. *Experimental and Applied Acarology,* **30,** 29-105.

Cook, P.E., McMeniman, C.J. & O'Neill, S.L. (2008) Modifying insect population age structure to control vector-borne disease. *Transgenesis and the Management of Vector-Borne Disease,* **627,** 126-140.

Cox-Foster, D.L., Conlan, S., Holmes, E.C., Palacios, G., Evans, J.D., Moran, N.A., Quan, P.L., Briese, T., Hornig, M., Geiser, D.M., Martinson, V., vanEngelsdorp, D., Kalkstein, A.L., Drysdale, A., Hui, J., Zhai, J.H., Cui, L.W., Hutchison, S.K., Simons, J.F., Egholm, M., Pettis, J.S. & Lipkin, W.I. (2007) A metagenomic survey of microbes in honey bee colony collapse disorder. *Science,* **318,** 283-287.

Clay, K., Klyachko, O., Grindle, N., Civitello, D., Oleske, D. & Fuqua, C. (2008) Microbial communities and interactions in the lone star tick, *Amblyomma americanum*. *Molecular Ecology,* **17,** 4371-4381.

De Luna, C.J., Moro, C.V., Guy, J.H., Zenner, L. & Sparagano, O.A.E. (2009) Endosymbiotic bacteria living inside the poultry red mite (*Dermanyssus gallinae*). *Experimental and Applied Acarology,* **48,** 105-113.

Dergousoff, S.J. & Chilton, N.B. (2010) Detection of a new *Arsenophonus*-type bacterium in Canadian populations of the Rocky Mountain wood tick, *Dermacentor andersoni*. *Experimental and Applied Acarology,* **52,** 85-91.

DiBlasi, E., Morse, S., Mayberry, J.R., Avila, L.J., Morando, M. & Dittmar, K. (2011) New *Spiroplasma* in parasitic *Leptus mites* and their *Agathemera* walking stick hosts from Argentina. *Journal of Invertebrate Pathology,* **107**, 225-228.

Duron, O., Raymond, M. & Weill, M. (2011) Many compatible *Wolbachia* strains coexist within natural populations of *Culex pipiens* mosquito. *Heredity,* **106,** 986-993.

Durvasula, R.V., Sundaram, R.K., Kirsch, P., Hurwitz, I., Crawford, C.V., Dotson, E. & Beard, C.B. (2008) Genetic transformation of a Corynebacterial symbiont from the Chagas disease vector *Triatoma infestans*. *Experimental Parasitology,* **119,** 94-98.

Enigl, M. & Schausberger, P. (2007) Incidence of the endosymbionts *Wolbachia, Cardinium* and *Spiroplasma* in phytoseiid mites and associated prey. *Experimental and Applied Acarology,* **42,** 75-85.

Epis, S., Sassera, D., Beninati, T., Lo, N., Beati, L., Piesman, J., Rinaldi, L., McCoy, K.D., Torina, A., Sacchi, L., Clementi, E., Genchi, M., Magnino, S. & Bandi, C. (2008) *Midichloria mitochondrii* is widespread in hard ticks (Ixodidae) and resides in the mitochondria of phylogenetically diverse species. *Parasitology,* **135,** 485-494.

Ferrari, J., Darby, A.C., Daniell, T.J., Godfray, H.C.J. & Douglas, A.E. (2004) Linking the bacterial community in pea aphids with host-plant use and natural enemy resistance. *Ecological Entomology,* **29,** 60-65.

Floate, K.D., Kyei-Poku, G.K. & Coghlin, P.C. (2006) Overview and relevance of *Wolbachia* bacteria in biocontrol research. *Biocontrol Science and Technology,* **16,** 767-788.

Foster, J., Ganatra, M., Kamal, I., Ware, J., Makarova, K., Ivanova, N., Bhattacharyya, A., Kapatral, V., Kumar, S., Posfai, J., Vincze, T., Ingram, J., Moran, L., Lapidus, A., Omelchenko, M., Kyrpides, N., Ghedin, E., Wang, S., Goltsman, E., Joukov, V., Ostrovskaya, O., Tsukerman, K., Mazur, M.,

Comb, D., Koonin, E. & Slatko, B. (2005) The *Wolbachia* genome of *Brugia malayi*: Endosymbiont evolution within a human pathogenic nematode. *Plos Biology,* **3,** 599-614.

Fry, A.J., Palmer, M.R. & Rand, D.M. (2004) Variable fitness effects of *Wolbachia* infection in *Drosophila melanogaster*. *Heredity,* **93,** 379-389.

Fytrou, A., Schofield, P.G., Kraaijeveld, A.R. & Hubbard, S.F. (2006) *Wolbachia* infection suppresses both host defence and parasitoid counter-defence. *Proceedings of the Royal Society B-Biological Sciences,* **273,** 791-796.

Galvao, A.S., Melo, J.W.S., Monteiro, V.B., Lima, D.B., De Moraes, G.J. & Gondim, M.G.C., Jr. (2012) Dispersal strategies of *Aceria guerreronis* (Acari: Eriophyidae), a coconut pest. *Experimental and Applied Acarology,* **57,** 1-13.

Gerson, U. (2001) Trends in research on acarine biocontrol agents. *10th Int. Congr. Acarology* (eds R.B. Halliday, D.E. Walter, H.C. Proctor & e. al.), pp. 457-459. CSIRO, Melbourne.

Gerson, U., Smiley, R.L. & Ochoa, R. (2003) Mites (Acari) for pest control.Blackwell Science Ltd, Oxford.

Ghosha, S., Azhahianambia, P. & Yadavb, M. (2007) Upcoming and future strategies of tick control: a review. *Journal of Vector Borne Diseases***, 44**, 79–89.

Gisder, S., Aumeier, P. & Genersch, E. (2009) Deformed wing virus: replication and viral load in mites (*Varroa destructor*). *Journal of General Virology,* **90,** 463-467.

Gotoh, T., Noda, H. & Hong, X.Y. (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. (2007) *Cardinium* symbionts cause cytoplasmic incompatibility in spider mites. *Heredity,* **98,** 13-20.

Gotoh, T., Sugasawa, J., Noda, H. & Kitashima, Y. (2007) *Wolbachia*-induced cytoplasmic incompatibility in Japanese populations of *Tetranychus urticae* (Acari: Tetranychidae). *Experimental and Applied Acarology,* **42,** 1-16.

Graham, R.I., Grzywacz, D., Mushobozi, W.L. & Wilson, K. (2012) *Wolbachia* in a major African crop pest increases susceptibility to viral disease rather than protects. *Ecology Letters*, **15**, 993-1000

Grindle, N., Tyner, J.J., Clay, K. & Fuqua, C. (2003) Identification of *Arsenophonus*-type bacteria from the dog tick *Dermacentor variabilis*. *Journal of Invertebrate Pathology,* **83,** 264-266.

Groot, T.V.M. & Breeuwer, J.A.J. (2006) *Cardinium* symbionts induce haploid thelytoky in most clones of three closely related *Brevipalpus species*. *Experimental and Applied Acarology,* **39,** 257-271.

Hall, S. (2011) Novel control of the sheep scab mite, *Psoroptes ovis*, through the application of bacteriophage therapy. PhD, The University of Edinburgh.

Hedges, L.M., Brownlie, J.C., O'Neill, S.L. & Johnson, K.N. (2008) *Wolbachia* and virus protection in insects. *Science,* **322,** 702-702.

Heise, S.R., Elshahed, M.S. & Little, S.E. (2010) Bacterial Diversity in *Amblyomma americanum* (Acari: Ixodidae) with a focus on members of the genus *Rickettsia*. *Journal of Medical Entomology,* **47,** 258-268.

Helle, W. & Sabelis, M.W. (1985) Spider mites: their biology, natural enemies and control. Elsevier, Amsterdam, the Netherlands.

Hilgenboecker, K., Hammerstein, P., Schlattmann, P., Telschow, A. & Werren, J.H. (2008) How many species are infected with *Wolbachia*? - a statistical analysis of current data. *Fems Microbiology Letters,* **281,** 215-220.

Hoy, M.A. & Jeyaprakash, A. (2005) Microbial diversity in the predatory mite *Metaseiulus occidentalis* (Acari: Phytoseiidae) and its prey, *Tetranychus urticae* (Acari: Tetranychidae). *Biological Control,* **32,** 427-441.

Hulcr, J., Rountree, N.R., Diamond, S.E., Stelinski, L.L., Fierer, N. & Dunn, R.R. (2012) Mycangia of *Ambrosia*beetles host communities of bacteria. *Microbial Ecology*.

Jaenike, J., Polak, M., Fiskin, A., Helou, M. & Minhas, M. (2007) Interspecific transmission of endosymbiotic *Spiroplasma* by mites. *Biology Letters,* **3,** 23-25.

Jones, R.T., Knight, R. & Martin, A.P. (2010) Bacterial communities of disease vectors sampled across time, space, and species. *Isme Journal,* **4,** 223-231.

Kittayapong, P., Jamnongluk, W., Thipaksorn, A., Milne, J.R. & Sindhusake, C. (2003) *Wolbachia* infection complexity among insects in the tropical rice-field community. *Molecular Ecology,* **12,** 1049-1060.

Koga, R., Tsuchida, T. & Fukatsu, T. (2003) Changing partners in an obligate symbiosis: a facultative endosymbiont can compensate for loss of the essential endosymbiont *Buchnera* in an aphid. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **270,** 2543-2550.

Lalzar, I., Harrus, S., Mumcuoglu, K.Y. & Gottlieb, Y. (2012) Composition and seasonal variation of *Rhipicephalus turanicus* and *Rhipicephalus sanguineus* bacterial communities. *Applied and Environmental Microbiology,***78,** 4110-4116.

Macaluso, K.R., Sonenshine, D.E., Ceraul, S.M. & Azad, A.F. (2002) Rickettsial infection in *Dermacentor variabilis* (Acari: Ixodidae) inhibits transovarial transmission of a second *Rickettsia*. *Journal of Medical Entomology,***39,** 809-813.

Machtelinckx, T., Van Leeuwen, T., Van De Wiele, T., Boon, N., De Vos, W.H., Sanchez, J.-A., Nannini, M., Gheysen, G. & De Clercq, P. (2012) Microbial community of predatory bugs of the genus *Macrolophus* (Hemiptera: Miridae). *BMC Microbiology,* **12**.

Machtelinckx, T., Van Leeuwen, T., Vanholme, B., Gehesquiere, B., Dermauw, W., Vandekerkhove, B., Gheysen, G. & De Clercq, P. (2009) *Wolbachia* induces strong cytoplasmic incompatibility in the predatory bug *Macrolophus pygmaeus*. *Insect Molecular Biology,* **18,** 373-381.

Mattila, J.T., Burkhardt, N.Y., Hutcheson, H.J., Munderloh, U.G. & Kurtti, T.J. (2007) Isolation of cell lines and a rickettsial endosymbiont from the soft tick *Carios capensis* (Acari: Argasidae: Ornithodorinae). *Journal of Medical Entomology*, **44**, 1091-1101.

Mattila, J.T., Munderloh, U.G. & Kurtti, T.J. (2007) *Rickettsia peacockii*, an endosymbiont of *Dermacentor andersoni,* does not elicit or inhibit humoral immune responses from immunocompetent *D. andersoni* or *Ixodes scapularis* cell lines. *Developmental and Comparative Immunology,* **31,** 1095-1106.

McMeniman, C.J., Lane, R.V., Cass, B.N., Fong, A.W.C., Sidhu, M., Wang, Y.F.& O'Neill, S.L. (2009) Stable introduction of a life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti*. *Science,* **323,** 141-144.

Milan, J.D., Harmon, B.L., Prather, T.S. & Schwarzlander, M. (2006) Winter mortality of *Aceria chondrillae*, a biological control agent released to control rush skeletonweed (*Chondrilla juncea*) in the western United States. *Journal of Applied Entomology,* **130,** 473-479.

Miller, A.D., Umina, P.A., Weeks, A.R. & Hoffmann, A.A. (2012) Population genetics of the wheat curl mite (*Aceria tosichella* Keifer) in Australia: implications for the management of wheat pathogens. *Bulletin of Entomological Research,* **102,** 199-212.

Min, K.T. & Benzer, S. (1997) *Wolbachia*, normally a symbiont of *Drosophila*, can be virulent, causing degeneration and early death. *Proceedings of the National Academy of Sciences of the United States of America,* **94,** 10792-10796.

Mochiah, M.B., Ngi-Song, A.J., Overholt, W.A. & Stouthamer, R. (2002) *Wolbachia* infection in *Cotesia sesamiae* (Hymenoptera: Braconidae) causes cytoplasmic incompatibility: implications for biological control. *Biological Control,* **25,** 74-80.

Montllor, C.B., Maxmen, A. & Purcell, A.H. (2002) Facultative bacterial endosymbionts benefit pea aphids *Acyrthosiphon pisum* under heat stress. *Ecological Entomology,* **27,** 189-195.

Moro, C.V., De Luna, C.J., Tod, A., Guy, J.H., Sparagano, O.A.E. & Zenner, L. (2009a) The poultry red mite (*Dermanyssus gallinae*): a potential vector of pathogenic agents. *Experimental and Applied Acarology,* **48,** 93-104.

Moro, C.V., Thioulouse, J., Chauve, C., Normand, P. & Zenner, L. (2009b) Bacterial taxa associated with the hematophagous mite *Dermanyssus gallinae* detected by 16S rRNA PCR amplification and TTGE fingerprinting. *Research in Microbiology,* **160,** 63-70.

Moro, C.V., Thioulouse, J., Chauve, C. & Zenner, L. (2011) Diversity, geographic distribution, and habitat-specific variations of microbiota in natural populations of the chicken mite, *Dermanyssus gallinae*. *Journal of Medical Entomology,* **48,** 788-796.

Nakamura, Y., Kawai, S., Yukuhiro, F., Ito, S., Gotoh, T., Kisimoto, R., Yanase, T., Matsumoto, Y., Kageyama, D. & Noda, H. (2009) Prevalence of *Cardinium* bacteria in planthoppers and spider mites and taxonomic revision of "*Candidatus Cardinium hertigii*" based on detection of a new *Cardinium*group from biting midges. *Applied and Environmental Microbiology,* **75,** 6757-6763.

Nakamura, Y., Yukuhiro, F., Matsumura, M. & Noda, H. (2012) Cytoplasmic incompatibility involving *Cardinium* and *Wolbachia* in the white-backed planthopper *Sogatella furcifera* (Hemiptera: Delphacidae). *Applied Entomology and Zoology,* **47,** 273-283.

Niebylski, M.L., Schrumpf, M.E., Burgdorfer, W., Fischer, E.R., Gage, K.L. & Schwan, T.G. (1997) *Rickettsia peacockii* sp nov, a new species infecting wood ticks, *Dermacentor andersoni*, in Western Montana. *International Journal of Systematic Bacteriology,* **47,** 446-452.

O'Neill, S.L., Hoffmann, A.A. & Werren, J.H. (1997) *Influential passengers*. Oxford University Press.

Oliver, K.M., Degnan, P.H., Hunter, M.S. & Moran, N.A. (2009) Bacteriophages encode factors required for protection in a symbiotic mutualism. *Science,* **325,** 992-994.

Oliver, K.M., Moran, N.A. & Hunter, M.S. (2005) Variation in resistance to parasitism in aphids is due to symbionts not host genotype. *Proceedings of the National Academy of Sciences of the United States of America,* **102,** 12795-12800.

Oliver, K.M., Moran, N.A. & Hunter, M.S. (2006) Costs and benefits of a superinfection of facultative symbionts in aphids. *Proceedings of the Royal Society B-Biological Sciences,* **273,** 1273-1280.

Oliver, K.M., Russell, J.A., Moran, N.A. & Hunter, M.S. (2003) Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proceedings of the National Academy of Sciences of the United States of America,* **100,** 1803-1807.

Panteleev, D.Y., Goryacheva, II, Andrianov, B.V., Reznik, N.L., Lazebny, O.E. & Kulikov, A.M. (2007) The endosymbiotic bacterium *Wolbachia* enhances the nonspecific resistance to insect pathogens and alters behavior of *Drosophila melanogaster*. *Russian Journal of Genetics,* **43,** 1066-1069.

Plantard, O., Bouju-Albert, A., Malard, M.-A., Hermouet, A., Capron, G. & Verheyden, H. (2012) Detection of *Wolbachia* in the tick *Ixodes ricinus* is due to the presence of the Hymenoptera endoparasitoid *Ixodiphagus hookeri*. *Plos One,* **7**.

Ramirez, J.L., Perring, T.M. & Miller, T.A. (2008) Fate of a genetically modified bacterium in foregut of glassy-winged sharpshooter (Hemiptera: Cicadellidae). *Journal of Economic Entomology,* **101,** 1519-1525.

Reeves, W.K., Loftis, A.D., Sanders, F., Spinks, M.D., Wills, W., Denison, A.M. & Dasch, G.A. (2006) *Borrelia, Coxiella*, and *Rickettsia* in *Carios capensis* (Acari: Argasidae) from a brown pelican (*Pelecanus occidentalis*) rookery in South Carolina, USA. *Experimental and Applied Acarology,* **39,** 321-329.

Reis, C., Cote, M., Paul, R.E.L. & Bonnet, S. (2011) Questing ticks in suburban forest are infected by at least six tick-borne pathogens. *Vector-Borne and Zoonotic Diseases,* **11,** 907-916.

Reynolds, K.T., Thomson, L.J. & Hoffmann, A.A. (2003) The effects of host age, host nuclear background and temperature on phenotypic effects of the virulent *Wolbachia* strain popcorn in *Drosophila melanogaster*. *Genetics,* **164,** 1027-1034.

Riegler, M., Sidhu, M., Miller, W.J. & O'Neill, S.L. (2005) Evidence for a global *Wolbachia* replacement in *Drosophila melanogaster*. *Current Biology,* **15,** 1428-1433.

Rodriguez-Navarro, S., Rodriguez Morell, H., Aleman Martinez, J.A., Flores-Macias, A. & Gustavo Torres-Martinez, J. (2011) Valuation of quality parameters for rearing *Aceria malherbae*

Nuzzaci (Acari: Eriophyidae), a biological control agent of field bindweed, *Convolvulus arvensis* L. *International Journal of Acarology,* **37,** 235-243.

Rodriguez-Navarro, S., Torres-Martinez, G. & Olivares-Orozco, J. (2004) Biological control of field bindweed (*Convolvulus arvensis* L.) using *Aceria malherbae* (Acari: Eriophyidae) in Mexico. *International Journal of Acarology,* **30,** 153-155.

Ros, V.I.D. & Breeuwer, J.A.J. (2009) The effects of, and interactions between, *Cardinium* and *Wolbachia* in the doubly infected spider mite *Bryobia sarothamni*. *Heredity,* **102,** 413-422.

Ros, V.I.D., Fleming, V.M., Feil, E.J.& Breeuwer, J.A.J. (2009) How diverse is the genus *Wolbachia*? Multiple-gene sequencing reveals a putatively new *Wolbachia* supergroup recovered from spider mites (Acari: Tetranychidae). *Applied and Environmental Microbiology,* **75,** 1036-1043.

Ros, V.I.D., Fleming, V.M., Feil, E.J. & Breeuwer, J.A.J. (2012) Diversity and recombination in *Wolbachia* and *Cardinium* from *Bryobia* spider mites. *BMC Microbiology,* **12**.

Sassera, D., Beninati, T., Bandi, C., Bouman, E.A.P., Sacchi, L., Fabbi, M. & Lo, N. (2006) '*CandidatusMidichloria mitochondrii*', an endosymbiont of the tick *Ixodes ricinus* with a unique intramitochondrial lifestyle. *International Journal of Systematic and Evolutionary Microbiology,***56,** 2535-2540.

Schuler, H., Arthofer, W., Riegler, M., Bertheau, C., Krumboeck, S., Koeppler, K., Vogt, H., Teixeira, L.A.F. & Stauffer, C. (2011) Multiple *Wolbachia* infections in *Rhagoletis pomonella*. *Entomologia Experimentalis Et Applicata,* **139,** 138-144.

Schütte, C. (2006) A novel bacterial disease of the predatory mite *Phytoseiulus persimilis*: disease syndrome, disease transmission and pathogen isolation. PhD, Thesis Wageningen University.

Scoles, G.A. (2004) Phylogenetic analysis of the *Francisella*-like endosymbionts of *Dermacentor* ticks. *Journal of Medical Entomology,* **41,** 277-286.

Skoracka, A., Smith, L., Oldfield, G., Cristofaro, M. & Amrine, J.W. (2010) Host-plant specificity and specialization in eriophyoid mites and their importance for the use of eriophyoid mites as biocontrol agents of weeds. *Experimental and Applied Acarology,* **51,** 93-113.

Sonthayanon, P., Peacock, S.J., Chierakul, W., Wuthiekanun, V., Blacksell, S.D., Holden, M.T.G., Bentley, S.D., Feil, E.J. & Day, N.P.J. (2010) High rates of homologous recombination in the mite endosymbiont and opportunistic human pathogen *Orientia tsutsugamushi*. *Plos Neglected Tropical Diseases,* **4**.

Sprong, H., Wielinga, P.R., Fonville, M., Reusken, C., Brandenburg, A.H., Borgsteede, F., Gaasenbeek, C. & van der Giessen, J.W.B. (2009) *Ixodes ricinus* ticks are reservoir hosts for *Rickettsiahelvetica* and potentially carry flea-borne *Rickettsia* species. Parasites & Vectors, **2**.

Stoeva, A., Harizanova, V., de Lillo, E., Cristofaro, M. & Smith, L. (2012) Laboratory and field experimental evaluation of host plant specificity of *Aceria solstitialis*, a prospective biological control agent of yellow starthistle. *Experimental and Applied Acarology,* **56,** 43-55.

Su, H.H., Jiang, F., Yu, M.Z., Yang, X.M., Yang, Y.Z. & Hong, X.Y. (2012) Effects of *Wolbachia* on rDNA-ITS2 variation and evolution in natural populations of *Tetranychus urticae* Koch. *Systematic and Applied Acarology,* **17,** 45-52.

Taroura, S., Shimada, Y., Sakata, Y., Miyama, T., Hiraoka, H., Watanabe, M., Itamoto, K., Okuda, M. & Inokuma, H. (2005) Detection of DNA of *'Candidatus Mycoplasma haemominutum'* and *Spiroplasma sp* in unfed ticks collected from vegetation in Japan. *Journal of Veterinary Medical Science,* **67,** 1277-1279.

Taylor, M., Mediannikov, O., Raoult, D. & Greub, G. (2012) Endosymbiotic bacteria associated with nematodes, ticks and amoebae. *Fems Immunology and Medical Microbiology,* **64,** 21-31.

Teixeira, L., Ferreira, A. & Ashburner, M. (2008) The bacterial symbiont *Wolbachia* induces resistance to RNA viral infections in *Drosophila melanogaster*. *Plos Biology,* **6,** 2753-2763.

Tully, J.G., Rose, D.L., Yunker, C.E., Carle, P., Bove, J.M., Williamson, D.L. & Whitcomb, R.F. (1995) *Spiroplasma ixodetis sp.-nov*, a new species from *Ixodes pacificus* ticks collected in Oregon.*International Journal of Systematic Bacteriology,* **45,** 23-28.

Turley, A.P., Moreira, L.A., O'Neill, S.L. & McGraw, E.A. (2009) *Wolbachia*infection reduces blood-feeding success in the dengue fever mosquito, *Aedes aegypti*. *Plos Neglected Tropical Diseases,* **3**.

Vala, F., Egas, M., Breeuwer, J.A.J. & Sabelis, M.W. (2004) *Wolbachia* affects oviposition and mating behaviour of its spider mite host. *Journal of Evolutionary Biology,* **17,** 692-700.

Vala, F., Weeks, A., Claessen, D., Breeuwer, J.A.J. & Sabelis, M.W. (2002) Within- and between-population variation for *Wolbachia*-induced reproductive incompatibility in a haplodiploid mite. *Evolution,* **56,** 1331-1339.

van Overbeek, L., Gassner, F., van der Plas, C.L., Kastelein, P., Rocha, U.N.D. & Takken, W. (2008) Diversity of *Ixodes ricinus* tick-associated bacterial communities from different forests. *Fems Microbiology Ecology,* **66,** 72-84.

Vasquez, C.J., Stouthamer, R., Jeong, G. & Morse, J.G. (2011) Discovery of a CI-inducing *Wolbachia* and its associated fitness costs in the biological control agent *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae). *Biological Control,* **58,** 192-198.

Walker, T., Johnson, P.H., Moreira, L.A., Iturbe-Ormaetxe, I., Frentiu, F.D., McMeniman, C.J., Leong, Y.S., Dong, Y., Axford, J., Kriesner, P., Lloyd, A.L., Ritchie, S.A., O'Neill, S.L. & Hoffmann, A.A. (2011) The wMel *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. *Nature,* **476,** 450-U101.

Wang, S., Ghosh, A.K., Bongio, N., Stebbings, K.A., Lampe, D.J. & Jacobs-Lorena, M. (2012) Fighting malaria with engineered symbiotic bacteria from vector mosquitoes. *Proceedings of the National Academy of Sciences of the United States of America,* **109,** 12734-12739.

Weeks, A.R. & Breeuwer, J.A.J. (2001) *Wolbachia*-induced parthenogenesis in a genus of phytophagous mites. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **268,** 2245-2251.

Weeks, A.R., Marec, F. & Breeuwer, J.A.J. (2001) A mite species that consists entirely of haploid females. *Science,* **292,** 2479-2482.

Weeks, A.R., Velten, R. & Stouthamer, R. (2003) Incidence of a new sex-ratio-distorting endosymbiotic bacterium among arthropods. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **270,** 1857-1865.

Welbourn, W.C. & Jennings, D.T. (1991) Two new species of Erythraeidae (Acari: Prostigmata) associated with the spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae), in Maine. *Canadian Entomologist,* **123,** 567-580.

Wu, K. & Hoy, M.A. (2012) Extended starvation reduced and eliminated *Wolbachia*, but not *Cardinium*, from *Metaseiulus occidentalis* females (Acari: Phytoseiidae): A need to reassess *Wolbachia*'s status in this predatory mite? *Journal of Invertebrate Pathology,* **109,** 20-26.

Xie, L., Miao, H. & Hong, X.Y. (2006) The two-spotted spider mite *Tetranychus urticae* Koch and the carmine spider mite *Tetranychus cinnabarinus* (Boisduval) in China mixed in their *Wolbachia* phylogenetic tree. *Zootaxa***,** 33-46.

Xie, R.-R., Liu, Y., Hong, X.-Y. & Gotoh, T. (2006) Effect of infection rate of *Wolbachia* on the reproduction in *Tetranychus kanzawai* Kishida (Acari: Tetranychidae) in China. *International Journal of Acarology,* **32,** 407-415.

Zabalou, S., Apostolaki, A., Livadaras, I., Franz, G., Robinson, A.S., Savakis, C. & Bourtzis, K. (2009) Incompatible insect technique: incompatible males from a *Ceratitis capitata* genetic sexing strain. *Entomologia Experimentalis Et Applicata,* **132,** 232-240.

Zchori-Fein, E. & Perlman, S.J. (2004) Distribution of the bacterial symbiont *Cardinium* in arthropods. *Molecular Ecology,* **13,** 2009-2016.

Zhang, X., Norris, D.E. & Rasgon, J.L. (2011) Distribution and molecular characterization of *Wolbachia* endosymbionts and filarial nematodes in Maryland populations of the lone star tick (*Amblyomma americanum*). *Fems Microbiology Ecology,* **77,** 50-56.

Zhong, J., Jasinskas, A. & Barbour, A.G. (2007) Antibiotic Treatment of the Tick Vector *Amblyomma americanum* Reduced Reproductive Fitness. *Plos One,* **2**.

Zindel, R., Gottlieb, Y. & Aebi, A. (2011) Arthropod symbioses: a neglected parameter in pest- and disease-control programmes. *Journal of Applied Ecology,* **48,** 864-872.

Zug, R. & Hammerstein, P. (2012) Still a Host of Hosts for *Wolbachia*: Analysis of Recent Data Suggests That 40% of Terrestrial Arthropod Species Are Infected. *Plos One,* **7**.

Appendix 1 Documented *Wolbachia, Cardinium, Spiroplasma* and *Rickettsia* infections in herbivorous mite species. The letters after the + (present) or –(not found) indicate the studies finding these results. An asterix (*) at the mite species name indicates, that there is more information on associated bacteria available, which is not seen in the table. Superscript after the reference indicates ES-effects on the host, with a "-" if the effect was not detected: CI = Reproductive incompatibility; F = feminization; P = parthenogenesis induction; O = other effect.

phytophagous mite species	**mite family**	***Wolbachia***	***Cardinium***	***Spiroplasma***	***Rickettsia***
Aculops lycopersici	Eryiophyidae	**- a**	**- a**	**- a**	**- a**
Amphitetranychus quercivorus	Tetranychidae	- m	+ c^{-CI}, b		
Amphitetranychus viennensis	Tetranychidae	- m			
Aponychus corpuzae	Tetranychidae	- m			
Aponychus firmianae	Tetranychidae	- m			
Brevipalpus californicus	Tenuipalpidae		+ c^{+F}		
Brevipalpus obovatus	Tenuipalpidae	- e	+ e		
Brevipalpus phoenicis	Tenuipalpidae	- e	+d^{+F}, g^{+PI}, e		
Bryobia berlesei	Tetranychidae	+ i	(- i)		
Bryobia graminum	Tetranychidae	+ f			
Bryobia kissophila	Tetranychidae	+ f, i	(- i)		
Bryobia neopraetiosa	Tetranychidae	+ f			
Bryobia praetiosa	Tetranychidae	+ f^{+PI}, i, j	(- i)		
Bryobia rubrioculus	Tetranychidae	+ k, f	+ i, k	- k	
Bryobia sarothamni	Tetranychidae	+ j^{-CI}, i, l^{-CI+O}	+ j^{+CI}, i, l^{+CI+O}		
Bryobia species I	Tetranychidae	+ i, j	(- i)		
Bryobia species V	Tetranychidae	+ i, j	(- i)		
Carpoglyphus lacti	Carpoglyphidae	**- a**	**- a**	**- a**	**- a**
Cenopalpus pulcher	Tenuipalpidae	- k	- k	- k	
Eotetranychus asiaticus	Tetranychidae	- m			
Eotetranychus cornicola	Tetranychidae	- m			

phytophagous mite species (cont.)	**mite family**	***Wolbachia***	***Cardinium***	***Spiroplasma***	***Rickettsia***
Eotetranychus dissectus	Tetranychidae	- m			
Eotetranychus rubricans	Tetranychidae	- m			
Eotetranychus smithi	Tetranychidae	- m			
Eotetranychus suginamensis	Tetranychidae	- m	+ c^{+Cl}, b		
Eotetranychus tiliarium	Tetranychidae	- m			
Eotetranychus uchidai	Tetranychidae	- m			
Eotetranychus uncatus	Tetranychidae	- m, k	+ k	- k	
Eutetranychus banksii	Tetranychidae	- s			
Eutetranychus orientalis	Tetranychidae	+ s			
Eryophyes vitis	Eryiophyidae	**- a**	**- a**	**- a**	**- a**
Halotydeus destructor	Penthaleidae	- e	- e		
Lepidoglyphus destructor	Glyciphagidae	**- a**	**- a**	**- a**	**- a**
Oligonychus afrasiaticus	Tetranychidae	- n	- n		
Oligonychus biharensis	Tetranychidae	+ s; - m			
Oligonychus coffeae	Tetranychidae	- m			
Oligonychus formosanus	Tetranychidae	- m			
Oligonychus gotohi	Tetranychidae	+ m^{+Cl}, m^{-Cl}			
Oligonychus ilicis	Tetranychidae	- m	+ b, c^{-Cl}		
Oligonychus persea	Tetranychidae	**- a**	**- a**	**- a**	**- a**
Oppiella nova	Oppiidae	+ e	+ e		
Panonychus bambusicola	Tetranychidae	- m			
Panonychus citri	Tetranychidae	- e, m	- e		
Panonychus mori	Tetranychidae	+ m^{+Cl}			
Panonychus osmanthi	Tetranychidae	- m			
Panonychus thelytokus	Tetranychidae	- m			

phytophagous mite species (cont.)	**mite family**	***Wolbachia***	***Cardinium***	***Spiroplasma***	***Rickettsia***
Panonychus ulmi	Tetranychidae	**- a**	**+ a**	**- a**	**- a**
Penthaleus major	Penthaleidae	- e	- e		
Petrobia harti	Tetranychidae	+ e, (- i)	+ e, i		
Sancassania berlesei	Acaridae	**- a**	**- a**	**- a**	**- a**
Sasanychus akitanus	Tetranychidae	- m			
Schizotetranychus bambusae	Tetranychidae	- m			
Schizotetranychus cercidiphylli	Tetranychidae	+ m			
Schizotetranychus leguminosus	Tetranychidae	- m			
Schizotetranychus longus	Tetranychidae	- m			
Schizotetranychus recki	Tetranychidae	- m			
Schizotetranychus schizopus	Tetranychidae	- m			
Tetranychus cinnabarinus	Tetranychidae	+ p; -e	- e		
T. eozensis	Tetranychidae	- m			
T. kanzawai	Tetranychidae	+ m^{-Cl}, v, $r^{+Cl, -Cl}$, s			
T. ludeni	Tetranychidae	- m			
T. neocaledonicus	Tetranychidae	+ s; -m			
T. parakanzawai	Tetranychidae	+ m^{-Cl}			
T. phaselus	Tetranychidae	- m			
T. piercei	Tetranychidae	- m			
T. puericola	Tetranychidae	+ m^{-Cl}	+ c^{-Cl}, b		
T. takafujii	Tetranychidae	- m			
T. turkestani	Tetranychidae	+ s			
T. urticae[u]	Tetranychidae	+ **a**, m^{-Cl}, s, t^{+Cl}, v^{+Cl}, o^{+O}, u, i, k, p, q, j, a, e; **- a**, n, m	+ **a** , c^{-Cl} ,b, l, **- a**, k, n, l, e	+ k; **- a**	+ u; **- a**
Rhizoglyphus robini[z]	Acaridae	**- a**, n	**- a**, n	**- a**	**- a**

phytophagous mite species (cont.)	**mite family**	***Wolbachia***	***Cardinium***	***Spiroplasma***	***Rickettsia***
Tyrophagus sp.	Acaridae	- k	- k	+ k	
Tyrophagus putrescentiae	Acaridae	- **a**, n	- **a**, n	**- a**	**- a**
Yezonychus sapporensis	Tetranychidae	- m			

a = chapter 3, this thesis; b = Nakamura *et al.* 2009; c = Gotoh, Noda & Ito 2007; d = Groot & Breeuwer 2006; e = Weeks, Velten & Stouthamer 2003; f = Weeks & Breeuwer 2001; g = Weeks, Marec & Breeuwer 2001; h = Wu & Hoy 2012; i = Ros *et al.* 2012; j = Ros *et al.* 2009; k = Enigl & Schausberger 2007; l = Ros & Breeuwer 2009; m = Gotoh, Noda & Hong 2003; n = Zchori-Fein & Perlman 2004; o = Vala *et al.* 2004: p = Xie, Miao & Hong 2006; q = Su *et al.* 2012; r = Xie *et al.* 2006; s = Breeuwer & Jacobs 1996; t = Vala *et al.* 2002; u = Hoy & Jeyaprakash 2005; v = Gotoh *et al.* 2007 ; w = De Luna *et al.* 2009; x = DiBlasi *et al.* 2011; y = Jaenike *et al.* 2007; z = chapter 4, this thesis ; *in this species there were more associated bacteria described.

Appendix 2 Endosymbiont infections in predatory mite species. The letters after the + (present) or –(not found) indicate the studies finding these results. An asterix (*) at the mite species name indicates, that there is more information on associated bacteria available, which is not seen in the table. Superscript after the reference indicates found ES-effects on the host: CI = Reproductive incompatibility; F = feminization; O = other effect; . In brackets if the effect was investigated, but the results are unclear. R was not included in the table because only our study investigated its presence and obtained only negative results.

predatory mite species	mite family	*Wolbachia*	*Cardinium*	*Spiroplasma*
Amblyseius andersoni	Phytoseiidae	- s		
Amblyseius cucumeris	Phytoseiidae	- **a**, s, k	- **a**, k	- **a**, k
Amblyseius herbicolus	Phytoseiidae	- s		
Amblyseius swirskii	Phytoseiidae	**- a**	**- a**	**- a**
Euseius finlandicus	Phytoseiidae	- k	+ k; -k	- k
Euseius scutalis	Phytoseiidae	**- a**	**- a**	**- a**
Galendromus annectens	Phytoseiidae	+ e	- e	
Galendromus helveolus	Phytoseiidae	- e	- e	
Galendromus occidentalis	Phytoseiidae	+ s		
Hypoaspis aculeifer	Laelapidae	- **a**	+ **(a)**	+ **(a)**
Hypoaspis miles	Laelapidae	- **a**	- **a**	+ **a**
Iphiseius degenrans	Phytoseiidae	- s		
Kampimodromus aberrans	Phytoseiidae	- k	- k	- k
Mesoseiulus longipes	Phytoseiidae	+ e	- e	
*Metaseiulus occidentalis**[u]	Phytoseiidae	+ e, u, n,	+ e, n, u, h[CI]	- (u)
Neoseiulus barkeri	Phytoseiidae	+ s; - k	- k	+ (k)
Neoseiulus bibens	Phytoseiidae	+ s		
Neoseiulus californicus	Phytoseiidae	- **a**, n, s, k	- **a**, n, k	+ **(a)**, k
Neoseiulus cucumeris	Phytoseiidae	+ n	- n	
Neoseiulus fallacis	Phytoseiidae	- e	- e	
Phytoseiulus macropilis	Phytoseiidae	- e	- e	
Phytoseiulus persimilis[aa]	Phytoseiidae	+ s, n, e; - **a**, k	- **a**, k, n, e	- **a**, k
Typhlodromus neotunus	Phytoseiidae	- s		
Typhlodromus pyri	Phytoseiidae	- s, k	- k	- k

a = chapter 3, this thesis; e = Weeks, Velten & Stouthamer 2003; h = Wu & Hoy 2012; k = Enigl & Schausberger 2007; n = Zchori-Fein & Perlman 2004; s = Breeuwer & Jacobs 1996; u = Hoy & Jeyaprakash 2005; aa = Schütte 2006; *in this species there were more associated bacteria described.

Appendix 3 Endosymbiont infections in parasitic mite species. The letters after the + (present) or –(not found) indicate the studies finding these results. An asterix (*) at the mite species name indicates, that there is more information on associated bacteria available, which is not seen in the table. Superscript after the reference indicates found ES-effects on the host: CI = Reproductive incompatibility; F = feminization; O = other effect; In brackets if the effect was investigated, but the results are unclear.

parasitic mite species	**family**	***Wolbachia***	***Cardinium***	***Spiroplasma***	***Rickettsia***
Balaustium sp.	Erythraeidae	+ e	+ e		
Chorioptes sp.	Psoroptidae	- **a**	- **a**	- **a**	- **a**
*Dermanyssus gallinae**[w]	Dermanyssidae	- w; + **a**	+w, **a**	+ w; - **a**	+ w; - **a**
Leptus lomani	Erythraeidae			+ x	
Leptus sayi	Erythraeidae			+ x	
Macrocheles robustus	Macrochelidae	- **a**	- **a**	- **a**	- **a**
Macrocheles subbadius	Macrochelidae			+ (y)	
Varroa destructor	Varroideae	- **a**	- **a**	- **a**	- **a**

a = chapter 3, this thesis; e = Weeks, Velten & Stouthamer 2003; w = De Luna *et al.* 2009 ; x = DiBlasi *et al.* 2011 ; y = Jaenike *et al.* 2007; *in this species there were more associated bacteria described

Appendix 4 Documented *Wolbachia, Cardinium, Spiroplasma* and *Rickettsia*, as well as *Francisella, Coxiella, Arsenophonus, Midichloria* and *Ehrlichia* infections in 30 tick species. The letters after the + (present) or – (not found) indicate the studies finding these results. An asterix (*) at the tick species name indicates, that there is more information on associated bacteria available, which is not seen in the table.

Tick species	**family**	***Wolbachia***	***Cardinium***	***Spiroplasma***	***Rickettsia***	***Francisella***	***Coxiella***	***Arsenophonus***	***Midichloria***	***Ehrlichia***
Amblyomma americanum	Ixodidae	+ E			+ I		+ I	+ I	- J	
Amblyomma maculatum	Ixodidae					+ C			- J	
Amblyomma tuberculatum	Ixodidae								+ J	
Carios capensis	Ornithodorina				+ S, T		+S			
Dermatocentor albipictus	Ixodidae					+ C			- J	
Dermatocentor andersoni	Ixodidae				+ D	+ C, Q		+ O		
Dermatocentor hunteri	Ixodidae					+ C				
Dermatocentor marginatus	Ixodidae								- J	
Dermatocentor nitens	Ixodidae					+ C				
Dermatocentor occidentalis	Ixodidae					+ C				
Dermatocentor reticulatus	Ixodidae								- J	
Dermatocentor variabilis	Ixodidae					+ C		+ P	- J	
Haemaphysalis leporispalustris	Ixodidae			+ R						
Haemaphysalis inermis	Ixodidae								- J	
Haemaphysalis palustris	Ixodidae								- J	
Haemaphysalis punctata	Ixodidae								+ J	
Hyalomma marginatum	Ixodidae								+ J	
Hyalomma truncatum	Ixodidae								+ J	
Ixodes hexagonus	Ixodidae								- J	
Ixodes pacificus	Ixodidae	- A	- A	+ L					- J	
*Ixodes ovatus** [M]	Ixodidae			+ M						
*Ixodes ricinus** [F, K]	Ixodidae	+ B, F			+ F, K, U	+ K			+ J, F	+ F
*Ixodes scapularis** [G]	Ixodidae	+ B, G			+ G				- J	+ G
Ixodes uriae	Ixodidae								+ J	
Ornithodoros porcinus	Ornithodorina					+ C				

Tick species (cont.)	**family**	***Wolbachia***	***Cardinium***	***Spiroplasma***	***Rickettsia***	***Francisella***	***Coxiella***	***Arsenophonus***	***Midichloria***	***Ehrlichia***
Rhipicephalus annulatus	Ixodidae								- J	
Rhipicelphalus bursa	Ixodidae								+ J	
Rhipicephalus microplus[H]*	Ixodidae	+ B, H					+ H			
Rhipicephalus sanguineus[N]*	Ixodidae	+ B			+ N		+ N	+ R	- J	
Rhipicephalus turanicus[N]*	Ixodidae				+ N		+ N		+ J	

A = Weeks, Velten & Stouthamer 2003; B = Plantard *et al.* 2012; C = Scoles 2004; D = Mattila, Munderloh & Kurtti 2007; E = Zhang, Norris & Rasgon 2011; F = van Overbeek *et al.* 2008; G = Benson *et al.* 2004; H = Andreotti *et al.* 2011; I = Clay *et al.* 2008; J = Epis *et al.* 2008; K = Reis *et al.* 2011; L = Tully *et al.* 1995; M = Taroura *et al.* 2005; N = Lalzar *et al.* 2012; O = Dergousoff & Chilton 2010; P = Grindle *et al.* 2003; Q = Niebylski *et al.* 1997; R = Brinton & Burgdorfer 1976; S = Reeves *et al.* 2006; T = Mattila *et al.* 2007; U = Sprong *et al*. 2009

Chapter 3

Arthropod endosymbionts: A survey for ´common suspects´ in agriculturally important mites

We aim to publish this paper in *Biological Control.*

Arthropod endosymbionts: A survey for ´common suspects´ in agriculturally important mites

Zindel R., Kuske S., Turlings T.C.J., Vorburger Ch. and Aebi A.

Abstract

The arthropod subclass Acari (mites and ticks) is economically important as it includes many pests, disease vectors and control agents, and can as parasites or allergenes significantly impact human- and animal health. Like insects, mites and ticks are often infected with endosymbiotic bacteria, which may affect their hosts' reproduction and modify fecundity, longevity, and susceptibility to natural enemies or pesticides. By changing the mites' life history traits, the symbionts may also influence biological control programs with or of their hosts. The aim of this study was to create an overview of the distribution of known symbionts in a selection of 21 mite species. We screened for 6 symbionts with specific PCR-primers. Besides confirming known endosymbiont infections, we report a new *Wolbachia* infection in *Dermanyssus gallinae* (De Geer), a *Cardinium* in the spider mite *Panonychus ulmi* (Koch) and a *Spiroplasma* strain in the predatory mite *Hypoaspis miles* (Berlese). Phylogenetic analysis revealed the first supergroup A – *Wolbachia* in a mite (*D. gallinae*). From an applied perspective, these findings on the widespread presence of endosymbionts in pest mites are important for control programs, as they can explain resistances against control agents and the may help to optimally exploit endosymbionts in rearing efforts and to enhance the control agents' efficiency.

Keywords: Acari, *Wolbachia, Cardinium, Spiroplasma, Stratiolaelaps scimitus*

Introduction

The view on organisms is slowly shifting from the classical „species“ paradigm to a modern multi genome arrangement concept, where many species contribute to the biology of a core organism. This new way of seeing an organism may have an impact on all research and applied fields depending on accurate knowledge of an organism's biology. Biological control (BC) in particular may be affected positively or negatively by the interactions between the biological control agent or the pest, and their potential symbionts (Zindel et al. 2011, chapter 2, this thesis). Here we focus on mites, which are among the most frequently used organisms in biological control, and on several of their maternally transmitted endosymbiotical bacteria (ES).

Mites (Acari) have, despite their economical importance, been largely neglected in ES research. While insects, their sister taxon within the arthropods, and their association with ES have been investigated in the context of fundamental symbiosis research (Werren et al. 2008), immunity related questions (Gross et al. 2009; Siozios et al. 2008; Wong et al. 2011), ES-based control strategies (Ahantarig and Kittayapong 2011; Brelsfoard and Dobson 2011; McMeniman et al. 2009) and evolutionary implications (Bull 1981; Charlat et al. 2003; Moran et al. 2008; Tortosa et al. 2010), is the number of studies conducted on mites and associated bacteria much lower, but has increased in the last few years. Mites are very diverse and include phytophagous, fungivorous, predatory and parasitic species, all of which can be of great importance to humans. Spidermites (Tetranychidae) and to a certain extent Acaridae (*Rhizoglyphus robini* as an example) can cause severe losses in agriculture, feeding on the leaves or other parts of crop plants. Dust mites (*Dermatophagoides* spp.) (to be more precise: their feces) are known to cause allergies in some people ranging from rashes to asthma (Ferres et al. 2011). The *Varroa* mites have received a lot of attention recently because of their potential role in the bee colony collapse disorder, impairing honey production (Potts et al. 2010). However, many mites are also beneficial. Predatory mites, such as *Phytoseiulus persimilis*, are commonly used to control thrips, spidermites and other pest species on crops or ornamentals and

are among the best selling products in biological control companies. Finally, phytophagous Eryiophyid mite species are regularly used in the control of weeds (Rodriguez-Navarro et al. 2004).

Endosymbiotic bacteria infect the majority of arthropod taxa (Duron et al. 2008; Hilgenboecker et al. 2008; Weinert et al. 2007; Zchori-Fein and Perlman 2004). Estimations of infection frequency for the two most studied maternally inherited bacterial ES, *Wolbachia* ssp. and *Cardinium* ssp. range between 40% and 66% and 6-7 and 21% respectively of insect species (Duron et al. 2008; Hilgenboecker et al. 2008; Zug and Hammerstein 2012). Given that arthropods represent a large part of our planet's biomass, maternally inherited ES are probably the most common bacteria occurring in association with living organisms on Earth. Endosymbiotic bacteria can be divided into obligatory and facultative symbionts. Obligatory symbionts are involved in, and sometimes fully responsible for, vital functions of their host (Baumann 2005). Facultative ES, on the other hand, are not essential for host survival and their presence can be neutral, beneficial or detrimental to their host (Oliver et al. 2003; Perotti et al. 2006). Both, obligatory and facultative ES can be involved in the feeding (Gunduz and Douglas 2009; Hosokawa et al. 2010), reproduction (O'Neill et al. 1997; Werren et al. 2008) or defense mechanisms of their host (Brownlie and Johnson 2009; Haine 2007). As reviewed by Zindel et al. (2011) the strong influence of these bacteria on their host's biology has implications for the use of such arthropods in pest or the efficiency of its own control.

With this study we wish to shift the general focus from endosymbionts in insects to those in mites and get an idea of their distribution in mites, across species with different lifestyles. In a total of 21 mite species we screened for the presence of six species of known bacterial endosymbionts (*Arsenophonus* (A)*, Cardinium* (C), *Flavobacterium* (F), *Rickettsia* (R), *Spiroplasma* (S) and *Wolbachia* (W)), using specific PCR-primers. Besides confirming known ES-infections, we report a new *Wolbachia* infection in the poultry red mite, *Cardinium* in the spider mite *Panonychus ulmi* and a *Spiroplasma* strain in the predatory mite *Hypoaspis miles* Berlese (recently renamed *Stratiolaelaps scimitus* Womersley). Phylogenetic analysis revealed the first supergroup A – *Wolbachia* in a mite (*D. gallinae*). From an applied perspective, these findings of ES in pest mites are important, as they can

be used to optimize control programs or to explain resistance in pests against certain control agents. Moreover, knowledge on ES infections in control agents can be used to explain and solve rearing problems such as skewed sex ratios and cytoplasmic incompatibility between populations of different origin.

Materials and methods

A total of 21 mites species (32 populations) were screened for the presence of (*Arsenophonus* (A)*, Cardinium* (C), *Flavobacterium* (F), *Rickettsia* (R), *Spiroplasma* (S) and *Wolbachia* (W). Samples were provided by acarologists and biological control companies. Most populations stemmed from established laboratory colonies.

Sample collection and DNA extraction: A minimum of 20 females and 10 males were killed and stored in 95% ethanol at -20°C until DNA was extracted. DNA of species larger than 2 mm was extracted in Chelex, for smaller mites the "hotshot" protocol by Montero-Pau and colleagues (Montero-Pau et al., 2008) was used. After extraction, DNA quality was evaluated by PCR using arthropod specific primers (Duron et al., 2008).

Endosymbiont screen: Specific primer sets for W, C, F, R, S and A were used (table 1). PCR reactions had a total volume of 25µl, containing 2µl of template DNA lysate, 5µl of PCR buffer (5x), 0.5µl of dNTP's (10mM), 2µl of $MgCl_2$ (25mM), 0.35µl of each primer (at 10mM) and 0.25µl of Taq polymerase (Promega, Flexi Go Taq). PCR conditions followed instructions in the literature (table 1), with slight modifications where necessary. A negative control (ddH_2O) and a positive control consisting of an infected arthropod individual were amplified along each batch of PCR reactions. PCR products were separated on a 1% agarose gel, stained with ethidium bromide and run at 75 volts for 45 minutes in 1% TAE buffer. The presence of bands was visualized and documented under UV light.

MLST *Wolbachia*: To ascertain strain similarity and to avoid misinterpretation due to recombinations, a multi-locus sequence typing approach (MLST) was used (Baldo et al., 2006). We additionally performed PCR and subsequent sequencing on five genes (gatB, fbpA, coxA, ftsZ and hcpA), following

protocol instruction on the MLST webpage (http://pubmlst.org/). Only for coxA we obtained consistent bands and sequences of all positive samples and hence, used coxA in combination with wsp in phylogenetic analyses.

Sequencing: Positive PCR products were cleaned enzymatically using Exonuclease I (New England Biolabs, through BioConcept, Allschwil, Switzerland) and Shrimp Alkaline phosphatase (Promega, Dübendorf, Switzerland) according to the following protocol: Remaining primers and nucleotides were removed by incubating 20 µl of PCR product with 0.8 µl of Shrimp Alkaline Phosphatase (SAP, 5000 U ml-1) and 1.2 µl of Exonuclease I (10 mM, with dilution buffer) for 1 hour at 37C°.
Alternatively they were cleaned by filtration (Millipore AG, Zug). Sequencing was carried out on an automated ABI3130xl Genetic Analyzer machine using ABI BigDye version 3.1 Terminator Sequencing chemistry. Chromatogram output quality was checked by eye and corresponding sequences (forward and reverse) aligned in Sequencher 4.9 (Gene Codes, Ann Arbor, USA). Sequences are deposited in GenBank (accession numbers JX844805-JX844824).

Phylogenetic analysis of identified bacteria (W, C and S): Obtained sequences and reference sequences from GenBank, (National Center for Biotechnology Information (NCBI)) were aligned using the alignment software Clustal X2 (Version 2.0, Larkin et al. 2007) and further adjusted by eye. The data set was analysed using the Neighbor-Joining method with maximum likelihood distance measure in PAUP (4.0 beta Win Swofford 2001). jModeltest version 0.1.1 (Posada, 2008) was used to choose the most likely model of nucleotide evolution. Bootstrap values for branches support were calculated in PAUP, performing 1000 replications. Trees were then graphically expressed (Tree view v1.6.6, Page 1996) and labeled (Adobe Illustrator v10.39, Adobe systems GMbH, München). Further details to individual trees are given below.

A phylogenetic tree, including available representatives of every *Wolbachia* supergroup, was constructed with the two housekeeping genes wsp and coxA. In the wsp- tree some additional mite sequences were included (*Bryobia sp.*) and representatives of supergroups C and F, which were not available for coxA (appendices 1 and 2). In the CoxA-tree supergroup H, J and E could be included

(figure 1 and appendix 2). For *Cardinium* and *Spiroplasma,* phylogenetic trees were constructed including as much variability of strains a possible (based on published trees), focusing on mite symbionts (figures 2 and 3; appendices 3 and 4).

Results

Twenty-one mite mites from 32 populations were screened for a total of 6 species of bacterial endosymbionts, using species-specific primers (table 2). *Wolbachia* was detected in a population of *Tetranychus urticae* from our laboratory population (ART, Zürich, Switzerland) and in a population of *Dermanyssus gallinae* from the FiBL (Forschungsinstitut für biologischen Landbau, Frick, Switzerland). *Cardinium* was found in another swiss *T. urticae* population, a swiss *Panonychus ulmi* population and *D. gallinae. Spiroplasma* positives were only found in the predatory mite *Hypoaspis miles* (now renamed *Stratiolaelaps scimitus*), but there in all three tested populations. More positive samples were detected by PCR, but infection could not be confirmed by sequencing due to low quality or quantity of bacterial material (indicated in brackets in table 2).

Wolbachia*:* The W infection rate in the populations of the two mite species tested positively in this study was low (1/30 and 2/30 respectively). The phylogenetic trees based on wsp and coxA sequences (figure 1, appendix 1) shows that the *T. urticae* symbiont sequences obtained in this study (JX844805 and JX844806) are most similar to other sequences derived from *T. urticae* W-symbionts assigned to supergroup B. All but one supergroup B assigned sequences are within one monophyletic group with reasonable bootstrapping support (70%). The W symbiont sequenced from *Dermanyssus gallinae* seems to be closely related to a W strain infecting *Drosophila melanogaster* and *Nasonia longicornis*, assigned to supergroup A (Ros et al., 2009). This is to our knowledge the first supergroup A W-strain found in a mite. Interestingly, a W sequence included only in the coxA tree, obtained from an endosymbiont infecting the Acari family of Ixodidae (*Amblyomma americanum*, Ixodida* HM061159) in the phylogenetic analysis, does not seem to belong into one of the common supergroups A or B.

***Cardinium**:* In populations positive for C, 32/50 (*P. ulmi*), 15/30 (*T. urticae*) and 17/50 (*D. gallinae*) individuals were infected. As already described in Zchori-Fein and Perlman (Zchori-Fein and Perlman, 2004) based on their 16S rRNA gene sequences, mite - C symbionts seem to be closer related among themselves than to insect-C-symbionts (figure 2). However, this result has little bootstrap support (values below 50%). In general there seems to be little genetic variation in C sequences examined in this tree and accordingly nodes close to the root of the tree have bootstrap values below 50% (not indicated in figure 2). Interestingly, C sequences obtained from the poultry red mite in this study as well as a sequence from a *Chaetodactylus sp*. appear to form a potentially new clade outside the classical "mite" and the "insect"-groups.

***Spiroplasma**:* Infection rates of S in *H. miles* in all three populations tested were high (10/30, 41/30 and 12/30 respectively). The S-sequences from our 3 populations are equally similar between and among the three tested populations, suggesting an infection by a similar strain, possibly the same, although to answer this question more than one gene should be sequenced. *Spiroplasma* sequences obtained in this study were most similar to the species *S. platyhelix* according to BLAST. *Spiroplasma platyhelix* is sometimes associated with species of the Odonata (Williamson et al., 1997). In the phylogenetic tree based on the 16S rRNA gene (figure 3) our S sequences are situated within the "new" clade described by DiBlasi and colleagues (2011).

Discussion

Many arthropods are infected with endosymbionts (ES). According to newest estimations, 40%, 21% and 34%) of arthropod species are infected with W, C and S respectively (Zug and Hammerstein, 2012). Preliminary surveys of endosymbiont diversity and distribution in arthropods suggested that the arachnids harbor more W, C and maybe also S than other arthropod groups and may for some ES species even represent a diversity hotspot (Chang et al., 2010; Duron et al., 2008). Thus, we expected to find at least equally high infection rates in mites as in arthropods in general. However, few mite species we screened were found to be infected with W, C and S and we can report no new infections

of R, F and A, despite the fact that we included an average of 35 individuals per host population, if possible several populations per host species and 6 different ES species. This disprepancy could be due to many reasons.

The possibility of missing ES, which are in fact present in a host population, cannot be ruled out, even with 35 host individuals tested (Weinert et al., 2007). Duron and colleagues (2008) mention geographic sampling range as a source of finding differences in ES prevalence. For some mite species we did not distinguish between females and males and could have included a high percentage of males in a population with only infected females, which increases the chance of missing an potential ES infection. Also, only screening for insect key ES, we might have missed the typical and influential mite symbionts.

It is however unlikely, that an ES with very low infection percentage in a population has a significant influence on the host unless it is still in the process of establishment. Hence would low-prevalence infections not be relevant in the context of biological control. Population bottle necks in the establishment of laboratory strains should also be mentioned here: Almost all of the screened populations had been in the laboratory for several generations, with the result that the infection patterns we found may not represent ES infection rates in the wild. There is also the possibility of false negatives due to low bacterial titres, amplification inhibition or too specific primers (Simoes et al., 2011).

Wolbachia is the best described reproductive ES to date, infecting a diverse range of organisms and exhibiting an equally wide range of effects on its hosts (Adachi-Hagimori et al., 2008; Duplouy et al., 2010; Kageyama et al., 2002; Tagami et al., 2001; Vasquez et al., 2011 and many more). In mites, W-ES have been reported mainly from the family Tetranychidae, but also Oppiidae, Phytoseiidae and Erythraeidae (Breeuwer and Jacobs, 1996; Gotoh et al., 2003; Ros et al., 2009; Weeks et al., 2003). Possible reproductive effects of the W-ES on the mite hosts has only been investigated in tetranychids and some cases of Cytoplasmic incompatibility (CI) and Parthenogenesis induction (PI) could be shown (Gotoh et al., 2003; Vala et al., 2002; Weeks and Breeuwer, 2001; Xie et al., 2006).

Wolbachia-ES sequences from *Tetranychus urticae* have been published before, but this is to our knowledge the first record of a W-ES in *Dermanyssus gallinae*. At the same time, the W sequence from *D. gallinae* appears to be a supergroup A strain – the first one to be found in mites. Of 30 individual *D. gallinae* screened, we only had one positive for W. With such a low infection rate it is hard to speculate about possible effects on the host and more screening should be conducted. De Luna and colleagues (2009) screened several populations of *D. gallinae* and did not detect any W, but some C and S sp. among others. The W-ES sequences from *Tetranychus urticae* were found to be most similar to B-supergroup sequences confirming the presence of at least one supergroup B W strain in this mite (Ros et al., 2009) (figure 1).

Cardinium also covers a wide range of known reproductive manipulations (except male-killing) as well as a number of other effects on its different host species (Giorgini et al., 2009; Hunter et al., 2003; Ros and Breeuwer, 2009; Weeks et al., 2001; Weeks and Stouthamer, 2004; Zchori-Fein et al., 2001). In mites, mostly Tetranychidae have been tested for C (Enigl and Schausberger, 2007; Nakamura et al., 2009; Weeks et al., 2003) and some reproductive manipulations have been detected (for example Gotoh et al., 2007; Groot and Breeuwer, 2006). Our results confirm C-infections in *T. urticae* and *D. gallinae* (De Luna et al., 2009; Nakamura et al., 2009) and report a new incidence in *Panonychus ulmi*. The relatively low number of C-infections outside the spidermites we found in this study is surprising as the Arachnids had previously been presented as a C-infection hotspot (Chang et al., 2010; Duron et al., 2008; Gotoh et al., 2003). However, looking at literature this might be true mainly for the Tetranychidae (chapter 1, this thesis). Outside the spidermites only a few incidences of C-infection are documented. The phytoseiids *Metaseiulus occidentalis* (Hoy and Jeyaprakash, 2005) and *Euseius finlandiscus* (Enigl and Schausberger, 2007), the bird parasite *D. gallinae* (Dermanyssidae) (this study and (De Luna et al., 2009) and the false spidermite genus *Brevipalpus* (Tenuipalpidae) (Groot and Breeuwer, 2006) were shown to be infected with C. Negative evidence of C has been collected by several studies (Enigl and Schausberger, 2007; Ros et al., 2012); this study; (Zchori-Fein and Perlman, 2004) and at least 38 species of 12 mite families (chapter 1, this thesis).

Studies investigating diversity of C in arthropods have found a monophyletic insect-C-clade and a tendency towards a mite – C - clade, although only supported by low bootstrap values (Chang et al., 2010; Zchori-Fein and Perlman, 2004). Here we report a similar general pattern, although some insect-C-species appear within the mite-clade and vice-versa. Sequences obtained in a recent study of C in spiders and harvestmen, which are taxonomically closer related to mites than to insects (Opiliones and Araneae are along with Acari sub-groups of the class Arachnida within the Chelicerata) (Chang et al., 2010), appear distributed over most of the tree (figure 2).

Spiroplasma sp., in contrast to W and C, are often found associated with plants and most infections seem to be commensal, although there are some cases of pathogenicity (e.g., citrus stubborn, cornstunt disease, Whitcomb, 1975; Whitcomb and Williamson, 1979) and mutualism (Bové 1997, reviewed in Regassa and Gasparich, 2006). In arthropods, S *sp.* from tabanid flies are the best characterized (Whitcomb et al., 1997), but also from a wide range of other insect orders. The aphid S - symbiont is vertically transmitted and has a negative effect on growth, reproduction and longevity of its host (Fukatsu et al., 2001). Ladybirds and moths or butterflies S have been described as Male-Killers (Jiggins et al., 1998; Majerus et al., 1998; Tinsley and Majerus, 2006). *Spiroplasma sp*. have also been detected in some mite species recently. *Leptus sayi* (DiBlasi et al., 2011), *Neoseiulus californicus* (Zchori-Fein and Schausberger unpublished) and several ticks (Brinton and Burgdorfer, 1976; Taroura et al., 2005; Tully et al., 1995) were shown to be infected.

Spiroplasma infection rates in *Hypoaspis miles* are between 30 and 60% in all 3 populations tested. However, the number of infected *H. miles* individuals in a sample size of 30 can vary by 20% (unpublished data), either reflecting real infection fluctuations or sampling artefacts (males and females could for example tendencially be found in different parts of the rearing container and therefore the sample could be biased). Sequences of the sequences obtained from all 3 populations are over 99% identical. The closest match to our sequences in BLAST are cultured *S. platyhelix* (DQ860101) with 95% sequence similarity over the full length. The 16S rRNA gene is generally accepted for species-depth identification, but the 16S-23S rRNA gene spacer region or the gyrB gene

are suggested for further phylogenetic classification and resolution (strains) (Regassa and Gasparich, 2006).

DiBlasi et al. (2011) detect identical S - strains in a host and its parasite and hypothesized horizontal transmission via a shared plant food source. *Hypoaspis miles* as a predatory mite can be raised on *Tyrophagus putrescentiae* in the laboratory, which did not show any infection in our survey, suggesting, together with the high infection rate, a real symbiotic relationship between *H. miles* and S, rather than a gut infection stemming from the food. However, 2 out of 3 populations in this study have their origin in commercial rearings and might have different food sources. Preliminary surveys of infected and uninfected lineages did not reveal significant sex-bias in *H. miles* (unpublished) as found in other hosts (Jiggins et al., 1998; Montenegro et al., 2006), but further studies are required to confirm that these S do not distort sex ratio (by male killing or potentially parthenogenesis or feminization). Negative S-effects on growth rate, fecundity and longevity had been found in *Acyrthosiphon pisum* (Fukatsu et al., 2001). Similarly high infection rates and a lack of evidence for any effects of the S on its host have been found in the predatory mite, *Neoseiulus californicus* (Zchori-Fein and Schausberger, unpublished). Knowledge of *Spiroplasma*-effects on *H. miles* as well as *N. californicus* is of great importance due to the mites' high commercial use and value.

Conclusion: We found three novel infections: a *Wolbachia* in the bird parasite *D.gallinae, Cardinium* in the spider mite *Panonychus ulmi* and a *Spiroplasma* in the commercially important predatory mite *H. miles*. Although the diversity and distribution of known bacterial ES in the tested mites appears to be lower than expected, we could still show that they are present in several mite species. Knowing how severely they can influence their host's biology (Zindel et al., 2011) their potential presence should be kept in mind when working with arthropod biological control agents and pests. The characterization of ES-effects on their hosts and the localization and transmission modes of the bacteria would be the next step on the way to a successful integration or even use in biological control. In general, more sensitive methods such as Real Time or Long PCR or more general primers (Simoes et al., 2011) could possibly be used to detect potential low titer infections or broader

targets. More importantly, in order to capture infections of new endosymbiont species, with their own range of effects and manipulation potential, metagenomic analysis using next generation sequencing seems to be the right tool. Existing knowledge on the bacterial community of a certain arthropod species can also be useful working on other research problems such as pathogen isolation and population ecology. For practical application in biological control however, as stated by Zindel and colleagues (2011), the effort invested in the detection and characterization of ES should be proportional to the estimated risk they pose or potential benefit the biological program can gain from the presence of the ES.

Acknowledgements

This work was supported by the COST-SER project C08Æ0109 to AA, part of the COST Action FA0701.We would like to thank Dr. Eric Palevsky, Shira Gal, Dr. Simone Immler and Johannette Klappwijk at Koppert for mite samples and Olivier Duron and Odile Bruggisser for provision of positive controls.

References

Adachi-Hagimori, T., Miura, K., Stouthamer, R., 2008. A new cytogenetic mechanism for bacterial endosymbiont-induced parthenogenesis in Hymenoptera. Proc. R. Soc. Lond. B. Biol. Sci. 275**,** 2667-2673.

Ahantarig, A., Kittayapong, P., 2011. Endosymbiotic *Wolbachia* bacteria as biological control tools of disease vectors and pests. J. Appl. Entomol. 135**,** 479-486.

Baldo, L., Hotopp, J.C.D., Jolley, K.A., Bordenstein, S.R., Biber, S.A., Choudhury, R.R., Hayashi, C., Maiden, M.C.J., Tettelin, H., Werren, J.H., 2006. Multilocus sequence typing system for the endosymbiont *Wolbachia pipientis*. Appl. Environ. Microbiol. 72**,** 7098-7110.

Baumann, P., 2005. Biology of bacteriocyte-associated endosymbionts of plant sap-sucking insects. Annu. Rev. Microbiol. 59**,** 155-189.

Breeuwer, J.A.J., Jacobs, G., 1996. *Wolbachia*: Intracellular manipulators of mite reproduction. Exp. Appl. Acarol. 20**,** 421-434.

Brelsfoard, C.L., Dobson, S.L., 2011. *Wolbachia* effects on host fitness and the influence of male aging on cytoplasmic incompatibility in *Aedes polynesiensis* (Diptera: Culicidae). J. Med. Entomol. 48**,** 1008-1015.

Brinton, L.P., Burgdorfer, W., 1976. Cellular and subcellular organization of 277F-agent, a *Spiroplasma* from rabbit tick *Haemaphysalis leporipalustris* (Acari: Ixodidae). Int. J. Syst. Bacteriol. 26**,** 554-560.

Brownlie, J.C., Johnson, K.N., 2009. Symbiont-mediated protection in insect hosts. Trends Microbiol. 17**,** 348-354.

Bull, J.J., 1981. Coevolution of haplo-diploidy and sex determination in Hymenoptera. Evolution 35**,** 568-580.

Carletto, J., Gueguen, G., Fleury, F., Vanlerberghe-Masutti, F., 2008. Screening the bacterial endosymbiotic community of sap-feeding insects by terminal-restriction fragment length polymorphism analysis. Entomologia Experimentalis Et Applicata 129**,** 228-234.

Chang, J., Masters, A., Avery, A., Werren, J.H., 2010. A divergent *Cardinium* found in daddy long-legs (Arachnida: Opiliones). J. Invertebr. Pathol. 105**,** 220-227.

Charlat, S., Hurst, G.D.D., Mercot, H., 2003. Evolutionary consequences of *Wolbachia* infections. Trends Genet. 19**,** 217-223.

De Luna, C.J., Moro, C.V., Guy, J.H., Zenner, L., Sparagano, O.A.E., 2009. Endosymbiotic bacteria living inside the poultry red mite (*Dermanyssus gallinae*). Exp. Appl. Acarol. 48**,** 105-113.

DiBlasi, E., Morse, S., Mayberry, J.R., Avila, L.J., Morando, M., Dittmar, K., 2011. New *Spiroplasma* in parasitic *Leptus* mites and their *Agathemera* walking stick hosts from Argentina. J. Invertebr. Pathol. 107**,** 225-228.

Duplouy, A., Hurst, G.D.D., O'Neill, S.L., Charlat, S., 2010. Rapid spread of male-killing *Wolbachia* in the butterfly *Hypolimnas bolina*. J. Evol. Biol. 23**,** 231-235.

Duron, O., Bouchon, D., Boutin, S., Bellamy, L., Zhou, L.Q., Engelstadter, J., Hurst, G.D., 2008. The diversity of reproductive parasites among arthropods: *Wolbachia* do not walk alone. BMC Biology 6.

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.

Ferres, J., Justicia, J.L., Garcia, M.P., Munoz-Tuduri, M., Alva, V., 2011. Efficacy of high-dose sublingual immunotherapy in children allergic to house dust mites in real-life clinical practice. Allergologia Et Immunopathologia 39**,** 122-127.

Fukatsu, T., Tsuchida, T., Nikoh, N., Koga, R., 2001. *Spiroplasma* symbiont of the pea aphid, *Acyrthosiphon pisum* (Insecta: Homoptera). Appl. Environ. Microbiol. 67**,** 1284-1291.

Giorgini, M., Monti, M.M., Caprio, E., Stouthamer, R., Hunter, M.S., 2009. Feminization and the collapse of haplodiploidy in an asexual parasitoid wasp harboring the bacterial symbiont *Cardinium*. Heredity 102**,** 365-371.

Gotoh, T., Noda, H., Hong, X.Y., 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., 2007. *Cardinium* symbionts cause cytoplasmic incompatibility in spider mites. Heredity 98**,** 13-20.

Groot, T.V.M., Breeuwer, J.A.J., 2006. *Cardinium* symbionts induce haploid thelytoky in most clones of three closely related *Brevipalpus* species. Exp. Appl. Acarol. 39**,** 257-271.

Gross, R., Vavre, F., Heddi, A., Hurst, G.D.D., Zchori-Fein, E., Bourtzis, K., 2009. Immunity and symbiosis. Mol. Microbiol. 73**,** 751-759.

Gunduz, E.A., Douglas, A.E., 2009. Symbiotic bacteria enable insect to use a nutritionally inadequate diet. Proc. R. Soc. Lond. B. Biol. Sci. 276**,** 987-991.

Haine, E.R., 2007. Symbiont-mediated protection. Proc. R. Soc. Lond. B. Biol. Sci. 275**,** 353-361.

Hilgenboecker, K., Hammerstein, P., Schlattmann, P., Telschow, A., Werren, J.H., 2008. How many species are infected with *Wolbachia*? - a statistical analysis of current data. FEMS Microbiol. Lett. 281**,** 215-220.

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.

Hoy, M.A., Jeyaprakash, A., 2005. Microbial diversity in the predatory mite *Metaseiulus occidentalis* (Acari: Phytoseiidae) and its prey, *Tetranychus urticae* (Acari: Tetranychidae). Biological Control 32**,** 427-441.

Hunter, M.S., Perlman, S.J., Kelly, S.E., 2003. A bacterial symbiont in the Bacteroidetes induces cytoplasmic incompatibility in the parasitoid wasp *Encarsia pergandiella*. Proc. R. Soc. Lond. B. Biol. Sci. 270**,** 2185-2190.

Jiggins, F.M., Hurst, G.D.D., Majerus, M.E.N., 1998. Sex ratio distortion in *Acraea encedon* (Lepidoptera: Nymphalidae) is caused by a male-killing bacterium. Heredity 81**,** 87-91.

Kageyama, D., Nishimura, G., Hoshizaki, S., Ishikawa, Y., 2002. Feminizing *Wolbachia* in an insect, *Ostrinia furnacalis* (Lepidoptera: Crambidae). Heredity 88**,** 444-449.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23**,** 2947-2948.

Majerus, T.M.O., Majerus, M.E.N., Knowles, B., Wheeler, J., Bertrand, D., Kuznetzov, V.N., Ueno, H., Hurst, G.D.D., 1998. Extreme variation in the prevalence of inherited male-killing microorganisms between three populations of *Harmonia axyridis* (Coleoptera: Coccinellidae). Heredity 81**,** 683-691.

McMeniman, C.J., Lane, R.V., Cass, B.N., Fong, A.W.C., Sidhu, M., Wang, Y.F., O'Neill, S.L., 2009. Stable introduction of a life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti*. Science 323**,** 141-144.

Montenegro, H., Petherwick, A.S., Hurst, G.D.D., Klaczko, L.B., 2006. Fitness effects of *Wolbachia* and *Spiroplasma* in *Drosophila melanogaster*. Genetica 127**,** 207-215.

Montenegro, H., Solferini, V.N., Klaczko, L.B., Hurst, G.D.D., 2005. Male-killing *Spiroplasma* naturally infecting *Drosophila melanogaster*. Insect Mol. Biol. 14**,** 281-287.

Montero-Pau, J., Gomez, A., Munoz, J., 2008. Application of inexpensive and high throughput genomic DNA extraction method for the molecular ecology of zooplanctonic diapausing eggs. Limnology and Oceanography: Methods 6**,** 218-222.

Moran, N.A., McCutcheon, J.P., Nakabachi, A., 2008. Genomics and evolution of heritable bacterial symbionts. Annu. Rev. Genet., pp. 165-190.

Nakamura, Y., Kawai, S., Yukuhiro, F., Ito, S., Gotoh, T., Kisimoto, R., Yanase, T., Matsumoto, Y., Kageyama, D., Noda, H., 2009. Prevalence of *Cardinium* bacteria in planthoppers and spider mites and taxonomic revision of "*Candidatus Cardinium hertigii*" based on detection of a new *Cardinium* group from biting midges. Appl. Environ. Microbiol. 75**,** 6757-6763.

O'Neill, S.L., Hoffmann, A.A., Werren, J.H., 1997. Influential passengers. Oxford University Press.

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.

Page, R.D.M., 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. Comput. Appl. Biosci. 12**,** 357-358.

Perotti, M.A., Clarke, H.K., Turner, B.D., Braig, H.R., 2006. *Rickettsia* as obligate and mycetomic bacteria. FASEB J. 20**,** 2372-2374.

Posada, D., 2008. jModelTest: Phylogenetic model averaging. Mol. Biol. Evol. 25**,** 1253-1256.

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E., 2010. Global pollinator declines: trends, impacts and drivers. Trends Ecol. Evol. 25**,** 345-353.

Regassa, L.B., Gasparich, G.E., 2006. *Spiroplasmas*: evolutionary relationships and biodiversity. Frontiers in Bioscience 11**,** 2983-3002.

Rodriguez-Navarro, S., Torres-Martinez, G., Olivares-Orozco, J., 2004. Biological control of field bindweed (*Convolvulus arvensis* L.) using *Aceria malherbae* (Acari: Eriophyidae) in Mexico. Int. J. Acarol. 30**,** 153-155.

Ros, V.I.D., Breeuwer, J.A.J., 2009. The effects of, and interactions between, *Cardinium* and *Wolbachia* in the doubly infected spider mite *Bryobia sarothamni*. Heredity 102**,** 413-422.

Ros, V.I.D., Fleming, V.M., Feil, E.J., Breeuwer, J.A.J., 2009. How Diverse Is the Genus *Wolbachia*? Multiple-Gene Sequencing Reveals a Putatively New *Wolbachia* Supergroup Recovered from Spider Mites (Acari: Tetranychidae). Appl. Environ. Microbiol. 75**,** 1036-1043.

Ros, V.I.D., Fleming, V.M., Feil, E.J., Breeuwer, J.A.J., 2012. Diversity and recombination in *Wolbachia* and *Cardinium* from *Bryobia* spider mites. BMC Microbiology 12.

Simoes, P.M., Mialdea, G., Reiss, D., Sagot, M.F., Charlat, S., 2011. *Wolbachia* detection: an assessment of standard PCR Protocols. Mol. Ecol. Resources 11**,** 567-572.

Siozios, S., Sapountzis, P., Ioannidis, P., Bourtzis, K., 2008. *Wolbachia* symbiosis and insect immune response. Insect Sci. 15**,** 89-100.

Tagami, Y., Miura, K., Stouthamer, R., 2001. How does infection with parthenogenesis-inducing *Wolbachia* reduce the fitness of *Trichogramma*? J. Invert. Pathol. 78**,** 267-271.

Taroura, S., Shimada, Y., Sakata, Y., Miyama, T., Hiraoka, H., Watanabe, M., Itamoto, K., Okuda, M., Inokuma, H., 2005. Detection of DNA of *'Candidatus Mycoplasma haemominutum*' and *Spiroplasma sp* in unfed ticks collected from vegetation in Japan. J. Vet. Med. Sci. 67**,** 1277-1279.

Tinsley, M.C., Majerus, M.E.N., 2006. A new male-killing parasitism: *Spiroplasma* bacteria infect the ladybird beetle *Anisosticta novemdecimpunctata (*Coleoptera: Coccinellidae). Parasitology 132**,** 757-765.

Tortosa, P., Charlat, S., Labbe, P., Dehecq, J.-S., Barre, H., Weill, M., 2010. *Wolbachia* age-sex-specific density in *Aedes albopictus*: a host evolutionary response to cytoplasmic incompatibility? Plos One 5.

Tully, J.G., Rose, D.L., Yunker, C.E., Carle, P., Bove, J.M., Williamson, D.L., Whitcomb, R.F., 1995. *Spiroplasma ixodetis sp. nov*, a new species from *Ixodes pacificus* ticks collected in Oregon. Int. J. Syst. Bacteriol. 45**,** 23-28.

Vala, F., Weeks, A., Claessen, D., Breeuwer, J.A.J., Sabelis, M.W., 2002. Within- and between-population variation for *Wolbachia*-induced reproductive incompatibility in a haplodiploid mite. Evolution 56**,** 1331-1339.

Vasquez, C.J., Stouthamer, R., Jeong, G., Morse, J.G., 2011. Discovery of a CI-inducing *Wolbachia* and its associated fitness costs in the biological control agent *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae). Biol. Control 58**,** 192-198.

Weeks, A.R., Breeuwer, J.A.J., 2001. *Wolbachia*-induced parthenogenesis in a genus of phytophagous mites. Proc. R. Soc. Lond. B. Biol. Sci. 268**,** 2245-2251.

Weeks, A.R., Marec, F., Breeuwer, J.A.J., 2001. A mite species that consists entirely of haploid females. Science 292**,** 2479-2482.

Weeks, A.R., Stouthamer, R., 2004. Increased fecundity associated with infection by a Cytophaga-like intracellular bacterium in the predatory mite, *Metaseiulus occidentalis*. Proc. R. Soc. Lond. B. Biol. Sci. 271**,** S193-S195.

Weeks, A.R., Velten, R., Stouthamer, R., 2003. Incidence of a new sex-ratio-distorting endosymbiotic bacterium among arthropods. Proc R. Soc. Lond. B. Biol. Sci. 270**,** 1857-1865.

Weinert, L.A., Tinsley, M.C., Temperley, M., Jiggins, F.M., 2007. Are we underestimating the diversity and incidence of insect bacterial symbionts? A case study in ladybird beetles. Biol. Lett. 3**,** 678-681.

Werren, J.H., Baldo, L., Clark, M.E., 2008. *Wolbachia*: master manipulators of invertebrate biology. Nat. Rev. Microbiol. 6**,** 741-751.

Whitcomb, R.F., 1975. Pathogenic effects of plant disease agents on vector insects - introduction. Ann. N. Y. Acad. Sci. 266**,** 259-259.

Whitcomb, R.F., French, F.E., Tully, J.G., Carle, P., Henegar, R., Hackett, K.J., Gasparich, G.E., Williamson, D.L., 1997. *Spiroplasma* species, groups, and subgroups from north American Tabanidae. Curr. Microbiol. 35**,** 287-293.

Whitcomb, R.F., Williamson, D.L., 1979. Pathogenicity of Mycoplasmas for Arthropods. Zentralblatt Fur Bakteriologie Mikrobiologie Und Hygiene Series a-Medical Microbiology Infectious Diseases Virology Parasitology 245**,** 200-221.

Williamson, D.L., Adams, J.R., Whitcomb, R.F., Tully, J.G., Carle, P., Konai, M., Bove, J.M., Henegar, R.B., 1997. *Spiroplasma platyhelix sp. nov*, a new mollicute with unusual morphology and genome size from the dragonfly *Pachydiplax longipennis*. Int. J. Syst. Bacteriol. 47**,** 763-766.

Wong, Z.S., Hedges, L.M., Brownlie, J.C., Johnson, K.N., 2011. *Wolbachia*-mediated antibacterial protection and immune gene regulation in *Drosophila*. Plos One 6.

Xie, R.-R., Liu, Y., Hong, X.-Y., Gotoh, T., 2006. Effect of infection rate of *Wolbachia* on the reproduction in *Tetranychus kanzawai* Kishida (Acari: Tetranychidae) in China. Int. J. Acarol. 32**,** 407-415.

Zchori-Fein, E., Gottlieb, Y., Kelly, S.E., Brown, J.K., Wilson, J.M., Karr, T.L., Hunter, M.S., 2001. A newly discovered bacterium associated with parthenogenesis and a change in host selection behavior in parasitoid wasps. Proc. Natl. Acad. Sci. USA 98**,** 12555-12560.

Zchori-Fein, E., Perlman, S.J., 2004. Distribution of the bacterial symbiont *Cardinium* in arthropods. Mol. Ecol. 13**,** 2009-2016.

Zhou, W.G., Rousset, F., O'Neill, S., 1998. Phylogeny and PCR-based classification of *Wolbachia* strains using wsp gene sequences. Proc. R. Soc. Lond. B. Biol. Sci. 265**,** 509-515.

Zindel, R., Gottlieb, Y., Aebi, A., 2011. Arthropod symbioses: a neglected parameter in pest- and disease-control programmes. J. Appl. Ecol. 48**,** 864-872.

Zug, R., Hammerstein, P., 2012. Still a host of hosts for *Wolbachia*: analysis of recent data suggests that 40% of terrestrial arthropod species are infected. Plos One 7.

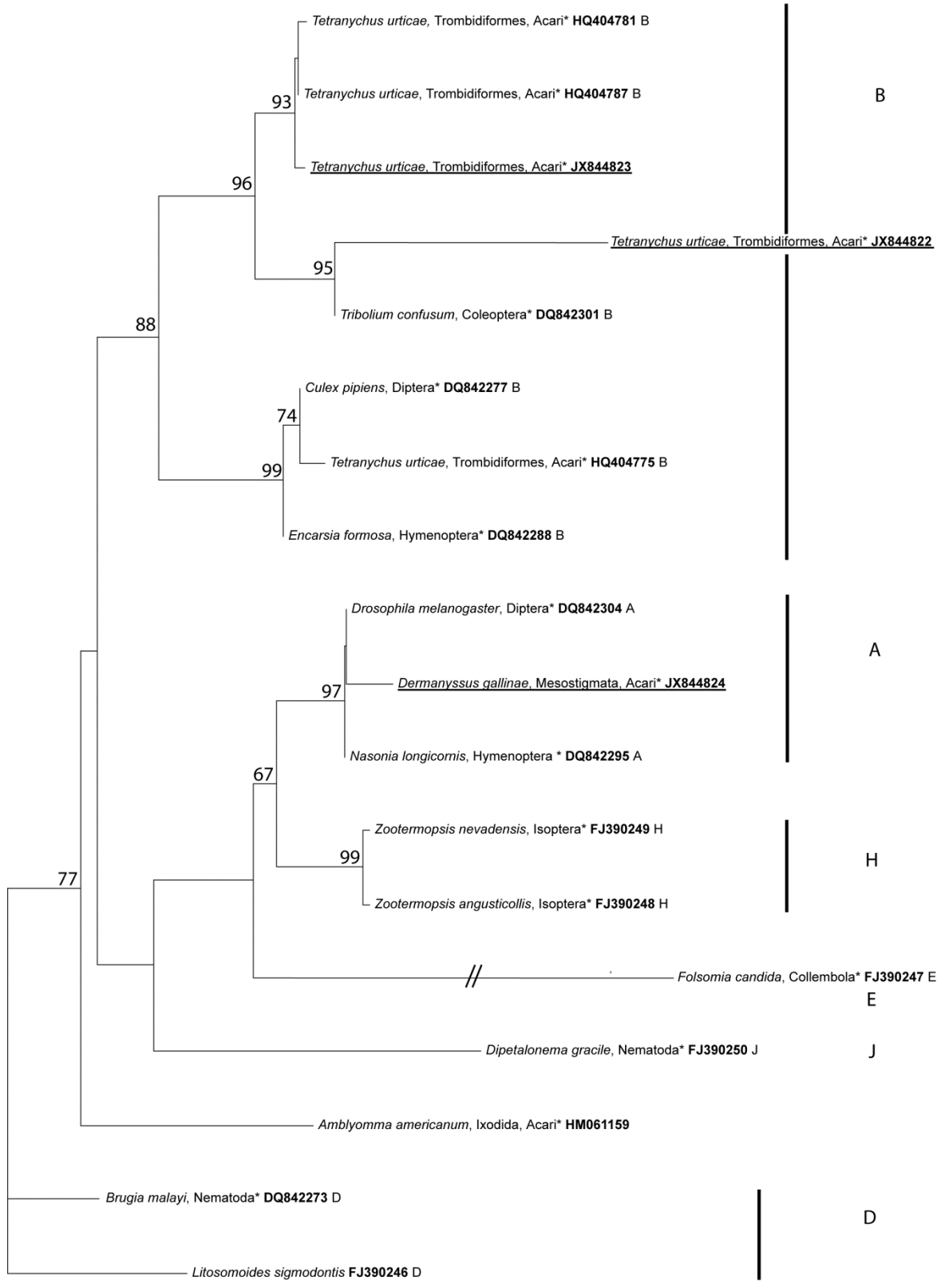


Figure 1 Diversity among mite- and other invertebrate *Wolbachia* endosymbionts presented in a tree based on the Cytochrome oxidase I gene, (coxA). Bacterial sequences are characterized by the bacterial species name if available or the name of their host species (*). Bootstrap support >50% based on 1000 replicates is indicated. Bacterial sequences obtained in this study are underlined.

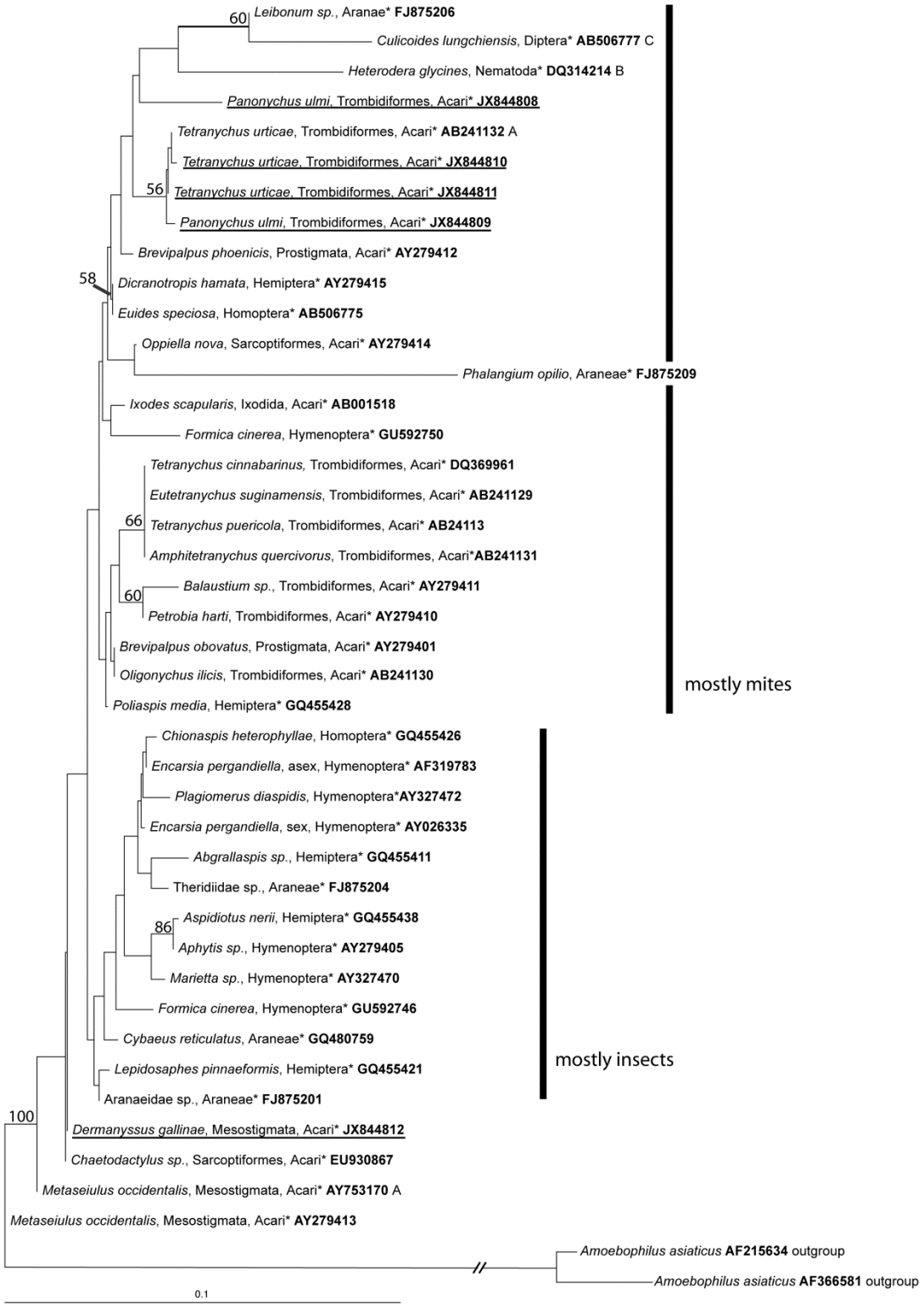


Figure 2 Diversity among mite- and other invertebrate *Cardinium* symbionts presented in a tree based on a 350 bp fragment of the ribosomal 16s DNA gene of *Cardinium*. Bacterial sequences are characterized by the bacterial species name if available or the name of their host species (*). Bootstrap support >50% based on 1000 replicates is indicated. Bacterial sequences obtained in this study are underlined. Sequences from *Amoebophilus asiaticus* were used for rooting of the tree.

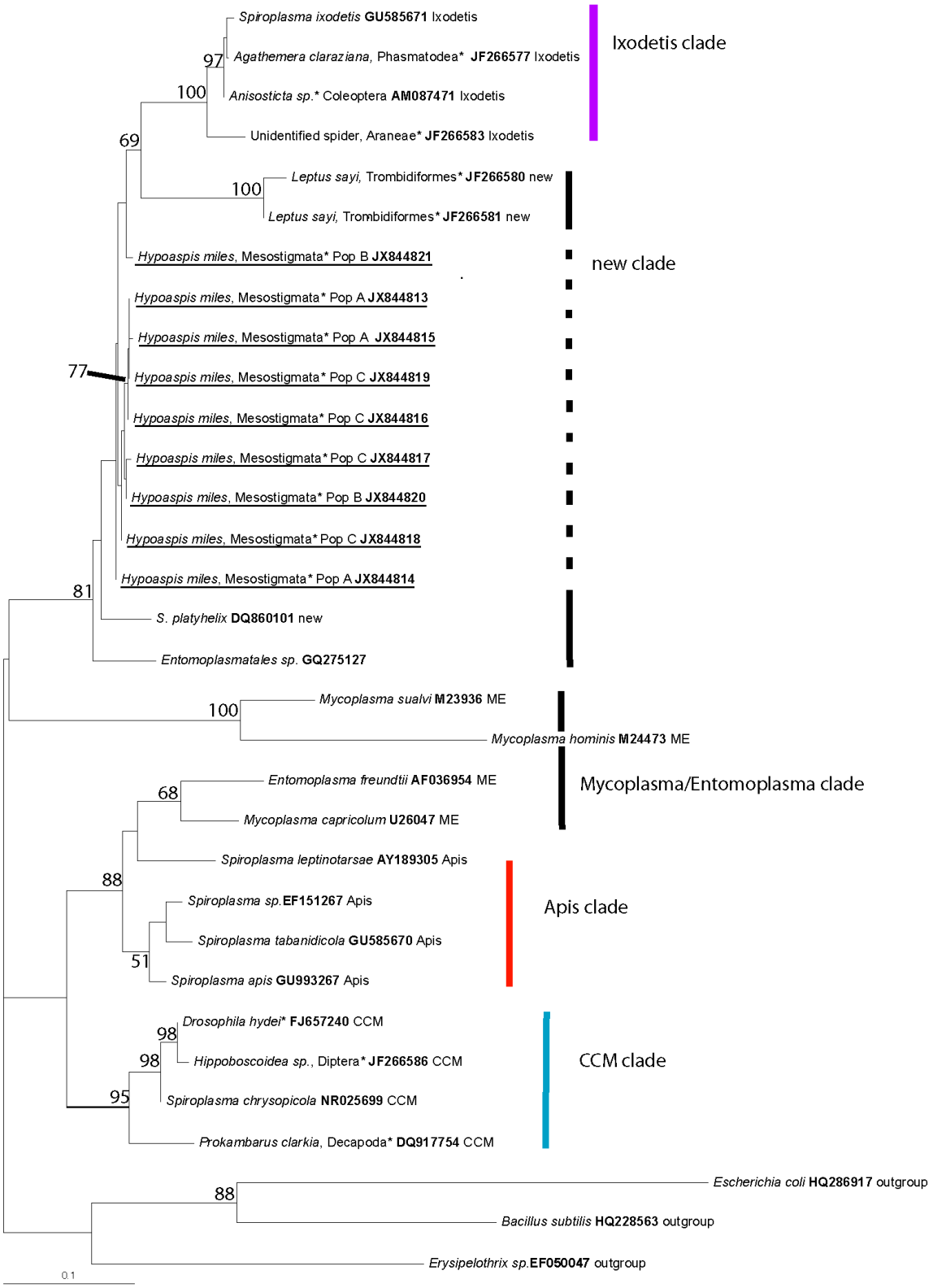


Figure 3 Diversity among *Spiroplasma* presented in a tree based on a 400- 450 bp fragment of the ribosomal 16s DNA gene of *Spiroplasma*. Bacterial sequences are characterized by the bacterial species name if available or the name of their host species (*). Bootstrap support >50% based on 1000 replicates are indicated. Bacterial sequences obtained in this study are underlined. Sequences from *Bacillus subtilis, Eschrichia coli* and *Erysiopelotrix sp.* were used for rooting of the tree.

Table 1 Primers used for the molecular detection and phylogenetic analyses of endosymbiontic bacteria. bp= base pairs, °C= degree Celsius, *a Touchdown-PCR program was used.

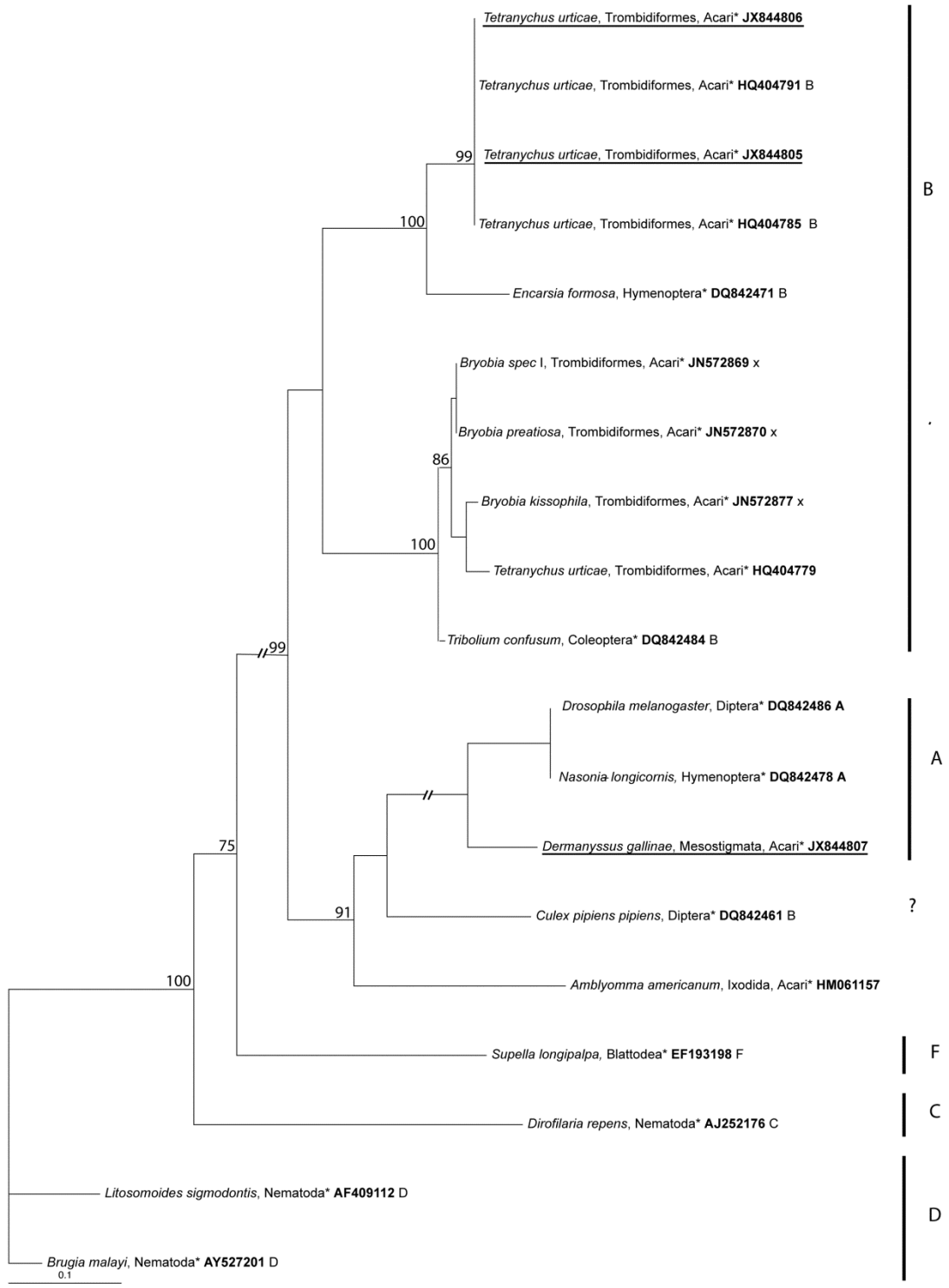
Organism	Primers	Target gene	Product size (bp)	Annealing temp (°C)	Reference
Arthropod 18S (DNA check)	**NSF4/18**: 5'-CTGGTTGATYCTGCCAGT-3' **NSR399/19**: 5'-TCTCAGGCTCCYTCTCCGG-3'	18S rDNA	400-450	54	Duron et al., 2008
General bacterial 16S	**Y2MOD**: 5'-ACTYCTACGGRAGGCAGCAGTRGG-3' **16SB1**: 5'-TACGGYTACCTTGTTACGACTT-3'	16S rDNA	variable	51	Carletto et al., 2008
Wolbachia	**Wsp_81F**: 5'-TGGTCCAATAAGTGATGAAGAAAC-3' **Wsp_691R**: 5'-AAAAAT TAAACGCTACTC CA-3'	*Wolbachia* outer surface protein	540	60-50*	Zhou et al., 1998; Tinsley and Majerus, 2006
	CoxA_F: 5'-TTG GRG CRA TYA ACT TTA TAG-3' **CoxA_R**: 5'-CT AAA GAC TTT KAC RCC AGT-3'	Cytochrome Oxidase I	487	54	Baldo et al., 2006
Cardinium	**CHF**: 5'-TACTGTAAGAATAAGCACCGGC-3' **CHR**: 5'-GTGGATCACTTAACGCTTTCG-3'	16S rDNA	450	65-55*	Zchori-Fein and Perlman, 2004
Flavobacterium	**Fl1**: 5'-ATTGTTAAAGTTCCGGCG-3' **Fl2**: 5'-CTGTTTCCAGCTTATTCGTAGTAC-3'	16S rDNA	800	60-50*	Tinsley and Majerus, 2006
Rickettsia	**RssuF**: 5'-CGG CTT TCA AAA CTA CTA ATC TA-3' **RssuR**: 5'-GAA AGC ATC TCT GCG ATC CG-3'	16S rDNA	450	60-50*	Tinsley and Majerus, 2006
Arsenophonus	**ArsF**: 5'-GGGTTGTAAAGTACTTTCAGTCGT-3' A**rsR2**: 5'-GTAGCCCTRCTCGTAAGGGCC-3'	16S rDNA	581-804	52	Duron et al., 2008
Spiroplasma general	**S27:** 5'- GAG AGT TTG ATC CTG GCT CAG-3' **SKSSspR**: 5'- TAG CCG TGG CTT TCT GGT AA-3'	16S rDNA	500bp	55	Enigl and Schausberger, 2007
S. poulsonii	**SpoulF**: 5'-GCTTAACTCCAGTTCGCC-3' **SpoulR**: 5'- CCTGTCTCAATGTTAACCTC-3'	16S rDNA	421	65-55*	Montenegro et al., 2005
S. ixodetis	**SpixoF**: 5'-TTAGGGGCTCAACCCCTAACC-3' **SpixoR**: 5'-TCTGGCATTGCCAACTCTC-3'	16S rDNA	810	65-55*	Duron et al., 2008

Table 2 Number and origin of mite populations (20 females, 10 males) screened and screen results. "> 3%" indicates, that at least 1 individual of the population tested positive in PCR, "> 20%" signifies an infection rate of over 20% in the population and "> 50%" over 50%. Values in brackets were not confirmed by sequencing the PCR positives.

taxonomic information	population origin	lifestyle	W (wsp_81F, wsp_691R[1])	C (16S, CHF and CHR[2] or Clof, Clor[3])	F FL1 and FL2[4]	R RSSUF and RSSUR[4]	S S27-F and STKSSsp-R[3]	A ArsF and ArsR2[5]
Trombidiformes		phytophagous						
Eryophyidae								
Aculops lycopersici	1. E. Palevsky, Israel		-	-	-	-	-	-
Eriophyes vitis	1. Affoltern, ZH		-	-	-	-	-	-
Tetranychidae								
Tetranychus urticae	1. ART Reckenholz		-	> 20%	-	-	-	-
	2. Ch. Schweizer, ART		> 3%	-	-	-	-	-
Panonychus ulmi	1. ACW Höhn 2008		-	> 20%	-	-	-	-
Oligonychus persea	1. E. Palevsky, Avocado, Israel		-	-	-	-	-	-
Sarcoptiformes								
Acaridae								
Rhizoglyphus robini	1. S. Immler, Sweden		-	-	-	-	-	-
	2. E.Palevsky, Lilium, Israel		-	-	-	-	-	-
	3. E.Palevsky, Allium, Israel		-	-	-	-	-	-
	4. E. Palevsky, Ruscus, Israel		-	-	-	-	-	-
Mesostigmata		predatory						
Phytoseiidae								
Amblyseius cucumeris	1. Koppert, Holland		-	-	-	(> 3%)	-	-
	2. Biobest, Belgium (Andermatt, CH)		-	-	-	-	-	-
Amblyseius swirskii	1. Koppert, Holland		-	-	-	(> 3%)	-	-
	2. BioBest, Belgium (Andermatt, CH)		-	-	-	-	-	-
Euseius scutalis	1. E. Palevsky, Israel		-	-	-	-	-	-
Neoseiulus californicus	1. Koppert, Holland		-	-	-	-	(> 20%)	-
Phytoseiulus persimilis	1. Koppert, Holland		-	-	-	-	-	-
	2. Biobest, Belgium (Andermatt, CH)		-	-	-	-	-	-

Laelapidae								
Hypoaspis aculeifer	1. S. Immler, Sweden		-	-	-	-	(> 3%)	-
	2. Koppert, Holland		-	(> 3%)	-	-	-	-
Hypoaspis miles	1. Koppert, Holland		-	-	-	-	> 20%	-
	2. S. Immler, Sweden		-	-	-	-	> 50%	-
	3. BioBest, Belgium		-	-	-	-	> 20%	-
Macrochelidae								
Macrocheles robustus	1. Koppert, Holland		-	-	-	-	-	-
Mesostigmata		ectoparasitic						
Dermanissidae								
Dermanissus gallinae	1. Veronika Maurer, FIBL		> 3%	> 20%	-	-	-	-
Varroideae								
Varroa destructor	1. Gilbert Dey		-	-	-	-	-	-
Chorioptes sp.	1. Petra Roosje, Veterinary, Bern		-	-	-	-	-	-
Sarcoptiformes		other (astigmatic mites)						
Acaridae								
Sancassania berlesi	1. S. Immler, Leeds		-	-	-	-	-	-
	2. S. Immler, Krakow		-	-	-	-	-	-
Tyrophagus putrescentiae	1. Koppert, Holland		-	-	-	-	-	-
Carpoglyphidae								
Carpoglyphus lacti	1. Koppert, Holland		-	-	-	-	-	-
Glyciphagidae								
Lepidoglyphus destructor	1. Koppert, Holland		-	-	-	-	-	-

[1)] Zhou et al., 1998; [2)] Zchori-Fein and Perlman, 2004; [3)] Enigl and Schausberger, 2007; [4)] Tinsley and Majerus, 2006; [5)] Duron et al., 2008. DNA presence was tested using general arthropod primers (NSF4 and NSR399) by Duron et al., 2008.



Appendix 1 Diversity among mite- and other invertebrate *Wolbachia* endosymbionts presented in a tree based on the *Wolbachia* surface protein gene (wsp). Bacterial sequences are characterized by the bacterial species name if available orthe name of their host species (*). Bootstrap support >50% based on 1000 replicates is indicated. Bacterial sequences obtained in this study are underlined.

Appendix 2 Wsp and coxA gene sequences used to construct phylogenetic tree for *Wolbachia*. Sequences obtained in this study are in bold red. The other sequences were downloaded from GenBank (http://www.ncbi.nlm.nih.gov/genbank).

Host class	Host order	Host species	Acc. No. wsp	Acc. No. coxA	Supergroup*	Species
Arachnida	Trombidiformes	*Tetranychus urticae*	HQ404785	HQ404781	B	*Wolbachia sp.*
		Tetranychus urticae	HQ404791	HQ404787	B	*Wolbachia sp.*
		Tetranychus urticae	HQ404779	HQ404775	B	*Wolbachia sp.*
		Tetranychus urticae	**JX844805**	**JX844822**		*Wolbachia sp.*
		Tetranychus urticae	**JX844806**	**JX844823**		*Wolbachia sp.*
		Bryobia kissophila	JN572877	-		*Wolbachia sp.*
		Bryobia praetiosa	JN572870	-		*Wolbachia sp.*
		Bryobia spec. I	JN572869	-		*Wolbachia sp.*
	Ixodida	*Amblyomma americanum*	HM061157	HM061159	?	*Wolbachia sp.*
	Mesostigmata	***Dermanyssus gallinae***	**JX844807**	**JX844824**		*Wolbachia sp.*
Insecta	Diptera	*Drosophila melanogaster*	DQ842486	DQ842304	A	*Wolbachia sp.*
		Culex pipiens pipiens	DQ842461	DQ842277	B	*Wolbachia sp.*
	Hymenoptera	*Nasonia longicornis*	DQ842478	DQ842295	A	*Wolbachia sp.*
		Encarsia formosa	DQ842471	DQ842288	B	*Wolbachia sp.*
	Coleoptera	*Tribolium confusum*	DQ842484	DQ842301	B	*Wolbachia sp.*
	Blattodea	*Supella longipalpa*	EF193198	-	F	*Wolbachia sp.*
	Isoptera	*Zootermopsis angusticollis*	-	FJ390248	H	*Wolbachia sp.*
		Z. nevadensis		FJ390249	H	*Wolbachia sp.*
Nematoda		*Dirofilaria repens*	AJ252176	-	C	*Wolbachia sp.*
		Onchocerca gibsoni	AJ252178	-	C	*Wolbachia sp.*
		Brugia malayi[+]	AY527201	DQ842273	D	*Wolbachia sp.*
		Litosomoides sigmodontis[+]	AF409112	FJ390246	D	*Wolbachia sp.*
		Dipetalonema gracile	-	FJ390250	J	*Wolbachia sp.*
Enthognata		*Folsomia candida*	-	FJ390247	E	*Wolbachia sp.*

*Supergroup information from genbank directly or Ros et al., 2009

[+]Not corresponding clones for wsp and coxA

Appendix 3 Sequences used to construct phylogenetic tree for *Cardinium*. Sequences obtained in this study are in bold red. Additional sequences were downloaded from GenBank (http://www.ncbi.nlm.nih.gov/genbank).

Host class	Host order	Host species	Acc. No.	Supergroup*	Species
Arachnida	Araneae	*Aranaeidae sp.*	FJ875201		*Cardinium sp.*
		Cybaeus reticulatus	GQ480759		*Cardinium sp.*
		Leibonum sp.	FJ875206		*Cardinium sp.*
		Phalangium opilio	FJ875209		*Cardinium sp.*
		Theriidae sp.	FJ875204		*Cardinium sp.*
	Trombidiformes	*Amphitetranychus quercivorus*	AB241131	A	*Cardinium sp.*
		Balaustium sp.	AY279411		*Cardinium sp.*
		Eutetranychus suginamensis	AB241129	A	*Cardinium sp.*
		Oligonychus ilicis	AB241130	A	*Cardinium sp.*
		Panonychus ulmi	**JX844808**		*Cardinium sp.*
		P. ulmi	**JX844809**		*Cardinium sp.*
		Petrobia harti	AY279410		*Cardinium sp.*
		Tetranychus cinnabarinus	DQ369961		*Cardinium sp.*
		T. puericola	AB241135	A	*Cardinium sp.*
		T. urticae	AB241132	A	*Cardinium sp.*
		T. urticae	**JX844810**		*Cardinium sp.*
		T. urticae	**JX844811**		*Cardinium sp.*
	Ixodida	*Ixodes scapularis*	AB001518	A	*Cardinium sp.*
	Mesostigmata	***Dermanyssus gallinae***	**JX844812**		*Cardinium sp.*
		Metaseiulus occidentalis	AY753170	A	*Cardinium sp.*
		M. occidentalis	AY279413		*Cardinium sp.*
	Sarcoptiformes	*Chaetodactylus sp.*	EU930867		*Cardinium sp.*
		Oppiella nova	AY279414		*Cardinium sp.*
	Prostigmata	*Brevipalpus obovatus*	AY279401		*Cardinium sp.*
		Brevipalpus phoenicis	AY279412		*Cardinium sp.*
Insecta	Diptera	*Culicoides lungchiensis*	AB506777	C	*Cardinium sp.*
	Hymenoptera	*Aphytis sp.*	AY279405		*Cardinium sp.*
		Encarsia pergandiella asex.	AF319783	A	*Cardinium sp.*
		Encarsia pergandiella sex.	AY026335		*Cardinium sp.*
		Formica cinerea	GU592746		*Cardinium sp.*
		Formica cinerea	GU592750		*Cardinium sp.*
		Marietta sp.	AY327470		*Cardinium sp.*
		Plagiomerus diaspidis	AY327472	A	*Cardinium sp.*
	Hemiptera	*Abgrallaspis sp.*	GQ455411		*Cardinium sp.*
		Aspidiotus nerii	GQ455438		*Cardinium sp.*
		Dicranotropis hamata	AY279415		*Cardinium sp.*
		Lepidosaphes pinnaeformis	GQ455421		*Cardinium sp.*
		Poliaspis media	GQ455428		*Cardinium sp.*
	Homoptera	*Chionaspis heterophyllae*	GQ455426		*Cardinium sp.*
		Euides speciosa	AB506775	A	*Cardinium sp.*
Nematoda		*Heterodera glycines*	DQ314214		*Cardinium sp.*
-		*Acanthamoeba*	AF215634	outgroup	*Amoebophilus asiaticus*
-		*Acanthamoeba*	AF366581	outgroup	*A. asiaticus*

*Supergroup information: Nakamura et al., 2009

Appendix 4 Sequences used to construct a phylogenetic tree for *Spiroplasma*. Sequences obtained in this study are in bold red. Other Sequences were downloaded from GenBank (http://www.ncbi.nlm.nih.gov/genbank). Host taxonomy is presented in parantheses if the sequence is not directly originating from the mentioned host organism, but stems from a culture.

Host class	Host order	host species	Acc. No.	Clade*	species
Arachnida	Araneae	Unidentified spider	JF266583	Ixodetis	*Spiroplasma sp.*
	Trombidiformes	*Leptus sayi*	JF266580	new	*Spiroplasma sp.*
		L. sayi	JF266581	new	*Spiroplasma sp.*
	Mesostigmata	***Hypoaspis miles***	**JX844813**		*Spiroplasma sp.*
		Hypoaspis miles	**JX844814**		*Spiroplasma sp.*
		Hypoaspis miles	**JX844815**		*Spiroplasma sp.*
		Hypoaspis miles	**JX844816**		*Spiroplasma sp.*
		Hypoaspis miles	**JX844817**		*Spiroplasma sp.*
		Hypoaspis miles	**JX844818**		*Spiroplasma sp.*
		Hypoaspis miles	**JX844819**		*Spiroplasma sp.*
		Hypoaspis miles	**JX844820**		*Spiroplasma sp.*
		Hypoaspis miles	**JX844821**		*Spiroplasma sp.*
	Ixodida	*(Ixodes pacificus)*	GU585671	Ixodetis	*S. ixodetis*
Insecta	Coleoptera	*Anisosticta sp.*	AM087471	Ixodetis	*Spiroplasma sp.*
	(Coleoptera)	*Cicindela campestris*	AF036954	ME	*Entomoplasma freundtii*
	(Coleoptera)	*(Leptinotarsus decemlineata)*	AY189305	Apis	*S. leptinotarsae*
	Diptera	*Drosophila hydei*	FJ657240	CCM	*Spiroplasma sp.*
	Diptera	*Hippoboscoidea sp*	JF266586	CCM	*Spiroplasma sp.*
	Diptera	Tabanidae sp.	NR025699	CCM	*S. chrysopicola*
	Diptera	Tabanidae sp.	GU585670	Apis	*S. tabanidicola*
	(Odonata)	*(Pachydiplax longipennis)*	DQ860101	New	*S platyhelix*
			GQ275127		*Entomoplasmatales sp.*
			EF151267	Apis	*Spiroplasma sp.*
	Phasmatodea	*Agathemera claraziana*	JF266577	Ixodetis	*Spiroplasma sp.*
			GU993267	Apis	*S. apis*
Malacostraca	Decapoda	*Prokambarus clarkia*	DQ917754	CCM	*Spiroplasma sp.*
Mammalia		Goats, sheep (pathogen)	U26047	ME	*Mycoplasma capricolum*
		(pigs)	M23936	ME	*Mycoplasma sualvi*
		(humans)	M24473	ME	*Mycoplasma hominis*
			HQ228563	outgroup	*Bacillus subtilis*
			EF050047	outgroup	*Erysiopelothrix sp.*
			HQ286917	outgroup	*Escherichia coli*

*Clade information: DiBlasi et al., 2011; Regassa and Gasparich, 2006

Chapter 4

The role of the bacterial community in the nutritional ecology of the bulb mite *Rhizoglyphus robini* (Acari: Astigmata: Acaridae)

This article was accepted at the *FASEB journal* in December 2012.

The role of the bacterial community in the nutritional ecology of the bulb mite *Rhizoglyphus robini* (Acari: Astigmata: Acaridae)

Renate Zindel[1*+], Maya Ofek[2+], Dror Minz[2], Eric Palevsky[3], Einat Zchori-Fein[3] and Alexandre Aebi[1,4]

[*]Corresponding author: Phone: +41 44 3377 74 59, Fax: +41 44 337 01 72

[+]These authors contributed equally to this study

[1]Agroscope Reckenholz-Tänikon, Research Station ART, Reckenholzstr. 191, 8046 Zürich, Switzerland

[2] Institute of Soil, Water and Environmental Sciences, Agricultural Research Organization, Bet Dagan, Israel

[3] Department of Entomology, Newe Ya'ar Research Center, Agricultural Research Organization, Ramat Yishay, Israel

[4] Currently at: University of Neuchâtel, Laboratory of Soil Biology, Emile-Argand 11, 2000 Neuchâtel, Switzerland

Running title: *Rhizoglyphus robini* microbiome

Abstract

The biology of many arthropods can only be understood when their associated microbiome is considered. The nutritional requirements of the bulb mite *Rhizoglyphus robini* Claparede (Acari: Astigmata: Acaridae) in the laboratory have been shown to be very easily satisfied, and in the field the mites prefer fungus-infected over uninfected plants. To test whether symbiotic bacteria facilitate the survival of *R. robini* on a temporarily nutritionally-unbalanced diet, we investigated the composition of its microbiome. Using 454 pyrosequencing of 16S rRNA gene fragments, three genera were found to dominate the bacterial community: *Myroides* (41.4%), *Serratia* (11.4%) and *Alcaligenes* (4.5%); the latter two are known to include chitinase producing species. Laboratory experiments demonstrated that mite fecundity is significantly higher (2 times) on fungus than on controls (sterilized potato dextrose agar and filter paper). Also, when mite homogenate was applied to a chitin layer, the halo produced through degradation was clearly visible while the saline control did not produce a halo. We thus concluded that *R. robini* utilizes fungal chitin, at least to a certain extent, as a food source with the help of its associated bacteria. This information supports the general concept of multigenome organisms and the involvement of bacteria in the mite's nutritional ecology.

Keywords: bacterial symbiont/chitin digestion/DGGE/16S rRNA pyrosequencing

Introduction

Exploration of the microbiome associated with many arthropods has proven to be very important to understanding their biology (1, for examples). Among the many functions microbes can have in their hosts' biology, nutritional provision is of high importance (2). Specialized feeders as well as omnivorous arthropods may engage in obligate or facultative interactions with microbes. Arthropods with highly restricted diets may rely on bacterial symbionts for the provision of essential amino acids, vitamins, and digestive enzymes, as well as for the metabolism of fatty acids and nitrogen processing (3, 4, 5). However, omnivorous arthropods also maintain obligate and facultative bacterial associates, helping them use nutrients from different sources (6, 7). Overall, arthropods' highly divergent life strategies, from strict sap or blood feeding to omnivory, are facilitated by the association with microorganisms.

Rhizoglyphus robini Claparede (Acari: Astigmata: Acaridae), an important agricultural pest in Mediterranean countries, lives in the soil, and on the bulbs and tubers of its host plants (mainly ornamentals and crops such as lily, onion and garlic) and is therefore commonly referred to as the bulb mite. Initially described as a primary pest directly affecting its host plant (8), the mite's biology is poorly understood. In the laboratory, it is usually kept on water-soaked peanuts but will also use roots, ferns and decaying soil insects as a food source (9), and it should therefore be considered omnivorous. The extent to which this generalist lifestyle is supported by the activity of associated bacteria is unknown.

Field observations show that *R. robini* is almost exclusively found on plants which suffer from fungal infections (10). *R. robini* has been shown to choose fungus-infected over uninfected hosts in preference tests (10, 11) and to be attracted to alcohols extracted from cultures of *Fusarium oxysporum* (12). In addition, *R. robini* exhibits high fecundity on fungal food sources (10, 13). Survival of *R. robini* on wet filter paper alone was explained by the mite's ability to derive nutrients from fungal mycelia developing on the cellulosic matrix (14). This evidence suggests that fungi make up a considerable proportion of the mite's diet. Fungal cell walls consist of 3% to 60% chitin (15, 16). While most animals do not possess the

enzymes required to digest chitin, in most insects chitinases are found to be involved in molting processes (17). More importantly, also for some pyroglyphid mite species, endogenous chitinases have been reported. These chitinases are major allergens for dogs (McCall et al 2006) (18). However, fungus-feeding mites are thought to rely on chitinases provided by associated bacteria (19). The fact that *R. robini* readily feeds on fungal tissue—potentially with the help of bacteria—puts the pest status of *R. robini* into question.

Here we sought information on the nature of the *R. robini* microbiome and tested the hypothesis that symbiotic bacteria may be linked to *R. robini*'s ability to exploit fungi as a food source. The following approaches were used to describe the composition and role of the bacterial community associated with *R. robini*: denaturing gradient gel electrophoresis (DGGE) and subsequent fingerprinting methods to describe the bacterial community of individual mites, and 454 pyrosequencing to obtain an overview of the complete composition of the bacterial communities in the populations. The mite's ability to exploit fungal food sources and the consequent influence on its fecundity were tested in laboratory experiments, and chitinase activity of mite homogenate was examined in a bioassay. This combination of bioinformatic and ecological techniques revealed the importance of bacteria to understanding the biology of a major pest species.

Materials and methods

Mite origin and rearing

The mites used in this study stemmed from field populations originally collected on three different host plants [Lily (*Lilium candidum*), *Ruscus* sp. and garlic (*Allium cepa*)] and isolated in separate containers since 2005. Mites were reared in Petri dishes containing wet filter paper and non-sterilized ground peanuts, and kept in a closed box in the dark. Petri dishes were stacked on a rack standing in soapy water

to prevent escape of individual mites to other populations' boxes. Cultures were maintained by transferring 10 to 30 individual mites to a new Petri dish. Water and peanuts were added every 14 days.

Examination of bacterial community composition of individual mites

DGGE was used to describe the bacterial communities of mites stemming from different populations. Lysates from 18 individual mites (six individuals from each of the three populations) were prepared as previously described (20) and used as template for amplification of bacterial 16S rRNA gene fragments using the primer combination of 341F with a GC clamp (40-nucleotide, GC-rich sequence) and 907R (21) as described by Gottlieb *et al*. in 2010 (22).

The PCR-amplified fragments were separated by DGGE using a 6% (w/v) acrylamide gel (acrylamide-*N*/*N*' methylenebisacrylamide) prepared in 1× TAE buffer (2 M Tris base, 1 M glacial acetic acid, 50 mM EDTA) with a denaturing gradient of 20% to 60% (100% denaturant corresponding to 7 M urea and 40% v/v formamide). Electrophoresis was performed at 200 V overnight in 1% (v/v) TAE buffer at 60°C in a BIORAD D-Code system. The gel was stained with ethidium bromide and photographed using a Polaroid camera on a UV-table. The images were imported to Fingerprinting® II software (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and the DGGE patterns were aligned. Cluster analysis of the DGGE patterns was performed with Fingerprinting II using the unweighted pair-group average with arithmetic mean (UPGMA) algorithm based on 1-Pearson r distance matrix between patterns.

Tag-encoded pyrosequencing of 16S rRNA gene fragments and data analysis

To further investigate the bacterial community at the mite population level, DNA was extracted from three replicates of approximately 250 pooled individuals from each of the three populations (garlic, lily and *Ruscus*). The collected mites were surface-sterilized with 70% ethanol and stored in 100% ethanol until use. Immediately before the extraction, individuals were washed with sterile saline solution (0.85% NaCl) to remove traces of ethanol. The PowerSoil® DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad,

CA, USA) was used for DNA extraction according to the manufacturer's instructions. DNA concentrations were measured using a NanoDrop ND1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The extracted DNA was subjected to high-throughput sequencing. 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing was performed by the Research and Testing Laboratory (Lubbock, TX, USA) as described by Dowd *et al.* in 2008 (23). The resulting sequences were analyzed using MOTHUR (24). Low-quality sequences (quality score average of 30 over a 31 base window) along with fragments shorter than 200 bp (~15% of the sequences) were eliminated before further analysis was performed using the trim.seq module of MOTHUR. Sequences were aligned using a Silva-compatible alignment database. The Chimera.slayer module of MOTHUR was used to detect chimeras, which were subsequently removed. A 97% sequence-similarity threshold was used to group the sequences into operational taxonomic units (OTUs). Representatives of each OTU were selected using the MOTHUR otu.rep module and classified with the MOTHUR classify.seqs module using a Silva-compatible taxonomy database. The affiliation of each OTU representative was verified by ARB and by NCBI blast analyses. The sequences of representatives of the most abundant OTUs were imported into MEGA software (version 5.05, 25), along with sequences of closest relatives according to ARB and NCBI database searches. Sequences were aligned with the Muscle algorithm and a neighbor-joining tree was calculated, based on Tamura's 3-parameter model using complete deletion for treatment of gaps and missing data. Hence, the tree was based on 200 nucleotides, the minimal length threshold used for initial selection of sequence reads. Support of tree topology was examined by bootstrap analysis with 1000 replicates.

After classification of the sequences into OTUs, OTU counts were used for comparison of the bacterial community compositions. Sample coverage was estimated by calculating the Good coverage estimate (26). For each sample, the Chao1 richness estimate (27), and the dominance and Shannon indexes of diversity were calculated in Excel. To compare the relative abundances of different bacterial taxa, non-parametric Kruskal-Wallis ANOVA was performed with STATISTICA® (the Statistica 7.1 software package

(StatSoft Inc., Tulsa, OK)). Pyrosequencing reads were deposited in the GenBank Short Read Archive under accession no. SRA 049893.

Comparison of mite fecundity on different diets

Fecundity of same-age females was examined under four different dietary conditions: 1) no food; 2) standard peanut diet; 3) potato dextrose agar (PDA); 4) PDA with *Fusarium oxysporum* mycelia (FO). To obtain young females for fecundity measurements, mites were placed in a Petri dish with peanut food and left for 2 days for oviposition before adults were removed. After approximately 12 days, adults with a maximal age difference of 2 days had developed from eggs. To avoid any possible influence of bacteria adhering to the mite cuticle, mites were starved for 1 day, then washed with a 5% commercial bleach solution (2 min) and rinsed with 75% ethanol before each experiment. A polyspore isolate of the phytopathogenic fungus *F. oxysporum* (FO 070-041), originally isolated from wheat, was obtained from the local spore base (ART, Zürich, Switzerland) and cultivated on PDA at room temperature in the dark. The mites were divided into groups of 60 (40 females and 20 males) and placed on individual Petri dishes containing a wet filter paper with four different treatments: 1) no food (negative control); 2) non-sterilized ground peanuts; 3) a 1-cm^2 piece of PDA; 4) a 1-cm^2 piece of PDA with *F. oxysporum* mycelia (FO). Peanuts were used as a positive control, representing the diet on which laboratory populations are reared. Each treatment was replicated three times. After 2 days, 25 females from each treatment were individually placed in 25-mm diameter Petri dishes containing only a wet filter paper and were allowed to oviposit for 24 h. If a female was dead at that time, the measurement was excluded. The entire experiment was repeated three times. Data were analyzed using the Statistica 7.1 software package, applying one-way ANOVA

Chitinase activity of mite homogenates

Chitin-degrading ability of the mite holobiont was examined. Crabshell chitin (Sigma) was washed (28, 29), spread in a thin layer (3 ml per plate) and allowed to dry on minimal medium containing 10 ml of 5 mg/ml filter-sterilized nystatin solution per liter of medium, according to Faramarzi *et al.* in 2009 (30). Mites were grown with the four different feeding treatments described above-. Mites were starved and surface-sterilized, and sterility was examined by rolling the mites on the surface of Lysogeny broth (LB) agar plates. The plates were inspected 48 h later for the development of bacteria or fungi. Mite homogenates were prepared by grinding approximately 100 individual adult mites in 40 µl sterile saline solution. Of the homogenates, 4x lµ (per replicate) in five serial dilutions of 5% to 100% of the stock solution were pipetted in droplets of 1 µl on chitin agar medium. *Escherichia coli* and sterile saline solution (0.85% NaCl) served as negative controls and a chitinase-producing *Bacillus subtilis* strain was used as a positive control. Plates were incubated at 30°C and inspected after 12, 24 and 36 h for the presence of haloes. Bacteria were picked from the center and edge of the haloes with a wooden toothpick, transferred to liquid LB and nutrient broth (NB) medium, incubated at 28°C overnight and then re-pipetted onto chitin plates to confirm the bacterial origin of the chitinases.

Results

Bacterial community composition of individual mites

The bacterial community composition of *R. robini* was examined and compared among laboratory-reared populations. Complex DGGE profiles were found within all populations (Figure 1). Most of the individuals shared dominant bands, but variations in banding pattern between individuals from different treatments was significant (Figure 1). Similarity was higher within than among populations (Figure 1).

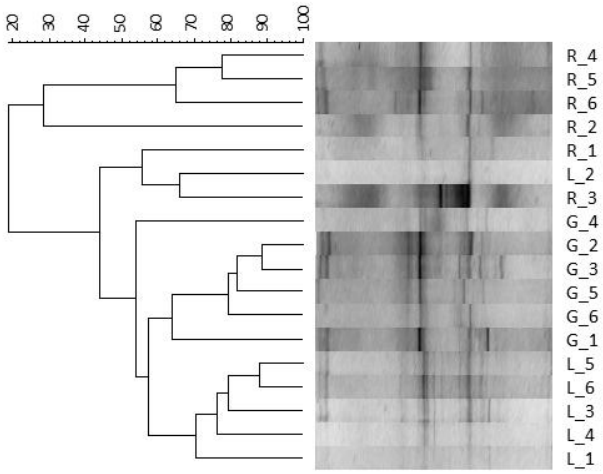


Figure 1 Comparison of bacterial community compositions of individual laboratory-reared *R. robini* from three populations: garlic (G), lily (L) and *Ruscus* (R). Compositions of bacterial communities were determined by PCR-DGGE using general bacterial primers. PCR-DGGE patterns were aligned using Fingerprinting II software and UPGMA cluster analysis was performed based on cosine similarity matrix.

Tag-encoded pyrosequencing of 16S rRNA gene fragments and data analysis

To further describe the bacterial community associated with *R. robini,* mass sequencing of 16S rRNA gene fragments was performed using 454-pyrosequencing technology. In total, 64,241 high-quality sequences were obtained. Sequences were classified into 709 OTUs using a 97% sequence-similarity threshold, and were taxonomically affiliated with 113 different genera. Table 1 presents the summary of sequence numbers and OTU classification for each population. A comparison of coverage values and diversity indices (Shannon H' and the derived evenness measure between the different populations

Table 1 Structure of *R. robini*-associated bacterial communities based on mass sequencing of 16S rRNA gene fragments.

Population	Σ sequences[1]	97% OTUs[2]	S_1[3]	%Rare[4]	Genera	Coverage[5]	Shannon[6]	Evenness[7]
Alium	20,247	556	113	96.6	63	0.966	3.49	0.636
Lilium	18,592	551	89	95.1	66	0.979	3.64	0.647
Ruscus	25,402	564	98	96.1	82	0.984	3.38	0.567
Neuman-Keuls critical range[7]					33	0.014	0.27	0.129

[1] Total number of sequences.
[2] Number of operational taxonomic units (OTUs) using a 97% sequence similarity threshold for classification. The number represents the total number of OTUs found for all replicates of the specified population.
[3] Number of singletons (OTUs represented by only one sequence).
[4] Percentage of the OTUs for which relative abundance was below 0.5% of the total number of sequences.
[5] Sample coverage estimate calculated after Good, 1953: C_{Good}= 1-F1/N F1: the number of singletons. N: the number of sequences; based on 97% OTUs classification.
[6] Average Shannon H' index of diversity H'= -Σ*pi*·ln(*pi*) : *pi* is the relative abundance of the *i*th OTU.
[7] Average Evenness, calculated as E= H'/Hmax. Hmax= H'/lnS where S is the total number of OTUs in the sample.
[8] Coverage, Chao1, dominance and Shannon indices were compared by non-parametric ANOVA and the critical range was determined.

revealed no significant differences. Relative abundances of most OTUs were below 0.5% of the total sequences, and between 16% and 20% of the OTUs were represented by a single sequence (Table 1).

The bacterial community of laboratory-reared *R. robini* was dominated by bacteria belonging to two phyla, Bacteroidetes and Proteobacteria, together accounting for 92% of the sequences (Figure 2).

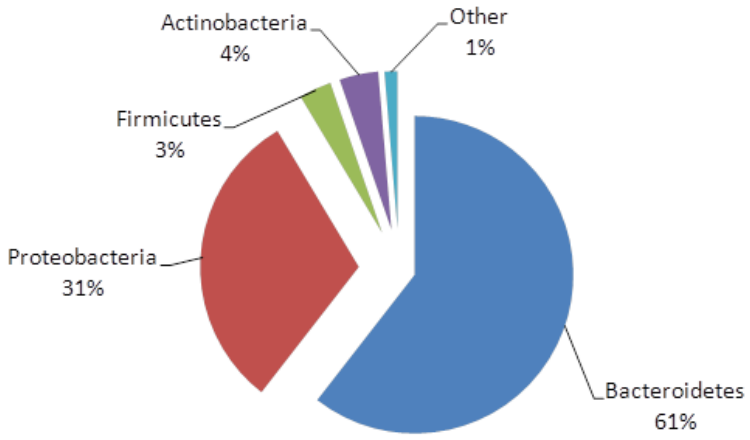


Figure 2 Composition of bacterial community associated with *R. robini*, based on 64,241 partial sequences of 16S rRNA genes pooled from the three laboratory-reared populations.

Moreover, sequences related to the Flavobacteriaceae (Bacteroidetes) and Enterobacteriaceae (γ-Proteobacteria) comprised 56% and 15.9% of the total sequences, respectively. Only 24 OTUs had relative abundances of over 0.5% and included 74.9% of the total sequences pooled from all populations. Closely related sequences of these 24 OTUs (according to ARB-Silva and NCBI databases) were previously retrieved from the gut of insects and other arthropods, the rhizosphere and endosphere of plants, compost, sludge and wastewater (Figures 3 and 4).

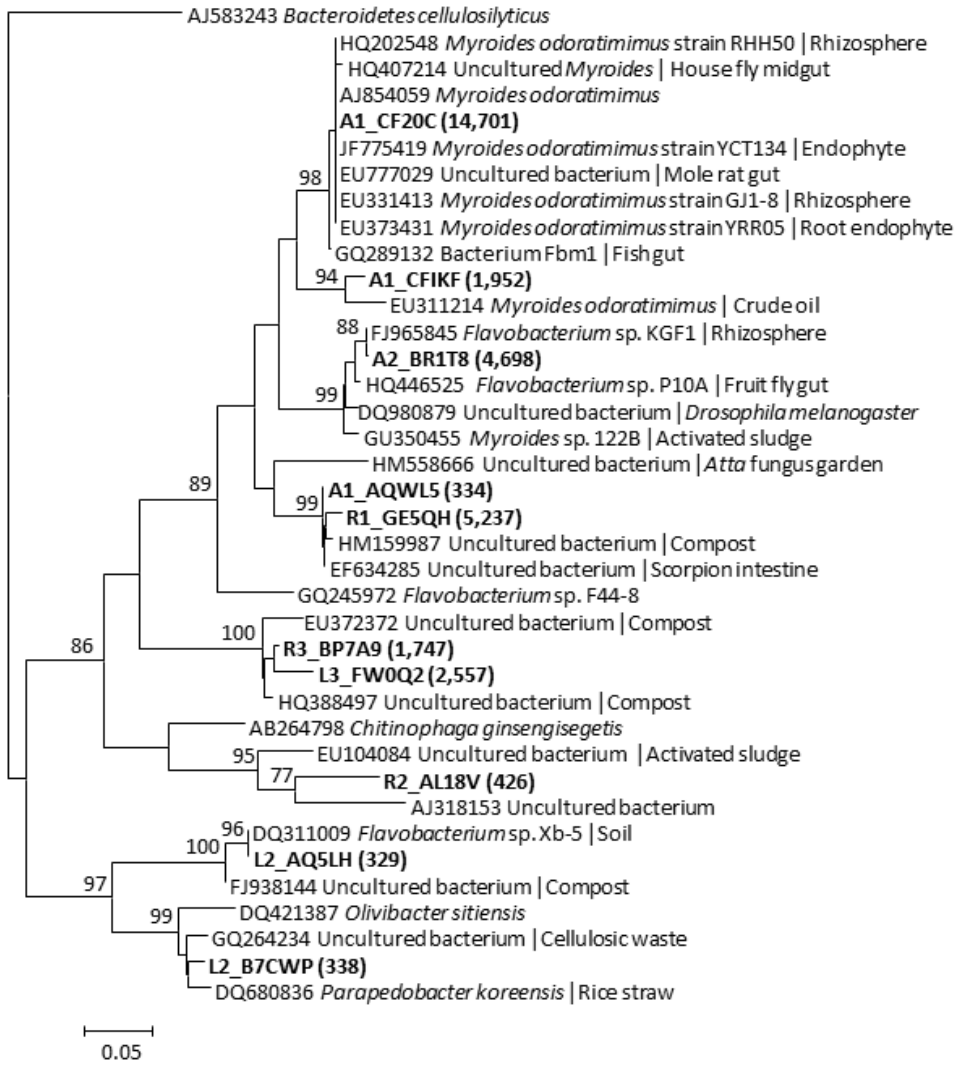


Figure 3 Phylogenetic tree of sequences representing the most abundant Bacteroidetes OTUs' 16S rRNA gene fragments obtained by mass sequencing and related sequences. A neighbor-joining tree was constructed using the ARB software package (31). Scale bar represents 0.05 substitutions per nucleotide position. Sequences recovered from this study are in boldface. Numbers in brackets indicate the number of sequences (out of 64,241 in total) included in the specific OTU. The origin sample from which related sequences were recovered is indicated after the | sign.

The single most abundant OTU, comprising 22.9% of the total sequences, was classified as *Myroides* (Flavobacteria) and was closely related (>99%) to *Myroides odoratimimus* (represented by sequence A1_CF20C in Figure 3). Two other OTUs were affiliated with the genus *Myroides*, which altogether accounted for 33.2% of the total sequences. The second most abundant OTU (9.8% of the total sequences) was affiliated with the genus *Serratia* (Enterobacteriaceae) and was closely related (>99%) to

Serratia marcescens (represented by sequence L2_BF92D in Figure 4). Together with a second *Serratia*-affiliated OTU (Figure 4), 11.4% of the total bacterial sequences were explained.

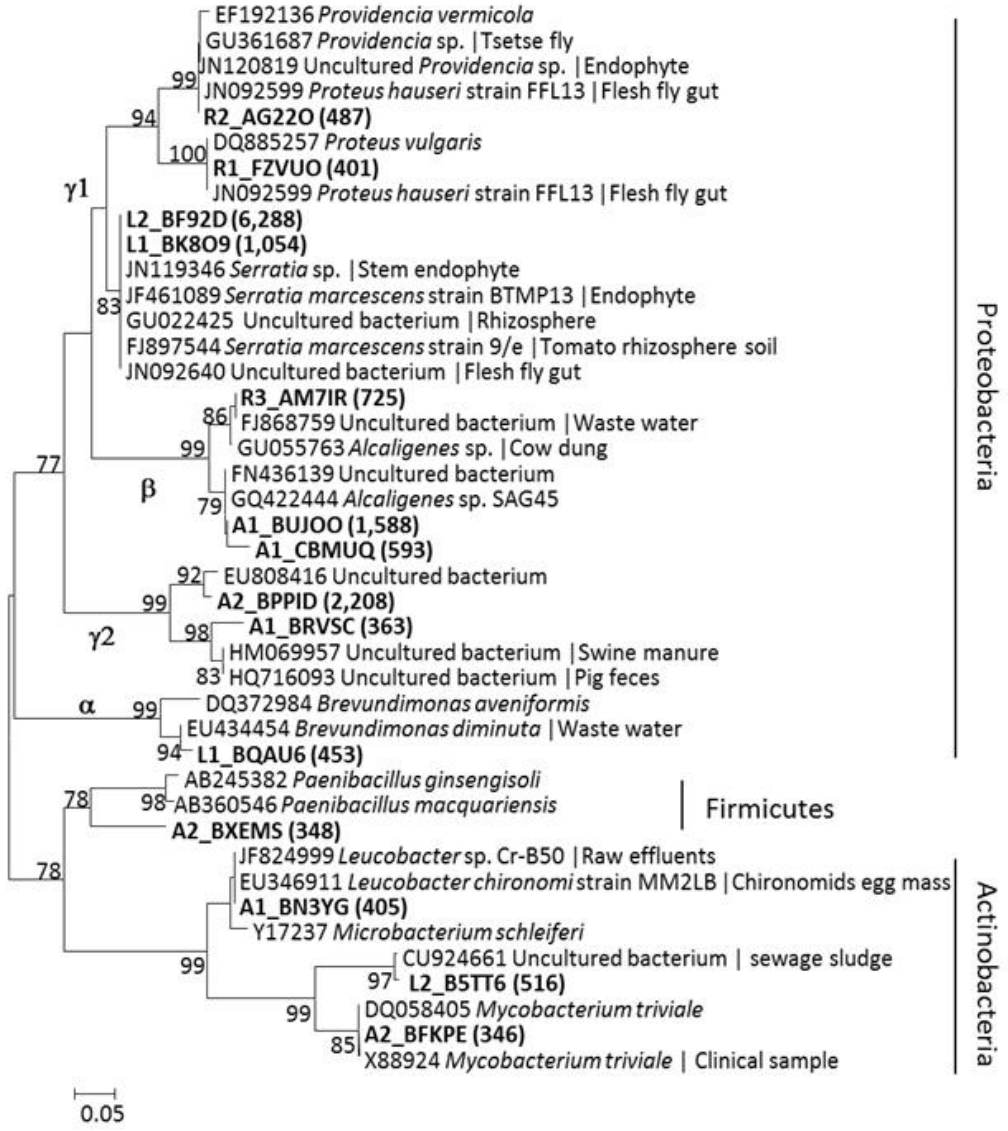


Figure 4 Phylogenetic tree of sequences representing the most abundant Actinobacteria, Firmicutes and Proteobacteria OTUs' 16S rRNA gene fragments obtained by mass sequencing and related sequences. A neighbor-joining tree was constructed using the ARB software package (31). Scale bar represents 0.05 substitutions per nucleotide position. Sequences recovered from this study are in boldface. Numbers in brackets indicate the number of sequences (out of 64,241 in total) included in the specific OTU. The origin sample from which related sequences were recovered is indicated after the | sign.

Differing bacterial community profiles were found between the three populations examined (Table 2). These populations shared 22 of the 24 dominant OTUs, but in some the relative abundance differed significantly. However, differences in composition occurred. OTUs related to an unclassified

Pseudomonadaceae and an unclassified Bacillaceae were among the dominant OTUs in the garlic population, but were close to or below the detection limit in the lily and *Ruscus* mite populations (Table 2).

Table 2 Comparison of relative abundances of the most dominant bacterial OTUs between three laboratory-reared populations of *R. robini*. Averages (*n* = 3) of relative abundances are presented for each OTU.

Phylogenetic affiliation		OTU representative[1]	Relative abundance (%)[2]		
			Allium	*Lilium*	*Ruscus*
Bacteroidetes					
Flavobacteria	*Myroides*	A1_CF20C	19.36 b	9.95 b	35.65 a
		A2_BR1T8	12.65 a	7.98 ab	4.14 b
		A1_CFIKF	2.85 ab	1.33 b	4.97 a
	Flavobacterium	R1_GE5QH	8.13	10.02	8.46
		L2_AQ5LH	0.22	0.76	0.64
	Flavobacteriacaea	L3_FW0Q2	1.14 c	7.82 a	3.40 b
		R3_BP7A9	0.81	4.68	1.94
		A1_AQWL5	0.38	0.51	0.54
Sphingobacteria	*Parapedobacter*	L2_B7CWP	0.03 b	1.40 a	0.51 ab
Unclassified	Unclassified	R2_AL18V	0.02 b	0.32 b	1.46 a
γ-proteobacteria					
	Serratia	L2_BF92D	7.78	14.74	1.38
		L1_BK8O9	1.49	2.54	0.18
	Providencia	R2_AG22O	0.06 b	0.54 ab	1.51 a
	Proteus	R1_FZVUO	0.08 b	0.48 b	1.21 a
	Alcanivoracaceae	A2_BPPID	6.22 a	2.26 b	1.86 b
	Pseudomonadaceae	A1_BRVSC	2.38 a	0.03 b	0 b
β-proteobacteria					
	Alcaligenes	A1_BUJOO	3.43	1.85	2.48
		R3_AM7IR	2.21	0.69	1.19
		A1_CBMUQ	1.95 a	0.23 b	0.97 ab
α-proteobacteria					
	Brevundimonas	L1_BQAU6	0.15	1.25	1.04
Firmicutes					
	Bacillaceae	A2_BXEMS	2.09 a	0.004 b	0 b
Actinobacteria					
	Mycobacterium	A2_BFKPE	0.47	0.19	0.69
	Leucobacter	A1_BN3YG	0.87	1.6	0.21
	Actinomycetales	L2_B5TT6	0.35 b	1.74 a	0.43 b
		∑	75.12	72.91	74.86

[1] The name of the sequence representing the OTU classified at 97% sequence similarity threshold. See also Figures 3 and 4.

Comparison of mite fecundity on different diets

Monitoring the fecundity of females fed on four different food sources, FO-fed females and peanut-fed females were found to have similar fecundity levels (Figure 5), which were approximately twice those of the negative control and PDA treatments. These effects of food source were highly significant (ANOVA, n = 60 per treatment; degrees of freedom = 3, $p < 0.01$).

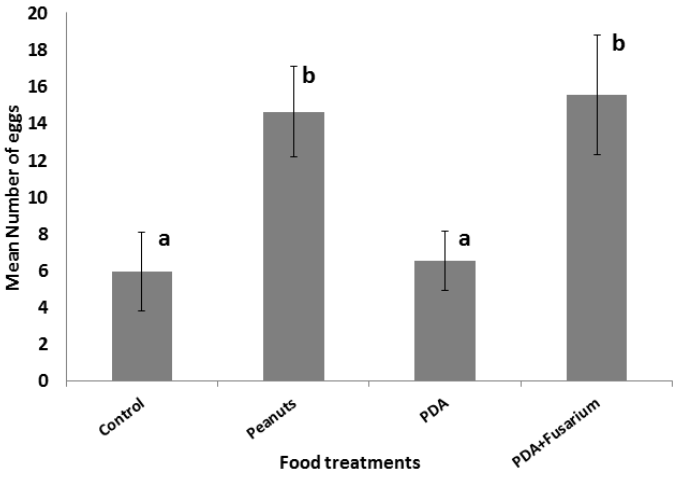


Figure 5 Number of eggs laid by same-age *R. robini* females after feeding on four different food sources for 2 days: Control—no food; Peanuts—nonsterilized ground peanuts; PDA; PDA + FO (an approximately 7-day-old culture of *F. oxysporum* on PDA) (ANOVA, n = 60 per treatment; degrees of freedom = 3, $p < 0.01$). All containers contained wet filter paper for moisture supply.

Chitinase activity of mite homogenates

To demonstrate the chitinolytic activity of the bacterial community associated with *R. robini*, we applied different concentrations of mite homogenate, prepared with surface-sterilized mites, on plates overlaid with a layer of chitin. The homogenate of all tested mites (from all four treatments) was able to clear chitin around the droplet (Figure 6), whereas neither saline solution alone nor *E. coli* bacterial cells produced a "halo". However, we did not quantify the effect of food source on presence or size of the cleared zones. Bacteria isolated from the center and outer circle of the halo showed the same strong chitinase activity when reapplied as colonies to a chitin-covered Petri dish (data not shown).

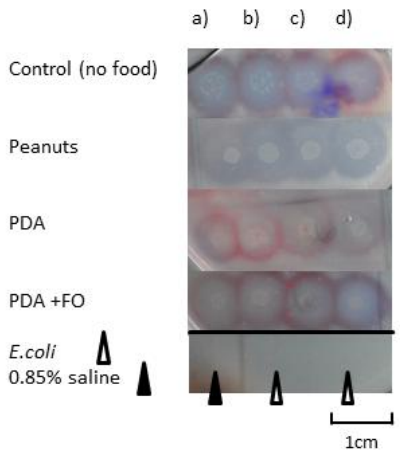


Figure 6 Demonstration of chitin degradation ("haloes") around 1µl droplets of mite homogenate diluted from 1/20 (a) to 1/4 (d). Mites were previously fed on different food sources: Control—no food; Peanuts—nonsterilized ground peanuts; PDA + FO (an approximately 7-day-old culture of *F. oxysporum* on PDA). The photo was taken after 24 h of incubation. *E. coli,* as well as pure saline solution were used as negative controls.

Discussion

The rationale for this study was the acknowledged importance of arthropod host-microbiome interactions, particularly with respect to host nutrition. While some studies have examined the ability of *R. robini* to transmit fungal and bacterial plant pathogens (32), bacterial symbionts of this plant-pest mite have never been studied. More importantly, the combination of culture-independent characterization of the associated microbiome with in vivo tests provides a unique view on the interactions between bacteria and their mite host.

A complex bacterial community was described in *R. robini* using DGGE. The analyzed mites were taken from three laboratory-reared populations, originally derived from infected material of three plant species. Initial comparison of the mites' bacterial profiles revealed shared dominant populations (Figure 1), indicating the presence of an autochthonous core community. In contrast, several bands were only present in a subset of the analyzed individuals, suggesting a species-rich, facultative interaction. Due to the laboratory background of the populations used for this study, differences between populations are difficult to interpret in an evolutionary context. In other generalist feeders a similarly complex DGGE

profile has been observed (33), which lead us to the idea that arthropods with diverse diets might harbor rich microbial communities to assist them in overcoming the nutritional challenges to which they are exposed. Gerson *et al.* (1991) (9) describe *R. robini* as a generalist, feeding on different types of decaying material and fungi, consistent with the observed complex bacterial profile. In a preliminary attempt to look at field collected populations we detected an even greater diversity in bacterial community composition (preliminary data, not shown) possibly reflecting the even greater diversity of food sources found under natural conditions.

A metagenomic approach was used to further describe the bacterial diversity associated with the examined populations. Such an approach has been recently used for comprehensive elucidation of bacterial community compositions and structures in different arthropods, including fleas (34), honey bees (35) and ticks (36). High-throughput sequencing of bacterial 16S rRNA gene fragments revealed high taxonomic richness in the *R. robini* bacterial community (Table 1). Indeed, over 700 OTUs, belonging to 113 different genera, were identified. However, for each of the populations examined, less than 5% of the OTUs had relative abundance values above 0.5% (Table 1), meaning that very few species represented the majority of bacterial cells in the community, while the rest were present in low relative abundance. The importance of rare species within this system cannot be neglected, as this genetic pool may become significant to the host following variations in environmental conditions (37). However, rare species may be non-resident (allochtonous) populations, ingested with the food and passing through the mite's elementary tract. Wong *et al.* (2011) (38) have stressed that allochtonous populations artificially inflate diversity in assessments of individual hosts as well as host populations. Due to the permissive feeding habits of *R. robini*, such a scenario is possible and we would expect this to be even more pronounced in field collected populations.

Bacteroidetes and Proteobacteria together represented 92% of the bacterial community (Figure 2). Thus, the core community of resident bacteria of the mite pest consists of a restricted number of phyla and is shared by all three examined populations. Indeed, variation between populations was related to

differences in relative abundance of the core populations, while differences in composition were uncommon (Table 2). Furthermore, the 24 most abundant OTUs, belonging to 14 genera, represented 74.9% of the bacterial community. A comparable magnitude of diversity has been found for adult blood-feeding fleas (34). In that study, the 23 most abundant OTUs represented ca. 50% of the bacterial community and included well-studied maternally transmitted endosymbionts belonging to the order Rickettisales, or the genera *Cardinium* and *Bartonella*. In the survey of laboratory-reared *R. robini* populations presented here, at least three bacterial genera previously reported as intracellular endosymbionts were detected: *Serratia sp*. (39), *Flavobacterium sp*. (40) and *Alcaligenes sp*. (41). However, PCR assays using species-specific primers did not reveal the presence of the insect-endosymbionts *Flavobacterium*, *Wolbachia*, *Cardinium*, *Rickettsia* or *Spiroplasma* in individual mites (Zindel et al. unpublished). Further studies are required to explore the localization (gut lumen or within the host, intra- or extracellular) and maternal transmission of the bacteria found in order to characterize their individual associations with the mite. Maternal transmission as well as specialized structures such as bacteriocytes within the host would indicate a very close association and an important role of the symbiotic bacterium for the host.

The genera *Myroides, Serratia, Flavobacterium, Alcaligenes, Providencia, Proteus* and *Brevundimonas* comprised the core bacterial community of the laboratory-reared *R. robini* populations tested (Figures 4 and 5). These genera have been reported to share habitats, such as the soil and rhizosphere of plants (42-46) and most relevant to this study, the gut of arthropods (47-51). *Serratia sp*. have previously been found associated with mites feeding on fungi (18). Some of the above mentioned genera include species and subspecies of bacteria that are pathogenic to insects and other arthropods (e.g. *Serratia* spp.; 52). Also, we find many species with a generalist lifestyle among them, which could suggest a rather loose association with the host, and possibly exchange with the surrounding soil and plant environment. Such an exchange could make a major contribution to the mite's ability to become established and grow on its host plant. As demonstrated by Okabe and Amano (1991) (11), *R. robini* will preferentially attack fungus-

infected or injured hosts, where its population establishment is considerably improved. While bulb injury physically facilitates the mite's movement through the plant tissue, exchange of bacteria with the host may support the mite's nutrition, particularly if the bacteria can efficiently pre-digest the fungal phytopathogen, the plant material, or both. Due to its high protein content (53, 54), a fungus-based diet may be favored by the mite. Besides the chitin, which requires the presence of chitinases, also other components from the cell content of the hyphae, such as trehalose, can be used by the mites (55).

The main taxonomic groups of the bacterial community associated with *R. robini* may differ in their chitinolytic activities. Chitinolytic activity of *Myroides* spp. has not been reported to date. However, the closest relative of the most dominant *Myroides*-related OTU in our dataset (Figure 3) exhibits *in-vitro* antagonism toward *F. oxysporum* as well as *Rhizoctonia solani* (56). This suggests that chitinolytic activity for these members is possible. In contrast to *Myroides*, members of the genus *Serratia* (Enterobacteriaceae), several *Alcaligenes* species (Alcaligenaceae) and *Flavobacterium* species are known for their chitinase activity (57-64). *Serratia* species are among the most effective chitin-degrading bacteria and the dominant species found here, *S. marcescens*, is able to produce at least four different chitinase enzymes as well as a chitin-binding protein (58). This set of biochemical capabilities has been shown to enable growth of this bacterium on fungal mycelium as a sole source of nutrition (64). Smrz and Catska (2010) (19) published a list of chitin-degrading bacteria found in association with mycophagous mites. This list comprises two *Serratia sp.*, *S. liquiefaciens* and *S. marcescens*, isolated from *Tyrophagous putrescentiae*, another acarid mite to *R. robini*.

Because the results showed that *R. robini*'s micobiome abounds in chitinolytic bacteria, we investigated the mite's fecundity on a fungal diet as well as the holobiont's chitinase activity. The standard peanut diet and the FO mycelium diet are similar in that they are both rich in fats and protein. However, chitinase activity is required for efficient utilization of FO mycelia. Transferring the mites from the peanut diet to the FO diet did not change their fecundity (Figure 5). This shows that *R. robini* readily feeds on fungus and apparently also possesses the ability to use it as a food source. The chitinase activity

of mites associated with their bacterial community was evaluated by application of mite homogenate on a thin chitin layer, where enzymatic decomposition of the purified carbohydrate could be observed directly. The fact that homogenate of surface-sterile mites was able to produce a clear "halo" in the chitin layer can be interpreted as the presence of chitinases in either the mite's body itself or its microbiome (Figure 6). Potential *R. robini* endogenous chitinases as well as present lysozyme, which can to a certain extent degrade chitin as well (65) cannot be distinguished from bacterial chitinases at this point. In mites, endogenous chitinases have to date only been isolated from Dermatophagoidae (66), where they were described as allergens to dogs and humans. However, bacteria picked from the haloes also grew on chitin as sole food source in subsequent cultivation and were able to produce a halo again. These results confirm that at least some of chitinases are of bacterial origin. The chitinolytic activity was obvious in all treatments, excluding the possibility that the food is the source of the activity. Moreover, this result shows that the potential to feed on fungi is maintained during periods of starvation (mites were starved for 2 days before being transferred to the different diets) or during periods of suboptimal nutrition (represented here by the no food control and PDA diets). This corresponds to the findings of Wooddy and Fashing (1993) (14) that the subsistence of *R. robini* on filter paper alone is dependent on the progressive development of cellulolytic fungi. The thriving of mites feeding on peanuts in the rearings as well as in this experiment could be explained by the fact that the peanuts fed to the mites are not sterilized, but kept dry and frozen. Prior to feeding, it only takes a few hours for fungi, probably vectored by the mites themselves, to start growing and serve as a food source for the mites. Also, *R. robini* has been shown to possess cellulose to digest plant material (67). (Myroides involvement)The results of the experiment further support the hypothesis that *R. robini* is a secondary pest, feeding at least to a certain extent, on the fungi growing on their host plant rather than on the host plant itself (Eric Palevsky and Uri Gerson, unpublished). Of course, it is highly likely that the mite actually feeds on a combination of fungal tissue and (pre-decomposed) plant material. Chitinase (along with trehalase and cellulase) activity has been used to classify the species of oribatid mites in feeding guilds as fungivorous

grazers or herbofungivorous grazers (55). Based on the results of this study and those of Bowman and Childs (1982) (67), *R. robini* should probably be classified in the latter feeding category. However, trehalase activity would still have to be confirmed and further studies would be needed to determine the relative importance of the different food components in the mite's nutrition. Moreover, both of the above mentioned studies did not distinguish between endogenous enzymes and enzymes of potential bacterial origins. Also, in our system we cannot exclude the presence of additional endogenous chitinases in the mite, besides the enzymatic activity we could assign to culturable bacteria. Finally we would like to mention the possibility of some of the associated bacteria themselves being a food source to the mite, as shown and discussed in Erban and Hubert (2008) (68).

We show here that the diverse bacteriome associated with an arthropod can be responsible for the host's ability to use a wide range of food sources, including fungi, which are otherwise mostly unavailable. The data support a growing number of studies stressing the importance of considering the multigenome organism (arthropod with its microbiome) when trying to unravel the complex life strategy of certain arthropods. Expanding our knowledge of this neglected aspect of arthropod biology and detailing the different species in the microbiome might open up possibilities for various manipulations of these biological systems towards, for example, eliminating essential bacteria, disrupting the community's composition and function, or controlling fungal infection, to achieve effective pest or disease-vector control. Also, the number of studies investigating bacterial communities associated with arthropods and other organisms is quickly increasing and will help with the general interpretation of diversity and composition and give a stronger base for extrapolation in general patterns.

Acknowledgements

This article was supported by the COST-SER project C08Æ0109 to AA, part of the COST Action FA0701. We thank Shira Gal and Netta Moses-Daube for help and support in the laboratory in Israel and Steven

Bacon, Mario Waldburger, Irene Bänziger and Franco Widmer in the laboratory in Switzerland. We also thank Max Kolton and Pilar Junier for their assistance and advice on experiments involving chitin.

References

1 Zindel, R., Gottlieb, Y. and Aebi, A. (2011) Arthropod symbioses: a neglected parameter in pest- and disease-control programmes. *J. Appl. Ecol.* **48**, 864–872

2 Dillon, R.J. and Dillon, V.M. (2004) The gut bacteria of insects: Nonpathogenic interactions. *Ann. Rev. Entomol.* **49**, 71–92

3 Guenduez, E.A. and Douglas, A.E. (2009) Symbiotic bacteria enable insect to use a nutritionally inadequate diet. *Proc. R. Soc. Lond. B Biol. Sci.* **276**, 987–991

4 Grünwald, S., Pilhofer, M. and Höll, W. (2010) Microbial associations in gut systems of wood- and bark-inhabiting longhorned beetles [Coleoptera: Cerambycidae]. *Syst. Appl. Microbiol.* **33**, 25–34

5 Snyder, A.K., Deberry, J.W., Runyen-Janecky, L. and Rio, R.V.M. (2010) Nutrient provisioning facilitates homeostasis between tsetse fly (Diptera: Glossinidae) symbionts. *Proc. R. Soc. Lond. B Biol. Sci.* **277**, 2389–2397

6 Feldhaar, H. and Gross, R. (2009) Insects as hosts for mutualistic bacteria. *Int J Med Microbiol.* **299**, 1–8

7 Sabree, A.L., Kambhampati, S. and Moran, N.A. (2009) Nitrogen recycling and nutritional provisioning by *Blattabacterium*, the cockroach endosymbiont. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 19521–19526

8 Manson, D.C.M. (1972) A contribution to the study of the genus *Rhizoglyphus* Claprède, 1869 (Acarina: Acaridae). *Acarologia* **13**, 621–650

9 Gerson, U., Cohen, E. and Capua, S. (1991) Bulb mite, *Rhizoglyphus robini* (Astigmata, Acaridae) as an experimental animal. *Exp. Appl. Acarol.* **12**, 103–110

10 Hanuny, T., Inbar, M., Tsror, L. and Palevsky E. (2008) Complex interactions between *Rhizoglyphus robini* and *Fusarium oxysporum*: implications on onion pest management. IOBC Proceedings of Integrated Control of Protected Crops, Temperate Climate. *IOBC/ WPRS Bulletin* **32**, 71–74

11 Okabe, K. and Amano, H. (1991) Penetration and pop growth of the robine bulb mite, *Rhizoglyphus robini* Claprède, on healthy and *Fusarium*-infested rakkyo bulbs. *Appl. Entomol. Zool.* **26**, 129–136

12 Okabe, K. and Amano, H. (1990) Attractancy of alcohols isolated from culture filtrates of *Fusarium* fungi for the robine bulb mite *Rhizoglyphus robini* Claprede (Acari, Acaridae) in sand. *Appl. Entomol. Zool.* **25**, 397–404

13 Okabe, K. and Oconnor, B.M. (2001) A method for both mass and individual rearing of fungivorous astigmatid mites (Acari). *Exp. Appl. Acarol.* **25**, 493–504

14 Wooddy, M.W. and Fashing N.J. (1993) The ability of *Rhizoglyphus robini* Claparède (Astigmata: Acaridae) to subsist solely on a diet of filter paper. *Int. J. Acarol.* **19**, 345–348

15 Hudson, H.J. (1986) Fungal Biology. Edward Arnold: London

16 Smrz, J. and Soukalova, H. (2008) Mycophagous mites (Acari: Oribatida and Acaridida) and their cooperation with chitinolytic bacteria. The 6th European Congress of Integrative Acarology pp. 374–377

17 Arakane, Y. and Muthukrishnan, S. (2010) Insect chitinase and chitinase-like proteins. *Cell. Mol. Life Sci.* **67**, 201-216

18 McCall, C., Hunter, S., Stedman, K., Weber, E., Hillier, A., Bozic, C., Rivoire, B., and Olivry, T. (2001) Characterization and cloning of a major high molecular weight house dust mite allergen (Der f 15) for dogs. *Vet. Immunol. Immunopathol.***78**, 231-247

19 Smrz, J. and Catska, V. (2010) Mycophagous mites and their internal associated bacteria cooperate to digest chitin in soil. *Symbiosis* **52**, 33–40

20 Frohlich, D.R., Torres-Jerez, I., Bedford, I.D., Markham, P.G. and Brown, J.K. (1999) A phylogeographical analysis of the *Bemisia tabaci* species complex based on mitochondrial DNA markers. *Mol. Ecol.* **8**, 1683–1691

21 Muyzer, G., Dewaal, E.C. and Uitterlinden, A.G. (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S ribosomal RNA. *Appl. Environ. Microbiol.* **59**, 695–700

22 Gottlieb, Y., Ghanim, M., Chiel, E., Gerling, D., Portnoy, V., Steinberg, S., Tzuri, G., Horowitz, A.R., Belausov, E., Mozes-Daube, N., Kontsedalov, S., Gershon, M., Gal, S., KatZir, N. and Zchori-Fein, E. (2006) Identification and localization of a *Rickettsia* sp. in *Bemisia tabaci* (Homoptera: Aleyrodidae). *Appl. Environ. Microbiol.* **72**, 3646–3652

23 Dowd, S.E., Callaway, T.R., Wolcott, R.D., Sun, Y., McKeehan, T., Hagevoort, R.G. and Edrington, T.S. (2008) Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). *B.M.C. Microbiol.* **8**, 125

24 Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J. and Weber, C.F. (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* **75**, 7537–7541

25 Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**, 2731–3739

26 Good, I.J. (1953) The population frequencies of species and the estimation of the population parameters. *Biometrika* **40**, 237–264

27 Chao, A. (1984) Non-parametric estimation of the number of classes in a population. *Scand. J. Stat.* **11**, 265–270

28 McBride, M.J. and Braun, T.F. (2004) GldI is a lipoprotein that is required for *Flavobacterium johnsoniae* gliding motility and chitin utilization. *J. Bacteriol.* **186**, 2295–2302

29 Reichenbach, H. (2006) The genus *Lysobacter*. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds). The Prokaryotes. Springer: New York, pp 939–957

30 Faramarzi, M.A., Fazeli, M., Tabatabaei Yazdi, M., Adrangi, S., Jami Al Ahmadi, K., Tasharrofi, N. and Aziz Mohseni, F. (2009) Optimization of cultural conditions for production of chitinase by a soil isolate of *Massilia timonae*. *Biotechnology* **8**, 93–99

31 Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S., Jobb, G., Forster, W., Brettske, I., Gerber, S., Ginhart, A.W., Gross, O., Grumann, S., Hermann, S., Jost, R., Konig, A., Liss, T., Lussmann, R., May, M., Nonhoff, B., Reichel, B., Strehlow, R., Stamatakis, A., Stuckmann, N., Vilbig, A., Lenke, M., Ludwig, T., Bode, A. (2004) ARB: a software environment for sequence data. *Nucleic Acids Res.* **32**, 1363–1371

32 Díaz, A., Okabe, K., Eckenrode, C.J., Villani, M.G. and Oconnor, B.M. (2000) Biology, ecology, and management of the bulb mites of the genus *Rhizoglyphus* (Acari: Acaridae). *Exp. Appl. Acarol.* **24**, 85–113

33 Mrázek, J., Štrosová, L., Fliegerová, K., Kott, T. and Kopečný, J. (2008) Diversity of insect intestinal microflora. *Folia Microbiol.* **53**, 229–233

34 Jones, R.T., Knight, R. and Martin, A.P. (2010) Bacterial communities of disease vectors sampled across time, space, and species. *ISME J.* **4**, 223–231

35 Cox-Foster, D.L., Conlan, S., Holmes, E.C., Palacios, G., Evans, J.D., Moran, N.A., Quan, P. L., Briese, T., Hornig, M., Geiser, D. M., Martinson, V., vanEngelsdorp, D., Kalkstein, A. L., Drysdale, A., Hui, J., Zhai, J., Cui, L., Hutchison, S. K., Simons, J. F., Egholm, M., Pettis, J. S. and Lipkin, W. I. (2007) A metagenomic survey of microbes in honey bee colony collapse disorder. *Science* **318**, 283–287

36 Andreotti, R., de León, A.A.P., Dowd, S.E., Guerrero, F.D., Bendele, K.G. and Scoles, G.A. (2011) Assessment of bacterial diversity in the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* through tag-encoded pyrosequencing. *B.M.C. Microbiol.* **11**, 6

37 Zilber-Rosenberg, I. and Rosenberg, E. (2008) Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiol. Rev.* **32**, 723–735

38 Wong, C.N.A., Ng, P. and Douglas, A.E. (2011) Low-diversity bacterial community in the gut of the fruitfly *Drosophila melanogaster*. *Environ. Microbiol.* **13**, 1889–1900.

39 Oliver, K. M., Moran, N. A., and Hunter, M. S. (2006) Costs and benefits of a superinfection of facultative symbionts in aphids. *Proc. R. Soc. Lond. Ser. B-Biol. Sci.* **273**, 1273-1280

40 Bandi, C., Damiani, G., Magrassi, L., Grigolo, A., Fani, R., and Sacchi, L. (1994) *Flavobacteria* as intracellular symbionts in cockroaches. *Proc. R. Soc. Lond. Ser. B-Biol. Sci.* **257**, 43-48

41 Bextine, B., Lauzon, C., Potter, S., Lampe, D. and Miller, T.A. (2004) Delivery of a genetically marked *Alcaligenes* sp. to the glassy-winged sharpshooter for use in a paratransgenic control strategy. *Curr. Microbiol.* **48**, 327–331

42 Tripathi, A.K., Verma, S.C. and Ron, E.Z. (2002) Molecular characterization of a salt-tolerant bacterial community in the rice rhizosphere. *Res. Microbiol.* **153**, 579–584

43 Berg, G., Krechel, A., Ditz, M., Sikora, R.A., Ulrich, A. and Hallmann, J. (2005) Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. *FEMS Microbiol. Ecol.* **51**, 215–229

44 Harichová, J., Karelová, E., Chovanová, K., Stojnev, T., Prokšová, M., Brindza, J., Brindza, P., Tóth, D., Pangallo, D. and Ferianc, P. (2006) Comparison of culturable Gram-negative bacterial community structures in the rhizosphere of three fruit plants. *Biologia* **61**, 663–670

45 Teixeira, L., Peixoto, R.S., Cury, J.C., Sul, W.J., Pellizari, V.H., Tiedje, J. and Rosado, A.S. (2010) Bacterial diversity in rhizosphere soil from Antarctic vascular plants of Admiralty Bay, maritime Antarctica. *ISME J.* **4**, 989–1001

46 Jin, F., Ding, Y., Ding, W., Reddy, M.S., Fernando, D.W.G. and Du, B. (2011) Genetic diversity and phylogeny of antagonistic bacteria against *Phytophthora nicotianae* isolated from tobacco rhizosphere. *Int. J. Mol. Sci.* **12**, 3055–3071

47 Spiteller, D., Dettner, K. and Boland, W. (2000) Gut bacteria may be involved in interactions between plants, herbivores and their predators: Microbial biosynthesis of N-acylglutamine surfactants as elicitors of plant volatiles. *Biol. Chem.* **381**, 755–762

48 Jaffe, K., Caetano, F.H., Sanchez, P., Hernandez, J., Caraballo, L., Vitelli-Flores, J., Monsalve, W., Dorta, B. and Lemoine, V.R. (2001) Sensitivity of ant (*Cephalotes*) colonies and individuals to antibiotics implies feeding symbiosis with gut microorganisms. *Can. J. Zool.* **79**, 1120–1124

49 Dharne, M.S., Gupta, A.K., Rangrez, A.Y., Ghate, H.V., Patole, M.S. and Shouche, Y.S. (2008) Antibacterial activities of multi drug resistant *Myroides odoratimimus* bacteria isolated from adult flesh flies (Diptera: Sarcophagidae) are independent of metallo beta-lactamase gene. *Braz. J. Microbiol.* **39**, 397–404

50 Zaspel, J.M. and Hoy, M.A. (2008) Microbial diversity associated with the fruit-piercing and blood-feeding moth *Calyptra thalictri* (Lepidoptera: Noctuidae). *Ann. Entomol. Soc. Am.* **101**, 1050–1055

51 Wang, H., Jin, L. and Zhang, H. (2011) Comparison of the diversity of the bacterial communities in the intestinal tract of adult *Bactrocera dorsalis* from three different populations. *J. Appl. Microbiol.* **110**, 1390–1401

52 Kleespies, R.G., Huger, A.M. and Zimmermann, G. (2008) Diseases of insects and other arthropods: results of diagnostic research over 55 years. *Biocontrol Sci. Technol.* **18**, 439–482

53 Christias, C., Couvarki, C., Georgopoulos, S.G., Macris, B. and Vomvoyanni, V. (1975) Protein content and amino acid composition of certain fungi evaluated for microbial protein production. *Appl. Microbiol.* **29**, 250–254

54 Srivastava, S., Pathak, N. And Srivastava, P. (2011) Identification of limiting factors for the optimum growth of *Fusarium oxysporum* in liquid medium. *Toxicol. Int.* **18**, 111–116

55 Siepel, H. and de Ruiter-Dijkman, E.M. (1993) Feeding guilds of oribatid mites based on their carbohydrase activities. *Soil Biol. Biochem.* **25**, 1491–1497

56 Seo, W.T., Lim, W.J., Kim, E.J., Yun, H.D., Lee, Y.H. and Cho, K.M. (2010) Endophytic bacterial diversity in the young radish and their antimicrobial activity against pathogens. *J. Korean Soc. Appl. Biol. Chem.* **53**, 493–503

57 Monreal, J. and Reese, E.T. (1969) The chitinase of *Serratia marcescens*. *Can. J. Microbiol.* **15**, 689–696

58 Brurberg, M.B., Synstad, B., Klemsdal, S.S., VanAalten, D.M.F., Sundheim, L. and Eijsink, V.G.H. (2001) Chitinases from *Serratia marcescens*. *Recent Res. Devel. Microbiol.* **5**, 187–204

59 Vaidya, R., Roy, S., Macmil, S., Gandhi, S., Vyas, P. and Chhatpar, H.S. (2003) Purification and characterization of chitinase from *Alcaligenes xylosoxydans*. *Biotechnol. Lett.* **25**, 715–717

60 McBride, M.J., Xie, G., Martens, E.C., Lapidus ,A., Henrissat, B., Rhodes, R.G., Goltsman, E., Wang, W., Xu, J., Hunnicutt, D.W., Staroscik, A.M., Hoover, T.R., Cheng, Y.Q. and Stein J.L. (2009) Novel features of the polysaccharide-digesting gliding bacterium *Flavobacterium johnsoniae* as revealed by genome sequence analysis. *Appl. Environ. Microbiol.* **75**, 6864–6875

61 Annamalai, N., Rajeswari, M.V., Vijayalakshmi, S. and Balasubramanian T. (2011) Purification and characterization of chitinase from *Alcaligenes faecalis* AU02 by utilizing marine wastes and its antioxidant activity. *Ann. Microbiol.* **61**, 801–807

62 Hariprasad, P., Divakara, S.T. and Niranjana, S.R. (2011) Isolation and characterization of chitinolytic rhizobacteria for the management of *Fusarium* wilt in tomato. *Crop Protection* **30**, 1606–1612

63 Someya, N., Ikeda, S., Morohoshi, T., Tsujimoto, M.N., Yoshida, T., Sawada, H., Ikeda T. and Tsuchiya K. (2011) Diversity of culturable chitinolytic bacteria from rhizospheres of agronomic plants in Japan. *Microbes Environ.* **26**, 7–14

64 Ordentlich, A., Elad Y. and Chet I. (1988) The role of chitinase of *Serratia marcescens* in biocontrol of *Sclerotium rolfsii*. *Phytopathology* **78**, 84–88

65 Childs, M., and Bowman, C. E. (1981) Lysozyme activity in 6 species of economically important astigmatid mites. *Comp. Biochem. Phys. B-Biochem. Mol. Biol.* **70**, 615-617

66 O'Neil, S. E., Heinrich, T. K., Hales, B. J., Hazell, L. A., Holt, D. C., Fischer, K., and Thomas, W. R. (2006) The chitinase allergens Der p 15 and Der p 18 from *Dermatophagoides pteronyssinus*. *Clin. Exp. Allergy* **36**, 831-839

67 Bowman, C.E. and Childs, M. (1982) Polisaccharidases in astigmatid mites (Arthropoda, Acari). *Comp. Biochem. Phys. B* **72**, 551–557

68 Erban, T., and Hubert, J. (2008) Digestive function of lysozyme in synanthropic acaridid mites enables utilization of bacteria as a food source. *Exp. Appl. Acarol.* **44**, 199-212

Chapter 5

Isolation of *Serratia marcescens* involved in chitin degradation in the bulb mite *Rhizoglyphus robini*

This article we aim to publish in *Symbiosis*.

Isolation of *Serratia marcescens* involved in chitin degradation in the bulb mite *Rhizoglyphus robini*

Zindel R, Ofek M and Aebi A.

Abstract

There is an increasing awareness of the importance of the microbiome of arthropods to understand their host's biology. In the bulb mite, *Rhizoglyphus robini*, associated bacteria have been found to be involved in its chitinolytic abilities. The bulb mite, a plant pest feeding on below-ground parts of mostly Liliaceae crops, prefers fungus-infested plants. Moreover its fitness is higher when feeding on a fungal food source than when feeding on non-infected plants. In this study we isolated a chitinolytic bacterium from mite homogenate and identified it molecularly as *Serratia marcescens*, which is a model organism for chitin degradation. To know the identity of the bacterium can be important for the development of biological control programs of the mite as well as for further studies investigating *Serratia marcescens* and its chitinolytic machinery.

Introduction

Exploration of the microbiome associated with many arthropods has proven to be very important to understanding their biology (Zindel *et al.* 2011; chapter 4, this thesis). Among the many functions microbes can have in their hosts' biology, nutritional provision is of high importance (Dillon& Dillon 2004). Arthropods may engage in obligate or facultative interactions with microbes and rely on bacterial symbionts for the uptake of essential amino acids, vitamins, and digestive enzymes, as well as for the metabolism of fatty acids and nitrogen processing (Grunwald *et al.* 2010; Guenduez& Douglas 2009; Snyder *et al.* 2010). For omnivorous arthropods, bacterial associates may be important to help them extract nutrients from variable sources (Feldhaar& Gross 2009; Sabree *et al.* 2009).

Overall, arthropods with highly divergent life strategies, from sap or blood feeders to omnivorous species, live in close association with microorganisms.

Rhizoglyphus robini lives in the soil, and on the bulbs and tubers of its host plants (mainly ornamentals and crops such as lily, onion and garlic) and is therefore commonly referred to as the bulb mite. Field observations show that *R. robini* is almost exclusively found on plants which suffer from fungal infections (Hanuny *et al.* 2008). Morover, it has been shown to choose fungus-infected over uninfected hosts in preference tests (Hanuny *et al.* 2008; Okabe & Amano 1991) and to be attracted to alcohols extracted from cultures of *Fusarium oxysporum* (Okabe & Amano 1990). In addition, *R. robini* exhibits high fecundity on fungal food sources (Hanuny *et al.* 2008; Okabe & Oconnor 2001; chapter 4, this thesis). Even the survival of *R. robini* on wet filter paper alone was explained by the mite's ability to derive nutrients from fungal mycelia developing on the cellulosic matrix (Wooddy & Fashing 1993). In a previous study we could already demonstrate very important aspects of the *R. robini*- holobiont (chapter 4, this thesis): We showed that the *R. robini* microbiome contains several bacterial genera known to contain chitin degraders. Also we confirmed that the mite's fecundity (number of eggs) is indeed higher on a fungal- than a non-fungal food sources. Last but maybe most importantly we demonstrated chitinolytic activity of mite homogenate through the production of a clear zone, a "halo", around a droplet of homogenate in a chitin layer. Slightly touching this clear zone with a pipette tip, growing it overnight, and re-applying it to a chitin-covered Petri-Dish resulted again in halo-formation, thus confirming bacterial origin of the chitinases (chapter 4, this thesis).

Rhizoglyphus robini is probably one of few pest species (chapter 4, this thesis) for which a thorough description of the microbiome has been achieved. However, only the precise identity of the most important associated bacteria can be used in the development or improvement of biological control programs of the mite. Here, a combined approach of selective media cultivation methods and DNA identification (sequencing of an approximately 1400bp fragment of the 16S rRNA gene) was used to isolate and identify the bacteria responsible for the chitin degradation.

Materials and methods

Mite origin and rearing: The mites used in this study stemmed from a field population originally collected on Lily (*Lilium candidum*) and kept in the laboratory since 2005. Mites were reared in Petri dishes containing wet filter paper and non-sterilized ground peanuts, and kept in a closed box in the dark. Petri dishes were stacked on a rack standing in soapy water to prevent escape of individual mites. Cultures were maintained by transferring 10 to 30 individual mites to a new Petri dish every 2 weeks approximately. Water and peanuts were added every 14 days.

Selective cultivation: Crabshell chitin (Sigma) was washed as described in the literature (McBride & Braun 2004; Reichenbach 2006), spread in a thin layer (2 ml per plate) and allowed to dry on minimal medium containing 50mg filter-sterilized nystatin solution per liter of medium, according to Faramarzi *et al.* (2009). Mites were starved and surface-sterilized by rinsing them with 95% ethanol and washing them for two minutes in a 1% bleach solution. Sterility was examined by rolling the mites on the surface of Lysogeny broth (LB) agar plates. The plates were inspected 48 h later for the development of bacteria or fungi. Mite homogenates were prepared by grinding approximately 100 individual adult mites in 150 µl sterile saline solution. Of the homogenate, 4x 1µl (per replicate) were pipetted in droplets of 0.5µl on chitin agar medium. Sterile saline solution (0.85% NaCl) served as negative control. Plates were incubated at 30°C and inspected after 12, 24 and 36 h for the presence of haloes. Bacteria were picked from the center and edge of the haloes with a autoclaved wooden toothpick and transferred to liquid chitin medium, which was, after a night of incubation at 30°C on a shaker, plated on non-selective LB Agar and incubated over night at 28°C. Colonies were visually checked for morphological consistency before three identical looking colonies per plate were used in colony-PCR with the primers Eub9_27 and Eub1542 (table 1) for preliminary assessment of diversity. PCR was performed in a total volume of 50µl containing: 2µl template DNA, 39.75µl water, 5µl 10x buffer, 1 µl 10µM of each primer, 1µl 1mM dNTP's and 0.25µl Taq polymerase at 5U/µl. Touchdown PCR cycling conditions were: 4.30min at 94°C, then 10 cycles of 94°C for 30s, 60°C-55°C for 30s, 72°C

for 1min 30s, decreasing annealing temperature by -0.5°C with each cycle, followed by another 20 cycles with a constant annealing temperature of 55°C and a final elongation of 10min at 72°C.

Table 1 Primers used in this study. bp= base pairs, °C= degree Celsius, *= touchdown PCR program

Primers		Annealing temp (°C)	Reference
Eub9_27:	5'-GAGTTTGATCCTGGCTCAG-3'	60-55*	Brosius *et al.* 1978
Eub1542:	5'-AGAAAGGAGGTGATCCAGCC-3'	60-55*	Brosius *et al.* 1978
27F:	5'-AGAGTTTGATCMTGGCTCAG-3'	54.5	Lane *et al.* 1991
907R:	5'-CCGTCAATTCMTTTGAGTTT-3'	50	Muyzer *et al*. 1993
341F:	5'-GCCTACGGGAGGCAGCAG-3'	62	Muyzer e*t al.* 1993
1513R:	5'-ACGGYTACCTTGTTACGACTT-3'	54.5	Russell *et al.* 2009

Clone culture: Preliminary identification of 20 bacterial colonies through comparison of a 600bp fragment in BLAST (as described above) confirmed the presence of just one bacterial genus: *Serratia*. Five colonies from 5 different plates were selected to be cultured. Prior to extraction, clones were picked with a pipette tip, which was dropped in 10ml of liquid LB, and incubated for 6h at 28°C on a shaker. DNA was extracted using GenElute™ bacterial Genomic DNA-Kit (Sigma-Aldrich) following the manufacturer's instructions. Of four out of five clones, a 1440bp fragment of the 16S rRNA gene was assembled from fragments sequenced using the primers 27F, 341F, 907F, 1543F, Eub9_27 and Eub1542 (table 1).

Sequencing: PCR products were cleaned either by filtration (Millipore) or the PCR product band was cut from the gel, frozen and the DNA was later extracted by compressing the agarose piece between two layers of Parafilm® (Sigma). Sequencing was carried out on an automated ABI3130xl Genetic Analyzer machine using ABI BigDye version 3.1 Terminator Sequencing chemistry. Chromatogram output quality was checked by eye and corresponding sequences (forward and reverse) were aligned. The different fragments of the 16S rRNA gene were assembled in Sequencher 4.9 (Gene Codes, Ann Arbor, USA). Assembled sequences are deposited in GenBank (JX872281- JX872284).

Sequences were queried using the online sequence databases; GenBank (National Center for Biotechnology Information (NCBI), using nucleotide Basic Local Alignment Search Tool (BLAST) and Sepsi-Test™ -BLAST, a tool designed to identify bacteria, yeast and fungi. We accepted positive identification to the species-level obtaining at least 99% "Max ident" at a coverage of 100% in BLAST and Sepsi-Test.

Results

Mite homogenate (0.5ul to 1ul) applied to chitin-covered minimal-medium resulted in haloes with 0.5cm radius around a central red colony within 12h and a complete digestion of the chitin layer after 48h. Colonies were picked, and incubated after 12h in liquid medium overnight. Colonies growing after plating liquid culture looked identical (shape, approximate size) apart from the color where they displayed a range from light red to dark red. The five colonies, c1 to c5, chosen for further sequencing represent a color range: c1 – lightest red; c2 – light red; c3 – dark red; c4 – red; c5 – red. All sequencing but with the primer 341F gave high quality results and initial BLAST queries indicated the isolation of a single species of the genus *Serratia*. The colony C4 probably contained a contamination and could not be used for further analysis.

The four assembled 1440bp sequences were identified as a *Serratia marcescens* strain, based on their 16S rRNA gene sequences that are at least 99% identical to previously characterized *Serratia marcescens*, and are most similar to *Serratia marcescens* subspecies *sakuensis* (AB061685) (99.4-99.8% identity in Sepsi-Test).

Discussion

Based on the 16S rRNA gene sequences we identify the chitinolytic bacteria isolated from the bulb mite *Rhizoglyphus robini* as *Serratia marcescens*. The genus *Serratia* contains many chitin degraders. *Serratia marcescens* is the most common among them and has the best studied chitinolytic machinery known to date. In addition to three chitinases (ChiA, ChiB and ChiC) it also produces a

chitobiase and a putative chitin-binding protein (Brurberg *et al.* 2001). Already in 1988, Ordentlich and colleagues could demonstrate that the bacterium can grow solely on fungal mycelium as a food source (Ordentlich *et al.* 1988).

Serratia spp. can be found in a variety of environments and are not seldom found associated with arthropods, as pathogens (*S. marcescens*, *S. liquefaciens* in a wide variety of animals), symbionts (*S. symbiotica* in aphids, a *Serratia* spp. in the fruit fly *Bactrocera dorsalis*), or with unknown status (Grimont & Grimont 1978; Wang *et al.* 2011). They have been reported from diseased and dead individuals, but have also been isolated from healthy organisms, as secondary and maybe even primary symbionts. The facultative symbiont *Serratia symbiotica* is vertically transmitted and provides defense against environmental heat-stress to its host, the aphid *Acyrtosiphon pisum* (Montllor *et al.* 2002). *Serratia symbiotica* is phylogenetically clearly identified as a *Serratia*, but has a strongly reduced genome size as many symbionts compared to their free-living sister species (Burke & Moran 2011). The genome size is being even more reduced in a *S. symbiotica* strain infecting the aphid *Cinara cedri*, where it is believed to be in the transition of becoming a primary symbiont (Lamelas *et al.* 2011). Several *Serratia* species can be pathogenic to higher animals and humans and are often involved in hospital-acquired infections (HAI) of the human urinary tract and general wound infections.

Three different chitinolytic *Serratia* species have previously been isolated from the mycophagous mites *Tyrophagus putrescentiae* and *Archegozetes longisetus* (Astigmata, Acarinae): *S.liquefaciens*, *S. rubidea* and *S. marcescens* (Smrz & Catska 2010). The authors mention that mites and bacteria form very efficient collaborations to reduce fungal cover, whereas the bacteria or the mite alone have little influence on an advanced fungal infestation. This observation is very valuable in the context of biological control of pathogenic fungi by chitinolytic organisms. Chitinolytic *Serratia* species have also by other studies been suggested as a control agent of fungi (Brurberg *et al.* 2001). However, in the case of the *Serratia* - host being a pest itself, a use of this system might be difficult.

The most similar sequence published stems from *Serratia marcescens* subspecies *sakuensis*. The subspecies *sakuensis* has been proposed after characterization of an endospore-forming isolate from activated sludge in a waste water treatment tank in Japan. Our sequences are more similar (on average 0.2%) to the *sakuensis* than to the *marcescens* subspecies (AJ233431). *Serratia marcescens sakuensis* is supposedly the first known endospore-forming *Serratia*. Endospores are bacterial cells resistant to unsuitable conditions which enable the bacterium to survive in a dormant state for long periods of time. I our case the capability to form endospores may be a strategy developed by the bacterium to survive long time periods outside of the host, without a food source, while spreading to new host individuals. Microscopy for endospore confirmation and other characterization tests (cell morphology, use of various carbon sources, biochemical tests, mass spectrometry or microarray assays) should be conducted in order to taxonomically assign the species found in *R. robini* to a subspecies (Ajithkumar *et al.* 2003 among others). Further sequence analysis, such as of the intergenic spacer region between 16S rRNA gene and 23S RNA gene, could also lead to a higher resolution of the taxonomic status.

In a previous article we described the bacterial community associated with *R. robini*. The genus *Serratia* was among the three dominant genera along with *Myroides* and *Alcaligenes* (chapter 4, this thesis). A total of 22 OTU's (operational taxonomic unit) assigned to *Serratia* were found, differing in at least 3% of the sequence, suggesting several *Serratia* species to be present (unpublished). In the present study we probably only identified one species. Other chitin-degrading *Serratia* species and chitin-degrading bacteria in general could have been missed in this study due to unsatisfied growth requirements other than carbon-source in this specific protocol. It is estimated that up to 99% of soil organisms are not detectable by conventional cultivation methods (Hugenholtz *et al.* 1998; Rondon *et al.* 2000). This percentage could be even higher in microorganisms adapted to the special environment of an organism's inside.

In conclusion we can say that the identification of *Serratia marcescens* as one of the main chitin-degrading bacteria in *Rhizoglyphus robini* may not be surprising, because of its known chitinolytic

qualities and previous findings of representatives of the genus in mycophagous mites. The information is nevertheless very interesting and important in many respects, of which I will mention two: 1) It is an important step in the characterization of a pest organism to identify the associated bacterial community and identify its impacts on the host's biology. We can now assume that the *R. robini* – host plant interaction includes two more players – the fungus and *S. marcescens*. For example, the elimination of either of the two could be an approach worth testing to decrease mite damage on crop plants. 2) *Serratia marcescens* is a model organism for chitin degradation and has, due to its efficiency, been discussed for fungal control. Smrz and Catska (2010) raise the idea of using chitinolytic bacteria in association with fungivorous mites in a combined approach, the mites being responsible for locomotion (grazing), whereas the bacteria supply the enzymes. The *Serratia marcescens* we found here already lives in a mite, although the pest status of *R. robini* is not yet fully understood and it is therefore hardly a candidate. However, the *S. marcescens* strain could potentially be used in combination with another mite. *Serratia marcescens* is also a human pathogen and hence all additional information on incidences and characteristics of potential value.

Acknowledgements

This work was supported by the COST-SER project C08Æ0109 to AA, part of the COST Action FA0701. We would like to thank Max Kolton for help with the chitin work and Pilar Junier, Matthieu Bueche and Franco Widmer for good advice with bacterial problems.

References

Ajithkumar B, Ajithkumar VP, Iriye R, Doi Y, Sakai T (2003) Spore-forming *Serratia marcescens* subsp *sakuensis subsp nov*., isolated from a domestic wastewater treatment tank. *International Journal of Systematic and Evolutionary Microbiology* **53**, 253-258.

Brosius J, Palmer ML, Kennedy PJ, Noller HF (1978) Complete nucleotide sequence of a 16S ribosomal-RNA Gene from *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America* **75**, 4801-4805.

Brurberg MB, Synstad B, Klemsdal SS*, et al.* (2001) Chitinases from *Serratia marcescens*. *Recent Research Develelopments in Microbiology* **5**, 187-204.

Burke GR, Moran NA (2011) Massive genomic decay in *Serratia symbiotica*, a recently evolved symbiont of aphids. *Genome Biology and Evolution* **3**, 195-208.

Dillon RJ, Dillon VM (2004) The gut bacteria of insects: nonpathogenic interactions. *Annual Review of Entomology* **49**, 71-92.

Faramarzi MA, Fazeli M, Tabatabaei Yazdi M*, et al.* (2009) Optimization of cultural conditions for production of chitinase by a soil isolate of *Massilia timonae*. *Biotechnology* **8**, 93–99.

Feldhaar H, Gross R (2009) Insects as hosts for mutualistic bacteria. *International Journal of Medical Microbiology* **299**, 1-8.

Grimont PAD, Grimont F (1978) The genus *Serratia*. *Annual Review of Microbiology* **32**, 221-248.

Grunwald S, Pilhofer M, Holl W (2010) Microbial associations in gut systems of wood- and bark-inhabiting longhorned beetles (Coleoptera: Cerambycidae). *Systematic and Applied Microbiology* **33**, 25-34.

Guenduez EA, Douglas AE (2009) Symbiotic bacteria enable insect to use a nutritionally inadequate diet. *Proceedings of the Royal Society B- Biological Sciences* **276**, 987-991.

Hanuny T, Inbar M, L. T, Palevsky E (2008) Complex interactions between *Rhizoglyphus robini* and *Fusarium oxysporum*: implications on onion pest management. IOBC Proceedings of Integrated Control of Protected Crops, Temperate Climate. *IOBC/ WPRS Bulletin* **32**, 71–74.

Hugenholtz P, Goebel BM, Pace NR (1998) Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *Journal of Bacteriology* **180**, 4765-4774.

Lamelas A, Jose Gosalbes M, Manzano-Marin A*, et al.* (2011) *Serratia symbiotica* from the aphid *Cinara cedri*: A missing link from facultative to obligate insect endosymbiont. *Plos Genetics* **7**.

Lane DJ (1991) 16S/23S rRNA sequencing. In: *Nucleic acid techniques in bacterial systematics* (eds. Stackebrandt E, Goodfellow M), pp. 115-175. John Wiley and Sons, New York, NY.

McBride MJ, Braun TF (2004) GldI is a lipoprotein that is required for *Flavobacterium johnsoniae* gliding motility and chitin utilization. *Journal of Bacteriology* **186**, 2295-2302.

Montllor CB, Maxmen A, Purcell AH (2002) Facultative bacterial endosymbionts benefit pea aphids *Acyrthosiphon pisum* under heat stress. *Ecological Entomology* **27**, 189-195.

Muyzer G, Dewaal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by Denaturing Gradient Gel Electrophoresis- analysis of polymerase chain reaction- amplified genes coding for 16S ribosomal-RNA. *Applied and Environmental Microbiology* **59**, 695-700.

Okabe K, Amano H (1990) Attractancy of alcohols isolated from culture filtrates of *Fusarium* fungi for the robine bulb mite, *Rhizoglyphus robini* Claprede (Acari: Acarinae) in sand. *Applied Entomology and Zoology* **25**, 397-404.

Okabe K, Amano H (1991) Penetration and population- growth of the robine bulb mite, *Rhizoglyphus robini* Claprede (Acari: Acaridae), on healthy and *Fusarium*-infected Rakkyo bulbs. *Applied Entomology and Zoology* **26**, 129-136.

Okabe K, Oconnor BM (2001) A method for both mass and individual rearing of fungivorous astigmatid mites (Acari). *Experimental and Applied Acarology* **25**, 493-504.

Ordentlich A, Elad Y, Chet I (1988) The role of chitinase of *Serratia marcescens* in biocontrol of *Sclerotium rolfsii*. *Phytopathology* **78**, 84-88.

Reichenbach H (2006) The genus *Lysobacter*. In: *The Prokaryotes* (ed. Dworkin M FS, Rosenberg E, Schleifer KH, Stackebrandt E), pp. 939–957. Springer, New York.

Rondon MR, August PR, Bettermann AD*, et al.* (2000) Cloning the soil metagenome: a strategy for accessing the genetic and functional diversity of uncultured microorganisms. *Applied and Environmental Microbiology* **66**, 2541-2547.

Russell JA, Moreau CS, Goldman-Huertas B*, et al.* (2009) Bacterial gut symbionts are tightly linked with the evolution of herbivory in ants. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 21236-21241.

Sabree ZL, Kambhampati S, Moran NA (2009) Nitrogen recycling and nutritional provisioning by *Blattabacterium*, the cockroach endosymbiont. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 19521-19526.

Smrz J, Catska V (2010) Mycophagous mites and their internal associated bacteria cooperate to digest chitin in soil. *Symbiosis* **52**, 33-40.

Snyder AK, Deberry JW, Runyen-Janecky L, Rio RVM (2010) Nutrient provisioning facilitates homeostasis between tsetse fly (Diptera: Glossinidae) symbionts. *Proceedings of the Royal Society B-Biological Sciences* **277**, 2389-2397.

Wang H, Jin L, Zhang H (2011) Comparison of the diversity of the bacterial communities in the intestinal tract of adult *Bactrocera dorsalis* from three different populations. *Journal of Applied Microbiology* **110**, 1390-1401.

Wooddy MW, Fashing NJ (1993) The ability of *Rhizoglyphus robini* Claparède (Astigmata: Acaridae) to subsist solely on a diet of filter paper. *International Journal of Acarology* **19**, 345–348.

Zindel R, Gottlieb Y, Aebi A (2011) Arthropod symbioses: a neglected parameter in pest- and disease-control programmes. *Journal of Applied Ecology* **48**, 864-872.

Appendices

Conclusions and future directions

Acknowledgements

Curriculum vitae

Certificate Doctoral School

Conclusions and future directions

Associated bacteria have an influence on many important biological characteristics of their arthropod hosts. For mites, where many species are of importance to humans because of their health implications, as pests or because of their use in biological control, the endosymbionts (ES) and their effects on the host are a significant piece of knowledge being used for example in their control or to make their application more efficient.

Screening 21 mite species for 6 well-known ES of insects (and mites) we realized, that this method leaves a large part of the diversity unexplored. For further studies of mite-associated bacterial communities we therefore recommend to apply more general approaches such as metagenomic analysis through 454 pyrosequencing or similar methods. However, metagenomics are expensive and for practical application in biological control, as stated in chapter 2 of this thesis, the effort invested in the detection and characterization of ES should be proportional to the estimated risk they pose or potential benefit the biological program can gain from the presence of the ES. For the bacteria we screened for there is already a large pool of information available (such as effective antibiotics for curing), which makes it easier to study their role in the mite host. The results of a metagenomic survey are usually very rich in information and therefore difficult to interprete.

In the bulb mite *Rhizoglyphus robini* we found a diverse bacterial community and could demonstrate a link to the mycophagous preferences of the mite. A *Serratia marcescens* bacterium seems to be involved in the degradation of chitin and hence the ability of the mite to use fungal carbohydrate. This information can now be used in the development of existing and new control strategies of the bulb mite, which is a serious pest on mostly lilicean crops and ornamentals. The fact that the mite probably mainly feeds on the fungus-infected plant-tissue degrades it from a primary to a secondary pest, significantly changing preconditions for biological or conventional control methods. Most arthropods have an associated bacterial community which may offer very useful possibilities to approach them.

A selection of open research questions, arisen during this 3-year project, are presented in the following paragraph:

1. Which location and function does the *Spiroplasma* have in the predatory mite *Hypoaspis miles* and how could this information be integrated in its rearing and application as biological control agent?

2. Why did the *Wolbachia* Multi-Locus-Screen-Typing (MLST) system work so poorly for the ES-positive mite samples? Could PCR protocol, primer specificity or DNA storage conditions be adapted to improve results? Or would phylogenetic analysis of mite-*Wolbachia* rely on other genes?

3. *Wolbachia* had not been detected in a screen of several populations of *Dermanyssus gallinae* by De Luna et al. (2009). How prevalent is this bacterium in the population screened in our project? Where does this low infection rate come from (food source – *D. gallinae* is parasitic)? The phylogenetic analysis of the wsp and coxA gene suggest a supergroup A *Wolbachia*, while all mite-W found so far can be assigned to supergroup B or were hard to classify. Could the analysis of more genes answer these questions?

4. The bacterial community of *Rhizoglyphus robini* mites from lab colonies is very diverse. A preliminary assay showed even more diversity in field populations (unpublished). Can this be confirmed in a more comprehensive approach, is thisa general pattern and what are the differences in composition and abundance of the microbiome in mite populations from different host plants/environments? How can this be related to the functions of the bacteria in the host?

5. Where is *Serratia marcescens* located in the *R. robini* mite host? Can it be removed? How would this change the fitness parameters of *R. robini*? Is it maternally transmitted or freshly acquired in each generation?

6. The *Serratia marcescens* strain found in *R. robini* is most similar to an endospore –forming subspecies named *S. marcescens sakuensis*. Can we detect endospores? Can we biologically and chemically further identify the isolated *Serratia marcescens* and assign it to a subspecies?

7. Are there several chitinolytic bacteria in *R. robini*?

8. Many *Myroides* spp. can digest cellulose. Can we show that the mite (homogenate?) digests cellulose? Can we show that the mite uses this activity and that cellulytic activity might even be more important than chitinolytic activity?

Acknowledgements

I want to thank everyone who helped me during these almost 4 years of work on my thesis, practically and mentally, supporting me in any kind of way. “Everyone” is among others:

Alexandre Aebi, my thesis advisor with plenty of good ideas and always an open door to his office (or a telephone line to his home office) and a freshly brewed espresso.

Ted Turlings, my thesis director for being my thesis director and his very wise words at our first meeting: “Stay focused”. (I tried).

Einat Zchori-Fein, Eric Palevsky, Netta Moses-Daube, Ayelet Caspi-Fluger, Shira Gal and all the others, who made my time in Israel in the summer of 2010 a great experience in the lab and outside of the lab.

Eric Palevsky for the mites, the mite help, and for being a co-author!

Einat Zchori-Fein for inviting me to her lab, good ideas and fruitful discussions, reading and editing manuscripts and good times.

My team of experts at the mid-thesis- and “real” defense: **Ted Turlings, Christoph Vorburger, Pilar Junier** and **Alexandre Aebi.**

Alexandre Aebi, Steven Bacon and **Mario Waldburger** for hours of cumbersome mite egg counting.

Mario Waldburger for all kinds of help with mites, bees, plants…

Maya Ofek, **Matthieu Bueche** and **Pilar Junier** for help with all work involving bacteria.

Irene Bänziger for the fungus, showing me how to grow it and for letting me use their facilities.

Max Kolton for chitin help.

Franco Widmer, Roland Kölliker, Jürg Enkerli, Salome Schneider, Fabienne Wichmann, Roy Kissling, Sonja Reinhard, Steffi Pfister and everyone else at the “Moekis” for advice and help with molecular work.

Laure-Emanuelle Perret-Aebi for getting me ART- inaccessible literature and for lending her chemical expertise to calculate solution concentrations.

All my co-authors for productive collaboration.

Steven Bacon for English-editing of several manuscripts (and for letting me copy part of his material and methods sections).

Christoph Lüthi, Manti (a tropical mantis) and Indian giant mantis Nr 1, 2 and 3 for a productive and entertaining office climate.

Silvio Blaser for keeping ART people fit (ART-sports) and well-fed (Beer reviews).

The biosafety group & co. for enjoyable and relaxing coffee, lunch and other breaks, off-work events etc.

COST (European Cooperation in Science and Technology), **ART Biosicherheit** (Agroscope Reckenholz-Tänikon), **SGPW** (Schweizerische Gesellschaft für Pflanzenbauwissenschaften) and the **Interuniversity Doctoral Program in Organismal Biology** in Neuchâtel for making this project and subprojects financially possible.

My parents and parents-in-law (although there is actually no law involved) **Salome**, **Christian, Luzia** and **Hans**, for looking extra often after Ida Maria in the last couple of months and support throughout the 4 years.

My mother **Salome**, who supported me in very many ways, but especially for 1) making it possible for me to focus through the last bit, taking very good care of Ida Maria and 2) for help with german translation of my English abstracts and articles.

My godmother **Eva** for help with the French for my public defense in Neuchâtel.

Pascal for many things and especially for bringing the great sacrifice of moving to Würenlos with me and Ida Maria, and supporting me despite this.

All friends and family for being patient with me and my oh so busy life lately.

Curriculum vitae

RENATE ZINDEL

1. Personal details

Name	Zindel Renate Christine
Date of birth	27.9.1982
Place of birth	Zürich
Citizenship	Zürich
Nationality	swiss
Partner	Pascal Stadler
Child	Ida Maria Salome Zindel (7.10.2011)
Work address	Agroscope Reckenholz-Tänikon Biodiversity and Environmental Management, Biosafety Reckenholzstrasse 191 8046 Zürich Switzerland +41 44 337 74 59 +41 76 596 64 19 renate.zindel@art.admin.ch, renatezindel@gmail.com www.agroscope.admin.ch/biosicherheit

2. Education

april 09 –december 12	PhD **Dissertation** with the title „**Mites and Endosymbionts – towards improved biological control**" at the agricultural research station Agroscope Reckenholz-Tänikon in Zürich-Affoltern and at the University of Neuchâtel with Dr. Alexandre Aebi and Prof. Dr. Ted Turlings.
august 08- june 09	**Teacher training college at the PH Bern**, studies to be a biology teacher at the gymnasium, teaching diploma June 2009
march 08-january 09	**Internship** the agricultural research station Agroscope Reckenholz-Tänikon in the biosafety group
2002-2008	**Studies in biology at the University of Fribourg, Switzerland** with a bachelors and a masters degree (Master of Science) in biology, January 2008
2006	**Courses and project work at** Umeå Universität, Sweden
2007	Master thesis at CABI Europe, Switzerland, in Delémont
1998-2002	Kantonsschule in Aarau (Typus B, old languages)
2000/01	Exchange year in the USA

3. Diplomas

2009	Teaching degree (biology at the Gymnasium)
2008	Master degree
2005	Bachelor degree
2002	Maturadiplom, Type B (languages)
2000	US Highschool diploma

4. Practical experiences

July/August 2010	**Short Term Scientific Mission (STSM**) at the laboratories of Dr. Einat Zchori-Fein and Dr. Eric Palevsky at Newe Ya'ar Research Station in Ramat Yishay, Israel.
January-June 2010	**Biology teacher** (replacement) at the Gymnasium Köniz-Lerbermatt, Bern, 8th and 9th grade
April - Juni 2009	**Biology teacher** (replacement) at the Gymnasium Köniz-Lerbermatt, Bern, 8th grade
März /April 2009	**Teaching internship** at the Gymnasium Köniz-Lerbermatt, Bern, with Janine Stüssi, teaching biology (in English)
2008-2009	**Internship in molecular ecology** with Dr. Alexandre Aebi at the agricultural research station Agroscope Reckenholz-Tänikon - DNA extraction (insects) - PCR for detection of endosymbionts in ladybirds, sequencing, sequencing analysis - Primer selection and design for gut content barcoding in *Harmonia axyridis*
2008	**Lab and field assistant** at the botanical institute in Bern - harvest and measurements of botanical field studies
2007	**Practical part of Master thesis with** Dr Marc Kenis at CABI Europe, Switzerland, in Delémont - insect sampling and determination - insect rearing, plant cultivation - data analysis
2006	**Field and lab assistant in aquatic ecology** (3 months, full time) at the Natural Science Research Station in Abisko, Northern Sweden - water samples, gradient measurements - filtrations, gas measurements
2005	**Bachelor thesis with Prof. Felix Mauch and Dr. Thomas Steinger in plant physiology and ecology at the University of Fribourg, Switzerland** - glycosinolate extraction - analysis of chemical components by HPLC
2004-2008	**Research and teaching assistant** at the University of Fribourg, Switzerland - botanical field studies, set-up, data collection, harvest, sample proceeding - courses: plant systematics and plant ecology

5. Attendence of specialized courses

2012	"Symposium in Fungi-Bacteria Interactions". Course organized by the CUSO Doctoral Program in Ecology and Evolution and the Interuniversity Doctoral Program in Organismal Biology in Neuchâtel, Switzerland. Local organizer: Prof. Dr. Pilar Junier. (duration: 1 day)
2011	"Bioinformatics workshop". COST Training School in Uppsala, Sweden. Local organizer: Dr. Lisa Klasson. 4th -8th of April 2011. (Duration: 5 days)
	"Aboveground and below-ground Community Ecology". Course organized by the CUSO Doctoral Program in Ecology and Evolution and the Interuniversity Doctoral Program in Organismal Biology in Neuchâtel, Switzerland. Local organizer: Prof. Dr. Pilar Junier. (duration: 1 day)
	"Minisymposium in metagenomics". Course organized by the CUSO Doctoral Program in Ecology and Evolution and the Interuniversity Doctoral Program in Organismal Biology in Neuchâtel, Switzerland. Local organizer: Prof. Dr. Pilar Junier. (duration: 3 days)
	"Introduction to chromatography and mass spectrometry for biologists". Course organized by the Interuniversity Doctoral Program in Organismal Biology in Neuchâtel, Switzerland. Local organizer: Gaetan Glauser and Armelle Vallat. (duration: 2 days)

2010	"Advances in Symbiosis research". COST Training School in Rehovot, Israel (duration: 7 days)
2009	"Electronic scanning microscopy". Course organized by the NCCR graduate school in Neuchâtel, Switzerland. (duration: 3 days)
	"Scientific writing clinic". Course organized by the NCCR graduate school with Dr. Heather Murray, in Neuchâtel, Switzerland. (duration: 3 days)
	"An Introduction to Plant Metabolomics". Course organized by the NCCR graduate school in Neuchâtel, Switzerland. (duration: 2 days)
2007	"An introduction to R". Course organized by the NCCR graduate school in Neuchâtel, Switzerland (duration: 6 days)
2005	„Evolutionary Biology". Workshop organized by Prof Dr. Dieter Ebert (University of Basel) and Dr. Tadeusz Kawecki (University of Fribourg, at the time) in Guarda, Switzerland. (duration: 1 week)
	"Tropical Ecology", an international 5-week course held at the Kibale Forest Research Station in Uganda, organized by the Tropical Biology Association (TBA) in Cambridge, UK. (duration: 5 weeks)
2004	"Feldstudien – Wissenschaftliches Arbeiten im Feld im Bereich Zoologie und Evolution", held at the ecological fieldstation in Tvärminne, Finland, organized by Prof. Dr. Dieter Ebert (University of Fribourg at the time). (duration: 2 weeks)

6. Publications

Peer-reviewed:

Poland, R.L., Adriaens, T., Brown P.M.J., Katsanis, A., San Martin, G., Branquart, E., Maes, D., Eschen, R., **Zindel, R.**, Van Vlaenderen, J., Babendreier, D., Roy, H. and Kenis, M. (2012). Assessing the ecological risk posed by a recently established invasive alien predator: The example of *Harmonia axyridis. Submitted to the Journal of Applied Ecology in July 2012*

Roy, H. E., Adriaens, T., Isaac, N. J. B., Kenis, M., Onkelinx, T., San Martin, G., Brown, P. M. J., Hautier, L., Poland, R., Roy, D. B., Comont, R., Eschen, R., Frost, R**., Zindel, R**., Van Vlaenderen, J., Nedved, O., Ravn, H. P., Gregoire, J.-C., de Biseau, J.-C., and Maes, D. (2012) Invasive alien predator causes rapid declines of native European ladybirds. *Diversity and Distributions* **18**, 717-725

Thomas, A., Trotman, J., Wheatley, A., Aebi, A., **Zindel, R**. Brown, P.M.J. (2012). Predation of native coccinellids by the invasive alien *Harmonia axyridis* (Coleoptera: Coccinellidae): detection in Britain by PCR-based gut analysis. Insect Conservation and Diversity. doi: 10.1111/j.1752-4598.2012.00184.x

Aebi, A., Brown, P. M. J., De Clercq, P., Hautier, L., Howe, A., Ingels, B., Ravn, H. P., Sloggett, J. J., **Zindel, R**. & Thomas, A. (2011) Detecting arthropod intraguild predation in the field. *Biocontrol,* **56**, 429-440.

Zindel, R., Gottlieb, Y. & Aebi, A. (2011) Arthropod symbioses: a neglected parameter in pest- and disease-control programmes. *Journal of Applied Ecology,* **48**, 864-872.

De Frenne, P., Graae, B. J., Kolb, A., Brunet, J., Chabrerie, O., Cousins, S. A. O., Decocq, G., Dhondt, R., Diekmann, M., Eriksson, O., Heinken, T., Hermy, M., Jogar, U., Saguez, R., Shevtsova, A., Stanton, S., **Zindel, R**., Zobel, M. & Verheyen, K. (2010) Significant effects of temperature on the reproductive output of the forest herb *Anemone nemorosa* L. *Forest Ecology and Management,* **259**, 809-817.

De Frenne, P., Kolb, A., Verheyen, K., Brunet, J., Chabrerie, O., Decocq, G., Diekmann, M., Eriksson, O., Heinken, T., Hermy, M., Jogar, U., Stanton, S., Quataert, P., **Zindel, R.,** Zobel, M. & Graae, B. J. (2009) Unravelling the effects of temperature, latitude and local environment on the reproduction of forest herbs. *Global Ecology and Biogeography,* **18**, 641-651.

Graae, B. J., Verheyen, K., Kolb, A., Van Der Veken, S., Heinken, T., Chabrerie, O., Diekmann, M., Valtinat, K., **Zindel, R**., Karlsson, E., Strom, L., Decocq, G., Hermy, M. & Baskin, C. C. (2009) Germination requirements and seed mass of slow- and fast-colonizing temperate forest herbs along a latitudinal gradient. *Ecoscience,* **16**, 248-257.

Kenis, M., Roy, H. E., **Zindel, R**. & Majerus, M. E. N. (2008) Current and potential management strategies against *Harmonia axyridis*. *Biocontrol,* **53**, 235-252.

General audience:

Aebi A, Linder C, Kenis M and **Zindel R** (2009). Quelles mesures de lutte?/Was bahnt sich an? UFA-revue 1: 38-39 (Landwirtschaftszeitung)

7. Attendance of scientific meetings, oral presentations and posters

2012 EU COST Action FA0701 Arthropod Symbiosis: From fundamental research to pest and disease management. Summit meeting. St. Pierre d'Oléron, France 12-13 of June 2012.
Oral presentation: "The microbiome of the pest mite *Rhizoglyphus robini*: implications for the pest's status." **Zindel R**, Ofek M, Minz D, Palvesky E., Zchori-Fein E and Aebi A.

2011 Third meeting of the IOBC working group "Integrated control of plant-feeding mites". Cesky Krumlov, Czech Republic, September 13-16 2011.
Oral presentation: "The bacterial community associated with the bulb mite *Rhizoglyphus robini* Claprede." **Zindel R**, Zchori-Fein E. Palevsky E and Aebi A.

2010 6th International *Wolbachia* Conference. Asilomar, California, USA, June 9-14.
Posters: 1. "Endosymbionts in a wide range of mite species". **Zindel R** and Aebi A.
2. "Can symbionts influence arthropod invasion". Aebi A and **Zindel R.**

2009 1st Meeting of the IOBC/WPRS Study Group "Benefits and risks asscociated with exotic biological control agents" on "*Harmonia axyridis* and other invasive ladybirds". Engelberg, Switzerland (September 6 -10th)
Poster: "Gut content barcoding on *Harmonia axyridis*". **Zindel R**, Katsanis A, Kenis M and Aebi A.
Oral presentation: „What can endosymbionts tell about the *Harmonia axyridis* invasion ?". Aebi A and **Zindel R**.
Oral presentation: "Impact of *Harmonia axyridis* on European ladybirds: which species are most at risk?" Kenis M, Katsanis A, Van Vlaenderen J, Eschen R, Golaz L, **Zindel R**, Babendreier D, Majerus M and Ware R.

Immunity and Symbioses Workshop (COST), Aussois, France (May 27-30th)

5th Annual Symposium of the PhD Program in Sustainable Agriculture (ASPSA) "Interactions between Plants and their Environment in a Changing World", Tänikon, Switzerland (August 27th)
Poster: „Mites and Endosymbionts: Towards imporved bioogical control". **Zindel R**, Kuske S, Kölliker U, Maurer V and Aebi A.

2006 GfÖ, Bremen, Germany

8. Languages

german	native language
englisch	read, written and spoken
french	read and spoken
swedish	read and spoken
italian	understanding, basic communication (school)
spanish	understanding, basic communication (school)

UNIVERSITÉ DE NEUCHÂTEL

Institut de biologie

Interuniversity Doctoral Program in Organismal Biology

Certificate of Completion

Renate Zindel

has fulfilled the requirements of the Doctoral Program.
From April 2009 to November 2012 she obtained 13 ECTS*.

Professor Ted Turlings
Director

Dr. Christiane Bobillier
Coordinator

Neuchâtel, 20 November 2012

** A minimum of 12 ECTS is required during the full period of the thesis*

UNIVERSITÉ DE NEUCHÂTEL

Institut de biologie

Interuniversity Doctoral Program in Organismal Biology

Certificate of Completion

From April 2009 to November 2012 **Renate Zindel** obtained 8.5 ECTS within the Doctoral Program (DP, minimum 8 required) and 4.5 ECTS outside the Doctoral Program (EX) with the following activities:

Communication activities:

DP	Scientific writing clinic	Oct.-Dec. 2009	2.0
EX	Wissenschaft verständlich vortragen, Agroscope	March 2010	0.5
EX	ASPSA 2010, 6th annual symposium of the PhD program in sustainable agriculture, Zurich (organization)	November 2010	0.5

Research tools activities:

DP	Introduction to plant metabolomics	November 2009	1.0
DP	Scanning electronic microscopy (SEM)	December 2009	1.0
EX	COST training school - Advances in symbiosis research, Israel	March 2010	1.5
DP	Introduction to chromatography and mass spectrometry for biologists	January 2011	1.0

Scientific activities:

EX	5th annual symposium of the PhD program in sustainable agriculture, Tänikon, Switzerland (poster)	August 2009	0.5
EX	Harmonia axyridis and other invasive ladybirds meeting, Engelberg, Switzerland (poster)	September 2009	0.5
DP	Annual Ph.D. students meeting 2010 (oral presentation)	April 2010	0.5
EX	6th international wolbachia conference, Asilomar, USA (poster)	June 2010	0.5
EX	ASPSA 2010, 6th annual symposium of the PhD program in sustainable agriculture, Zurich (poster)	November 2010	0.5
DP	Annual Ph.D. students conference 2011 (poster)	May 2011	0.5
DP	Minisymposium in metagenomics (attendance)	June 2011	1.5
DP	Aboveground and below-ground community ecology - a joint workshop with CUSO (attendance)	September 2011	0.5
DP	Fungi-bacteria interactions - a joint symposium with CUSO (attendance)	June 2012	0.5

Professor Ted Turlings
Director

Dr Christiane Bobillier
Coordinator

Neuchâtel, 21.11.2012 - Page 1/1