Charles University in Prague

Faculty of Science

Nela Gloríková

Impact of endosymbiotic bacteria on the capacity of host spiders (Araneae) to provide ecosystem services.

Vliv endosymbiotických baktérií na schopnost pavouků (Araneae) poskytovat ekosystémové služby.

Bachelor thesis

supervisor: **RNDr. Milan Řezáč, Ph.D.**   April 2022

Acknowledgement

I wish to express my gratitude to my supervisor RNDr. Milan Řezáč, PhD. for his constructive criticism and helpful consultations. I would like to thank my colleagues from the Crop Research Institute for their support and the suitable literature. I am grateful to my dear friends: Mr. (soon JUDr.) Radim Jakubec, Míňa Colaco, Veronika Surkovová, Bc. Eliška Burdová, Hanička Platková, Honza Dohnálek, Tomáš Ludvík and Erik Schmotzer in helping to fight the gloom. Finally, I would like to thank my family for their constant support.

This thesis is dedicated to my most beloved Vasília, Cecília, Prodlužka and in loving memory to Rozdvojka.

General affidavit

I declare that I prepared my bachelor thesis entitled *Impact of endosymbiotic bacteria on the capacity of host spiders (Araneae) to provide ecosystem services* independently. I used only the literature listed in the bibliography. Neither this work nor a substantial part of it has been submitted for another or the same academic degree.

Prague, 25/04/2022   Nela Gloríková

Abstract

The impact of the microbial community as well as individual strains of endosymbiotic bacteria on spider hosts has only begun to be increasingly studied in the last 20 years. This is primarily due to new molecular methods and devices, but on the other hand also to a growing desire to unravel the mechanisms behind the ability of spiders to provide ecosystem services. Their unique contribution lies in their high diversity, their generalist hunting strategy, and their efficient long-distance dispersal called ballooning. However, their abilities are possibly affected by the tiny organisms inhabiting their tissues - from the haemolymph to the digestive tract and the reproductive organs. In this work, attention is paid to the aspects that are influenced or manipulated by microbes for the purpose of their own transmission. Studies investigating known reproductive manipulators such as *Wobachia*, *Cardinium* or *Rickettsia* are summarized. The work summarizes recent findings in the areas of the impact of endosymbiotic bacteria on metabolic pathways and nutritional support, but also on behavioral aspects such as predatory/antipredatory behavior, defense against parasitoids or pathogens, and dispersal or avoidance of stress. Information about spiders is also related to knowledge about other invertebrates.

Keywords: endosymbiotic bacteria, spider, manipulation, facultative symbiont, *Wolbachia*, ecosystem services

Abstrakt

Vliv mikrobiálního společenstva i jednotlivých kmenů endosymbiotických bakterií na aspekty pavouka jako hostitele se začal intenzivněji studovat teprve v posledních 20 letech. Je to především díky novým molekulárním metodám a přístrojům, ale na druhé straně také díky rostoucí snaze odhalit mechanismy, které stojí za schopností pavouků poskytovat člověku užitečné ekosystémové služby. Jejich přínos spočívá ve vysoké diverzitě, v jejich generalistické strategii lovu a v efektivním šíření na velké vzdálenosti, tzv. ballooningu. Všechny tyto vlastnosti jsou však na pozadí dost možná ovlivňovány drobnými organismy obývajícími tkáně jejich hostitelů – od hemolymfy po trávicí soustavu a reprodukční orgány. V této práci je věnována pozornost právě těm životním aspektům, které jsou ovlivňovány nebo manipulovány mikroby za účelem jejich vlastní proliferace. Jsou zde shrnuty studie zkoumající notoricky známé manipulátory reprodukce, jako jsou *Wobachia, Cardinium* nebo *Rickettsia*. Práce shrnuje recentní poznatky v oblastech vlivu endosymbiotických bakterií a jejich společenstev na metabolické dráhy a výživu, ale také na behaviorální oblasti, jako je predační/anti-predační chování, obrana proti patogenům, disperse a vyhýbáni se stresu. Informace ze skupiny pavouků jsou dávány do kontextu poznatků o ostatních bezobratlých, kteří rovněž poskytují ekosystémové služby nebo jsou to naopak škůdci.

Klíčová slova: endosymbiotická bakterie, pavouk, manipulace, fakultativní symbiont, Wolbachie, ekosystémové služby

Table of Contents

Introduction

Host-symbiont relationships are very common among organisms worldwide. The endosymbiosis[1] was originally described as mutually beneficial relationship between two unrelated organisms, with one of them (usually the microorganism) inhabiting the host's body (* Buchner, 1965 in Batra, 1968). In the traditional sense host-endosymbiont mutual relations might be mutualistic (beneficial), commensal (neutral) or parasitic (negative). Nowadays, the line between pathogenesis and mutualism is becoming increasingly blurred as more examples of persistent bacterial infection are identified (Dale and Moran, 2006).

Infection by a certain endosymbiotic bacteria may be advantageous to a host in some situation, but detrimental in others according to manipulation of the bacterium (for example Wolbachia Li et al. (2020). Symbiotic bacteria to its proliferation affect host's life for example in development, reproduction, defence against natural enemies, nutrition, or immunity (summarised in Table 3). The intensity of this relationship depends on the evolutionary history with host and the degree to which the host and symbiont are obligately co-dependent (Dale and Moran 2006).

Obligate symbionts[2] (primary, P-symbionts, OS) usually share longer coevolutionary history with their host, with its history ranging from 30 to 270 million years. This is reflected for example in significant genome reduction reaching up to 90% of original genes and genome modifications (Moran et al., 2008). These irreversible adaptations have given rise to a unique dependence, which, however, in addition to positive improvements, brings many risks to both the host and the endosymbiont.

Moran et al. 2008

---
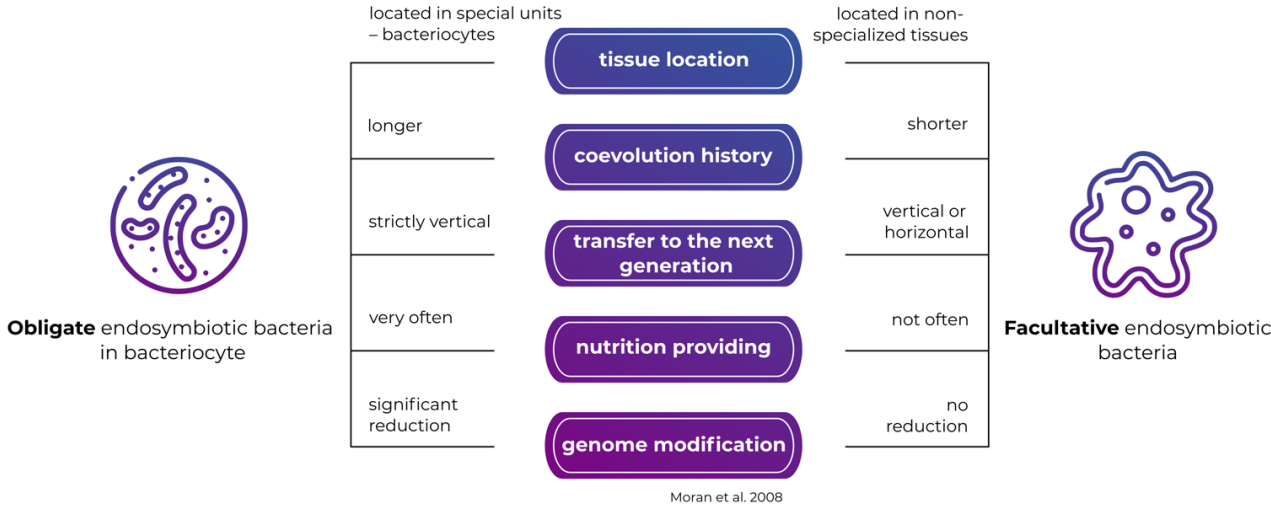
[1] There are several taxonomic groups in the endosymbiont position in this relationship (bacteria, protist, or fungi). However, the review will focus only on bacterial domain.
[2] Obligate symbionts are required for successful reproduction of the host; thus, the host is dependent on its persistence (in contrast with facultative symbionts).

Fig 1: Scheme of basic differences in biology of obligate and facultative endosymbiont in Arthropods (Author: NG). Obligate endosymbionts are microorganisms that have several characteristic features compared to facultative bacteria (1) they are located in special units in the host – bacteriocytes (2) they share a long coevolutionary history with the host (3) they are not strictly transferred horizontally (4) they usually provide the necessary nutrients and substances to the host (5) there is a significant reduction and modification of the genome in endosymbionts (The scheme was made based on the information in Moran et al., 2008).

Host-associated microorganisms location ranges from specialized cells to various tissues. The specialized cells, bacteriocytes, are inhabited by OS in some insect species in superorders like *Homoptera*, *Dictyoptera* or *Hemiptera* (Baumann, 2005; Bigliardi et al., 1995; Fukatsu and Nikoh, 1998; Sacchi et al., 1998). Morphology of bacteriocytes differs markedly between host groups (reviewd in Fukatsu and Nikoh 1998). OS microbiota is found to be a system-integrated organ of the host (Bäckhed et al., 2005). Since OS offer support to the host, the host is interested to passes them on to the next generation via ovarian passage (Koga et al., 2012).

Fig 2: Obligate endosymbiotic bacteria *Buchnera aphidicola* [3] of a Pea aphid *Acyrthosiphon pisum* [Harris, 1776]. HN – host nucleus, C – cytoplasm, B – bacteriocyte with *Buchnera* (Author: J. White and N. Moran, University of Arizona).

Fig 3: Spider from the family *Linyphiidae* providing ecosystem services by consuming aphid pests (Author: archive of the Czech Arachnological Society, provided by MŘ).

Compared to obligate endosymbionts, the host is not dependent on the presence of **facultative symbionts** (secondary, S-symbionts, FS). Relationship can be beneficial for a host, but deleterious as well. For this reason, symbionts had to evolve mechanisms to ensure efficient transmission to the next

---

[3] The endosymbiosis was studied by Paul Buchner (1886-1978) – „the founder of systematic symbiosis research "(Sapp, 2002). *Buchnera aphidicola* (Munson et al., 1991) is famous obligate endosymbiotic bacteria in aphids and was crutial model species in initial symbiotic research (Fig 2). This bacterium was proudly named after prof. Buchner by Paul Baumann and his student.

generation itself. There are two widespread strategies for doing this (1) facultative mutualism (giving infected matrilines advantage to self-transmit) and (2) reproductive manipulation (manipulates host behaviour to self-transmit) (Moran et al., 2008). Strategies of bacterial strains have been studied intensively in insect-host (summarised in Table 3, reviewed in Moran et al., (2008)), however, only partially reviewed in non-insect groups like spiders (*Araneae*, Fig 3).

The aim of this review study is to summarize the impact of the facultative endosymbiotic bacteria (mutualists as well as reproductive manipulators) on the efficiency of spiders to provide ecosystem services (Fig 4). These services are all the benefits that a man receives from the activities of healthy ecosystems.

This topic will allow focus on the bacteria-spider relationship in context beyond the individual borders. Endosymbiotic bacteria to their effective proliferation and transmission influence variety of spider-associated-behavioural aspects from the predation capacity, long-way dispersion, reproduction to the resistance against stress. Thus, it may modify the capacity of spiders to provide ecosystem services.

facultative
endosymbiotic bacteria

beneficial invertebrates
providing **ecosystem services**

methods
of transfer

spiders
predators

potentionally **affected aspects** of host life

predatory activity   mobility   nutrients   dispersion   reproduction   web construction   pesticide resistance (stress)
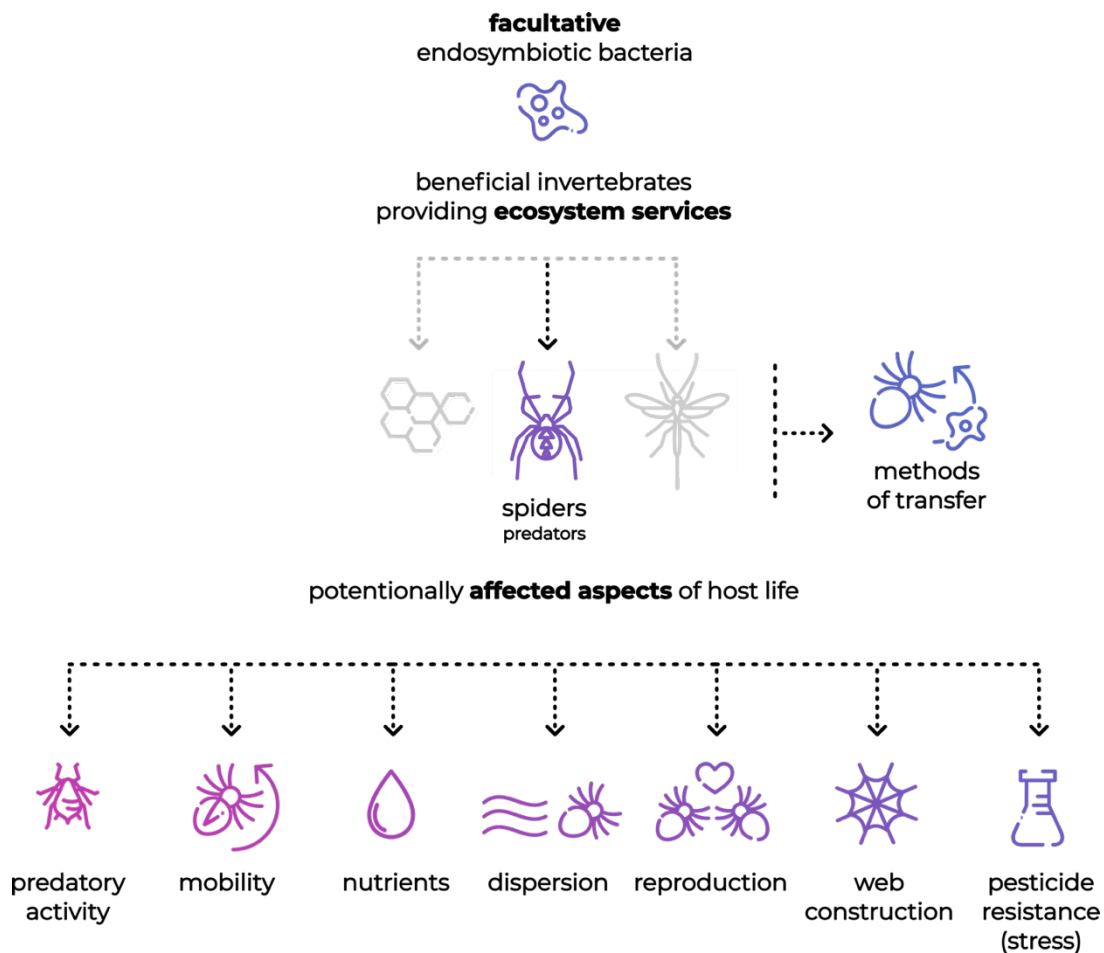
Fig 4: A scheme visualizing the theoretical objectives of this thesis: the impact of the facultative endosymbiotic bacteria on the capacity of **spiders** to provide ecosystem services. (Author: NG)

1 Terminology in the invertebrates´ endosymbiont biology

The overall terminology in the field of endosymbiosis seems unclear, as it describes phenomena with the same term that are not identical in other fields (Husnik and Keeling, 2019). This is the case, for example, of the terms "primary and secondary endosymbiont". In cell biology, the primary symbiont is an organelle, that was formed by phagotrophy of a cyanobacteria by a eukaryotic cell. The secondary symbiont was formed by swallowing red or green algae that already contained the primary plastid.

In contrast to this meaning, the primary symbiont could be a microorganism on which the host is obligatorily dependent. The secondary symbiont has a diametrically different meaning in this case. Paul Buchner described secondary symbiont as "morphologically different" to the primary symbionts that he observed in aphids (* Buchner, 1965).

It is important to note that some authors also distinguish between the symbiont and endosymbiont, while others do not. For example, in a study on the biology of bacteriocyte-associated endosymbionts, the author defines (S-) endosymbionts as microorganisms with a clearly established location in the host, whereas (S-) symbionts lack this clear location (Baumann, 2005). It is also important to look at the relationship between the endosymbiont and the host from both the host and microorganism perspectives. Bacteria described as obligatory and incapable of existing outside the host does not mean that the host is unable to live without it (Hosokawa et al., 2016). However, when the benefits to the symbiont are not as obvious as the benefits to the host, it is more important to talk about this association as a domestication or slavery (*Szathmáry and Smith, 1995 in Werren and O'Neill 1997).

*

Categorisations can often be misleading and unstable over evolutionary time (Husnik and Keeling, 2019). For the purposes of this work, the division from N. Moran into obligatory bacteriome-associated endosymbionts and facultative mutualists or facultative reproductive manipulators will be used. In this thesis, the terminology symbiont, endosymbiont, microbe, or bacteria will be used more loosely than the definition described by the authors above. Finally, terminology will be respected according to the authors of the scientific studies.

2   Spiders (*Araneae*) as a group of biological control agents

Spiders (*Arthropoda*, *Chelicerata*, *Araneae*) belong to a diversified and ubiquitous group of invertebrate predators. They are the second most numerous order of the class *Arachnida* and on the scale of most diverse organismal orders worldwide they reach top 10. There are over 50,000 species in the world in more than 4,200 genera (World Spider Catalog 2022).

Fig. 6a-6d: Examples of the diversity of spiders (Araneae) in ecosystems inhabiting epigeic (6a – family *Linyphiidae*, 6c – family *Lycosidae*) and epiphytic (6b – family *Philodromidae*, 6d – family *Theridiidae*) niches.

Spiders are distributed across continents (except Antarctica) in virtually all terrestrial habitats. These euryphagous predators consume a wide variety of invertebrates, including pests and invasive species, and can therefore serve as a natural defence for ecosystems and perform significant ecosystem service (Nyffeler, 1999). Using monoclonal antibodies, it has been found that spiders even minority represented in the population may have a high predation capacity rate on agriculture aphid pest. One such spider was long-jawed orb weaver *Pachygnatha degeeri* [Sundevall, 1830], which frequently prey on aphids in months when the colony is established (Harwood et al., 2005). This is probably the most effective month for biological control (Harwood et al., 2004). Even if spider species have no preference

for pests or have a low capture rate, they can have a significant non-consumptive effect, for example by increasing pest emigration from the host plant (Michalko et al., 2019; Rendon et al., 2016).

Spider communities have been found to include a higher percentage of positive indicator species for aphid biological control[4] than, for example, some predaceous beetle communities (Birkhofer et al., 2018). Furthermore, spiders represent variety of trophic niches in comparison with other generalist invertebrate predators. It is not surprising considering their wide diversity of hunting strategies (Mestre et al., 2013; Michalko et al., 2019). Not only feeding behaviour, but also body size, hunting strategies, or even age /in the genus *Philodromus,* fig. 6b/ significantly affect trophic position (Sanders et al., 2015).

On the other hand, their very slow quantitative functional response, which is caused by slow development, makes them unable to reduce the high population growth of many pests in agroecosystems (Pekár, 2012; Riechert, 1999). Some authors are sceptical of including such exclusive generalist predators among beneficial animals. Based on an experimental study, Schmidt-Entling and Siegenthaler 2009 believe that the wolf spiders (*Lycosidae*, fig. 6c) in the role of top predators reduce the density of intermediate predators and thus enhance the herbivorous insects such as plant-hoppers and leaf-hoppers.

Despite these discrepancies spiders play an irreplaceable role of predators in ecosystems. It is strengthened by their abundance, or ability to disperse and colonize new habitats. The influence of this group on pests and thus the function of spiders in the role of biological control agents is conditioned by factors such as the phenology of or environmental characteristics (reviewed in Michalko et al. 2019). The influence of endosymbiotic bacteria, which are still only beginning to be investigated in this group, probably also plays its role.

<p style="text-align:center">*</p>

The study of endosymbiotic bacterial strains in spiders, in contrast to insects, is a relatively recent matter. However, in the last 20 years, through the new technologies and molecular methods, research in this area is increasing. Study of the diversity of endosymbiotic bacteria progress and endosymbionts are document in groups where they have never been found (or searched) before from haplogyne to entelegyne spiders (Ceccarelli et al. 2016; Cordaux et al., 2001). Some studies have directly focused on the diversity of endosymbiotic bacteria in spiders inhabiting agroecosystems. This diversity showed to be enormous. Spiders are providing here ecosystem services by predating on serious crop pests.

Hu et al. (2019) compared the diversity of intestinal (gut) bacteria among the three spider species: *Pardosa laura* [Karsch, 1879], *Pardosa astrigera* [L. Koch, 1878] (both *Lycosidae*) and *Nurscia albofasciata* [Strand, 1907] (*Titanoecidae*). These spiders are common predators in cotton fields, however, differ in hunting strategies. All subjects were starved for one week prior to PCR analysis to minimize the proportion of non-native bacterial strains. A total of 230 genera from 23 phyla of bacteria were

---

[4] Indicator species is a health-ecosystem diagnostic organism signalling biological condition change. The abundance may reflect specific condition of ecosystem.

recorded, while the Proteobacteria was the most dominant. There was no significant difference in the microbial diversity between spider species. Proteobacteria are also dominant in butterflies (in Czech Republic endangered *Melitaea cinxia* [Linnaeus, 1758]) or in honeybees (Engel et al., 2012; Ruokolainen et al., 2016). It is important to study microbial diversity and abundance of bacteria in intestinal environment because gut epithelium is often used as an entry for pathogens (Hu et al., 2019).

White et al. (2020) investigated symbionts in 14 species (267 individuals) of spiders belonging to the families *Linyphiidae* (Fig. 6a), *Tetragnathidae* and *Oxyopidae*. Specimens were starved for approximately 5 days and surface sterilised to avoid contamination. Together, they detected 27 operational taxonomic units (OUT, 7 bacterial genera) of endosymbiotic bacteria. Among the genera detected were *Wolbachia, Rickettsia, Cardinium, Rickettsiella, Spiroplasma, Rhabdochlamydia* and one brand new strain from the *Rickettsiales* group in long-jawed orb weaver *Glenognatha foxi* [McCook, 1894]. Individual strains of endosymbionts were mostly widespread and characteristic of the spider species. There were 71% of individuals tested positive for at least one strain of endosymbionts, however spiders, which contained several different strains, were no exception. The record holder of this study was the species *Idionella rugosa* [Crosby, 1905] (*Linyphiidae*) with 8 OTUs (belonging to 5 bacterial strains).

Authors are discussing that these findings cope with knowledge of the microbial diversity typical for insect kingdom endosymbionts on genus level (Duron et al. 2008; Hu et al. 2019). However, we cannot reliably compare the results of such studies due to the different geographical and time scales in which they were conducted.

3 Distribution and transfer of endosymbiotic bacteria in spiders

Obligatory as well as facultative relationships of Arthropoda hosts with symbionts further requires a mechanism of intergenerational transmission to the offspring. Most intracellular symbionts are transmitted from parent to offspring vertically, leading to long-term coevolution (Sauer et al., 2002). Such a phenomenon has been termed "hereditary symbiosis" (Bright and Bulgheresi, 2010). Evidence of **vertical transfer (VT)** via egg cells in spiders is convincing.

The reproductive manipulator *Wolbachia* was discovered in the cytoplasm of an egg cell of *Nephila clavata* [L. Koch, 1878] and in vitelline body (VB) as well (Oh et al., 2000). The egg-cell-structure VB ostensibly contributes to yolk formation and mitochondria transmission (André and Rouiller, 1957; Sotelo and Trujillo-Cenóz, 1957)). In planthoppers, the bacterial reproductive manipulators enter reproductive tissues involving vitellogenin (VG) (Guo et al., 2018). This precursor of egg-yolk protein is synthetized by fat body, which was found to be metabolically influenced by *Wolbachia* in spiders (Li et al., 2020). VG is transferred with haemolymph to ovaries and via receptor-mediated endocytosis into the oocyte. *Wolbachia* and probably other reproductive manipulators are taking advantage of it and hitchhiking using VG/VB into the next generation. This transfer process may be similar in spiders and requires further investigation.
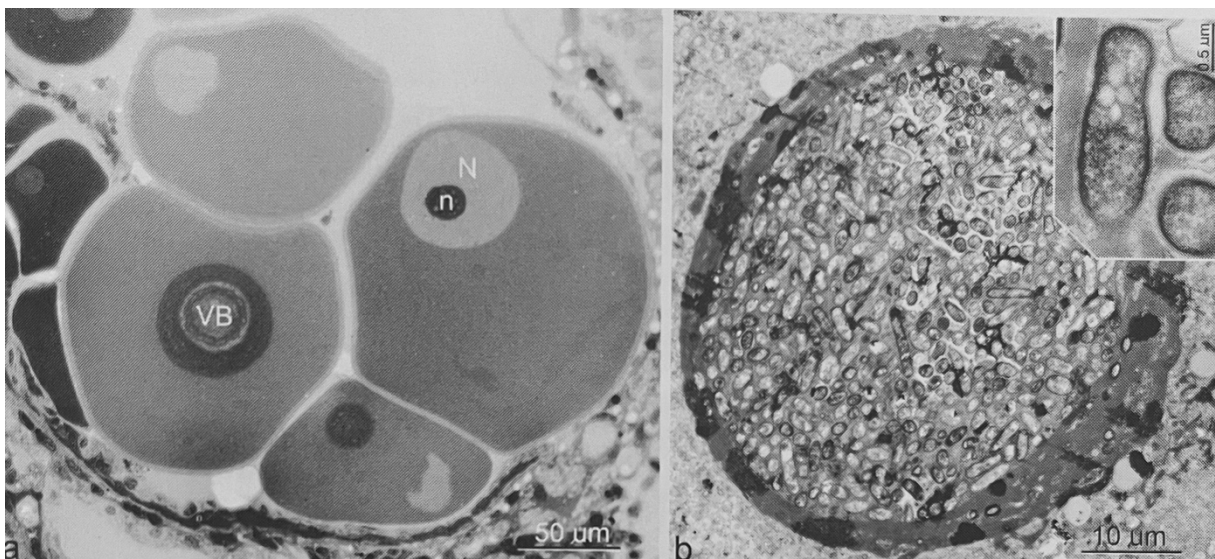

Fig 7a: Young egg cell in spider *Zygiella*. N – nucleus with nucleolus (n); VB – vitelline body.
Fig 7b: The dense symbiotic bacteria in vitelline body under higher magnification (Author: * Reimers in Foelix 2010).

There is the discordance between host and bacterial phylogenies in some cases suggesting that the **horizontal transfer (HT)** may play a role at least in the evolutionary time horizon (Goodacre, 2011; Goodacre et al., 2006). HT and successful coinfection between host species may bring an opportunity for the gene transfer and recombination, causing consequences in symbiont-population structure (reviewed in Moran et al., (2008)). In comparison to primary bacteriome-associates (strictly vertically

transferred), facultative symbionts (vertically or horizontally transferred) can have a lot of recombination between strains, as *Wolbachia* has shown (Baldo et al., 2006). Hereafter, some of the repetitive regions revealed in the published *Wolbachia* genome are likely adaptations for facilitating recombination within or across genomes (Wu et al., 2004).

HT of Wolbachia was discovered between the *Agelenopsis* spp. funnel-web spider species (9 species, n=128, *Agelenidae*) across continental USA. There were 3 *Wolbachia* strains found in spiders and none of them was found to be species specific. However, closely related *Wolbachia* clades tend to have HT more likely between related *Agelenopsis* spp. hosts than unrelated, suggesting some specificity in these bacteria-host relationships (Baldo et al., 2008).

*

In spiders, HT can occur during prey consumption. Majority of spider species belong to generalist predators with an extraintestinal digestion (Foelix, 2010)[5]. Expectedly, the microbiome diversity analyses often encountered endosymbiotic strains that are typical for insects. These strains are usually present in spider intestines only at low frequencies, however, the results from these analysis are not unambiguous (Kennedy et al., 2020; Zhang et al., 2018). Other study contradict that microbial communities in the spider gut undergo significant fluctuations that are dictated by the taxon of prey consumed (Kennedy et al., 2020).

Low frequencies of some non-native-endosymbiont strains in spiders can be cause by several ecophysiological adaptations. During the consumption, the liquefied prey is filtered two times. While the initial filtering effect is provided by numerous bristles at the edges of the mouth, the secondary filtration takes place in a specialized structure placed on a rostral plate in the flattened pharynx (Foelix, 2010). Experiments with India ink solution have shown experimentally that particles larger than 1 μm do not penetrate such a filter barrier (* Bartels, 1930 in Foelix, 2010). This finding indicates that filter barrier may serve to prevent for example pathogens from horizontal transmission to the spiders' digestive tract. Cell size of the ellipsoid bacterium *Cardinium* ranges from 4 to 5 μm in length, thus may be filter out by this barrier (Kitajima et al. 2007). Interestingly, *Cardinium* tends to appear to be more prevalent in spiders in comparison with other arthropods (Duron et al., 2008; Zchori-Fein and Perlman, 2004).

However, the reproductive parasite *Wolbachia* with the cell size from 0.2 to 4 μm may escape this mechanical barriers and infect novel host (Taylor et al., 2018). Probably other barriers like the antimicrobial peptides contained in the venom of spiders or inherited immune system, tends to prevent from HT infection of small pathogens (Kuhn-Nentwig, 2003). Here, it is still important to approach studies about endosymbiotic diversity critically, especially in cases where the model organisms were

---

[5] Spiders dispone with variable venom, which primarily serves to paralyze prey, contains both neurotoxic polypeptides and smaller biogenic amines and sometimes proteolytic enzymes (* Bachmann, 1976 in Foelix, 2010). These are the enzymes that digest the prey and the spider can suck the liguified prey tissues through the hole in the cuticle.

not starved before the actual (especially whole-body) analysis. There is for example a significant intestinal microbial variability between individuals of wolf spider *Pardosa laura* (Hu et al. 2019). Some studies show that even starvation may not be fully sufficient for elimination of alien bacterial strains (White et al., 2020a).

Regular HT of endosymbionts from infected prey to the host would be expected to result in the formation of geographical patterns where sympathrically occurring hosts would share identical bacterial strains, but studies show that this is not the case (reviewed in Goodacre, 2011). Population-specific patterns were also not evident in most species of social spiders of the genus *Stegodyphus*. This populations have very low intrinsic genetic variability and thus it is believed that the microbiome might support local adaptation to the environmental changes – different between localities (Busck et al., 2020). In comparison, another analysis of microbial structure of *Stegodyphus* nests differ between geographical locations. However, this findings are likely linked to environmental factors rather than microbes (Nazipi et al., 2021a).

<center>*</center>

The HT of facultative endosymbionts directly from the environment was observed and further experimentally tested in *Hemiptera* (Hosokawa et al., 2016). Similar phenomenon was highly unlikely to evolve in spiders, because both eggs and the first instars of the young spiderlings are never directly exposed to the environment and placed in the silken cocoon. A typical cocoon of entelegyne spiders is made up of several types of silken fibers (* Vollrath, 1992 in Foelix, 2010). Cocoons have been expected to have an antimicrobial effect in the past. Strong evidence of microbial effect of cocoon surface was proven in the common house spider *Parasteatoda tepidariorum* [C. L. Koch, 1841] (Babczyńska et al., 2019). In some cases, silk, or webs themselves also showed an anti-microbial effect (Sharma, 2014; Wright and Goodacre, 2012). However, this pattern is certainly not uniform and varies between groups of spiders (Alicea-Serrano et al., 2020).

4 Influence of endosymbiotic bacteria on the capacity of spiders to provide ecosystem services

4.1 Nutritional support and metabolism promotion

Nutritional supplementation by endosymbionts is known across the insect group, however, the effect on metabolism pathways is well studied mostly in *Wolbachia*. For examples in insects, *Wolbachia* may interference expression of protein ferritin[6] and thus the iron storage in wasp *Asobara tabida* [Nees, 1834], which is on *Wolbachia* obligately dependent species (Kremer et al., 2009). Nutritional supplementation is important benefit mainly in food specialists or organisms living on the edge of their ecological possibilities. The host is dependent on the presence of the symbiont and the benefits it provides. It can be crucial for proper growth, reaching maturity, and reproduction (Hosokawa et al., 2016). This phenomenon was first demonstrated in aphids that host *Buchnera aphidikola* gammaproteobacteria. Aphids benefit from this relationship in many ways, but one of the most important is the intake of essential amino acids and vitamins (Baumann, 2005; Douglas, 1998; Wilson et al., 2010). For other example, there is a bacteriocyte-resident *Wigglesworthia glossinidia* [Aksoy, 1995] in tse-tse flies synthetising B-vitamins or the specialized protist that allow the termite *Coptotermes* to digest wood as its only source (Akman et al., 2002; Hongoh et al., 2008). However, not all species that have obligate endosymbionts must be metabolically dependent on them. The gammaproteobacterial belonging to the "*Candidatus*[7] *Blochmania*" is assumed to be beneficial, because this strain is closely related to endosymbionts of aphids or tsetse flies. Nevertheless, the nutritional function of this endosymbiont in believed-to-be omnivore ants *Camponotus* is not known yet (Sauer et al., 2002).

Such nutrient supplementation could significantly affect the functioning of spiders in ecosystems and their role in providing ecosystem services. It could increase fitness, the ability to spread and occupy new habitats in individuals and populations. On the other hand, this could lead to dependence on endosymbiotic support. This close bond could be easily susceptible to the effects of pesticides in agroecosystems. In honeybees, laboratory experiments indicate that glyphosate-based herbicides may affect honeybee intestinal microbial community and reduce resistance to stress (Motta et al., 2018). Numerous studies showed that exposition to pesticides may be lethal or cause various sub-lethal effects to spiders (Evans et al., 2010; Gloríková and Řezáč, 2022; Lacava et al., 2021; Petcharad et al., 2018; Řezáč et al., 2021).

There were only few recent studies investigating metabolism and nutritional support of bacteria in spiders. The effect of *Wolbachia* and *Cardinium* on amino acid metabolism has been found in the

---

[6] The intracellular protein Ferritin is the main storage of iron in almost all eucaryota and procaryota species

[7] *Candidatus* is recommended name for non-cultivable organisms according to Murray and Schleifer, (1994)

small linyphiid spider *Hylyphantes graminicola* [Sundevall, 1830]. This spider is an important biological control agent of diverse pests in maize and cotton fields around the world. Li et al. (2020) showed in their gene-based research that co-infection with both bacteria increases the fat content and, in males, the content of free amino acids. This may be due to affecting the amount of glutamate, which is important not only for protein synthesis but also for amino acid transamination. Further, gene expression involved in thyroid hormone synthesis, lipocalin and hemocyanin genes were also affected. The authors believe that fat body (structure metabolically equivalent to vertebrate liver) in the host increases the nutritional richness of the environment and allows bacteria like *Wolbachia* to proliferate at high densities (Li et al., 2020).

For comparison, symbiotic microbiome (with *Wolbachia* and *Cardinium* presence) may be involved not only in amino acid metabolism, but also in carbohydrate and energy metabolism in two *Pardosa* species (*Lycosidae*) and one *Nurscia* species (*Titanoecidae*). Authors used for this suggestion the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) method, which is bioinformatics software useful in the field of metagenomic analysis allowing interference of a microbial community. All three species were positively tested for *Wolbachia* infection, however, there was *Cardinium* present only in spider *Nurscia albofasciata* (Hu et al., 2019).

These results follow another study where metabolic functional pathways of spiders' gut microbiome were predicted (Tyagi et al., 2021). The authors investigated the microbiome structure of 12 species of spiders from the family *Araneidae* (subfamilies: *Araneinae*, *Argiopinae* and *Gasteracanthinae*). The results showed that spiders of these three groups shared the microbiome community structure, which is not coherent with their phylogeny. Again, carbon metabolism and biosynthesis of amino acids were the most common, but the predicted metabolic pathways were very diverse. For example, pyruvate metabolism, glycolysis or arginine / proline metabolism appeared in the PICRUSt analysis as well. Authors assumes that the bacteria involved in the above-mentioned metabolisms have the highest relative abundance in the spider's gut (Tyagi et al., 2021).

These papers suggest that the influence of individual bacterial strains as well as entire microbial communities can have a significant impact on metabolism at several levels. This effect may not always be positive and, as examples from insect groups show, can lead to complete host dependence on the endosymbiotic bacteria.

4.2 Pathogens defence in social spiders

The relationship between host and parasites is one of the most widely spread type of interaction among living organisms (Durkin et al., 2021). The effectiveness of defence against parasites is a crucial ability for a spider to be able to provide ecosystem services. There are several ecophysiological way how spiders prevent from pathogens: (1) dual liquid filtration during prey consumption (2) antimicrobial

activity of silk and/or (3) endosymbiotic bacteria (Foelix, 2010; Nazipi et al., 2021a; Wright and Goodacre, 2012). According to studies, protection of the intestinal bacteria against pathogens might be very beneficial for their host (Koch and Schmid-Hempel, 2011). The resident microbiota provides protection against parasitic tripanosomatid (*Crithidia bombi*) in bumblebees (*Bombus terrestris*). Transmission of these beneficial bacteria is believed-to-be one of the most significant benefit of a sociality (Koch and Schmid-Hempel, 2011).

Transfer of specific pathogens might be more successful between social organisms (Wilson, 2000). The low genetic variability, sex ratio bias and frequent extinction-colonisation events are an aggravating factors causing susceptibility of populations (Settepani et al., 2017). In other social species, there are mutualistic endosymbiont-host relationships providing ants or termites antimicrobial defence (Chouvenc et al., 2018; Currie et al., 2006). Similar interactions can be expected also in social spiders but have not been studied yet.

There was detected an abundant presence of a potentially araneopathogenic fungus at elevated humidity in the nest of social spider *Stegodyphus dumicola* [Pocock, 1898]. These fungi are the best studied micro-pathogens of spiders (Durkin et al., 2021). Up today, it has not been possible to reveal whether any bacterial strain protects the nest of *S. dumicola*. The antimicrobial activity was reported in vitro for three strains from the cultured nest-associated bacteria so far (Nazipi et al., 2021a). Furthermore, there was a new strain of *Staphylococcus sciuri* discovered on the surface of *S. dumicola*. This strain shows extremophilicity, halotolerance and antimicrobial activity against related bacterial species. Although host non-specificity, this *Staphylococcus* might provide social spiders with a crucial competitive advantage (Nazipi et al., 2021b).

Peripherally, studies of bacterial interactions within the microbial community of non-social spiders revealed negative correlations of pathogen *Rickettsia* with the rest of the microbiome (Andersson et al., 1998; Hu et al., 2019). This may indicate interspecies competition or targeted elimination of the bacterium, which could reduce host thriving and thus threaten the proliferation of the whole community.

4.3 Reproductive barriers, manipulation of reproduction and parthenogenesis induced by endosymbiotic bacteria in spiders

Spiders have one of the highest incidence and diversity of endosymbiont bacteria among arthropods. There were up to five distinct endosymbiotic reproductive manipulators discovered in various spider families specifically *Spiroplasma, Rickettsia, Cardinium, Arsenophonus*, and *Wolbachia* (Duron et al., 2008; Goodacre et al., 2006; Oh et al., 2000; Vanthournout et al., 2011). They are known for manipulation host reproduction to its own proliferation and transmission. In Arthropods, for example, they can cause cytoplasmatic incompatibility (CI) or female-biased sex ratio distortion (SRD) driven by feminisation of males, embryonal male-killing, or parthenogenesis (reviewed in Hurst and Jiggins, 2000;

Kremer et al., 2009; Rosenwald et al., 2020; Veneti et al., 2005). In spiders, the effect of endosymbionts on both SRD and CI has been confirmed to date, further hypothesizing the induction of parthenogenesis.

4.3.1 Sex ratio distortion and male-killing

According to Fisher's sex-allocation theory, the equal sex ratio (1:1) of offspring in organisms with sexual reproduction is the evolutionary stable condition (Fisher, 1958). Female-biased sex ratio distortion (SRD) was observed in systems of social spiders where kin selection is hypothesized to favour selection. Such species are for example velvet spider *Stegodyphus dumicola*, crab spider *Diaea socialis* [Main, 1988], combweb spider *Anelosimus domingo* [Levi, 1963] or *A. eximius* [Keyserling, 1884] (Avilés and Maddison, 1991; Rowell and Main, 1992). In a social group, it is advantageous to invest energy in reproducing females at the expense of "unnecessary" males (Aviles, 1986).

With haplodiploid sex determination (SD), it is possible to directly regulate the sex of the offspring by fertilization (or the elimination of paternal genome). Surprisingly, it has been found that in social spiders with a diplodiploid SD with male heterogamety system, a similar regulation may exist. It was shown experimentally that the theoretical variability in the number of male embryos per egg sac was significantly higher than that observed. Cytological preparations showing 24 chromosomes for female and 22 chromosomes for male embryo in *Anelosimus domingo* (Avilés et al., 2000). In males of related species, there were observed two types of spermatids: with and without sex chromosomes. The mechanism of SRD in favour of females in *Anelosimus* is unknown, but it seems to be post-meiotic, according to equal ratio of both spermatid types (Avilés and Maddison, 1991).

On the other hand, primary SRD of solitaire spider species is typically predicted because of reproductive manipulation by endosymbiotic bacteria (reviewed in Hurst and Vollrath, 1992). Reproductive manipulator *Spiroplasma poulsonii* can selectively kill male embryo in *Drosophila melanogaster* [Meigen, 1830] by interaction with DCC (dosage compensation complex), which is involved in sex determination (Veneti et al., 2005). The male-killing phenotype may provide indirect benefit for example lower number of offspring reduce competition between female youths (reviewed in Charlat et al., 2003). Causal relationship between SRD of the solitaire spider and the infection of endosymbiotic bacteria is suggested in few linyphiid spiders.

Dwarf spider *Oedothorax gibbosus* [Blackwall, 1841] is a palearctic cosmopolitan species inhabiting mashes, wet meadows, or littorals near the water. Interestingly, this species shows a significant sexual dimorphism, which in males differentiated into two phenotypes (1) form *tuberosus* with conical hump (Fig 8b) and (2) form *gibbosus* with high rounded bulge (Fig 8a) (Kůrka et al., 2015).

Several groups of reproductive manipulators, namely *Wolbachia, Rickettsia* and *Cardinium*, have been detected in *O. gibbosus*. Of these, it is *Wolbachia* that has been shown to cause SRD. It was experimentally shown, that after antibiotic treatment the unbiased SR was restored. Possibly, the embryo

male-killing is likely to be the manipulation way of the bacteria (Vanthournout et al., 2011). However, its mechanism may be different from that of *Spiroplasma* in *D. melanogaster*, as *Wolbachia* and *Spiroplasma* are unrelated bacteria (*Oedothorax* and *Drosophila* are unrelated model organisms as well). *Wolbachia* belongs to the class *Gammaproteobacteria* whereas *Spiroplasma* belongs to the distant class *Molicutes*. Authors suggest that SRD impact of Wolbachia might be implicated in the observed coexistence of two different male phenotypes by the influence on sexual selection (Fig. 8a - 8b; Vanthournout et al., 2011; Goodacre, 2011).
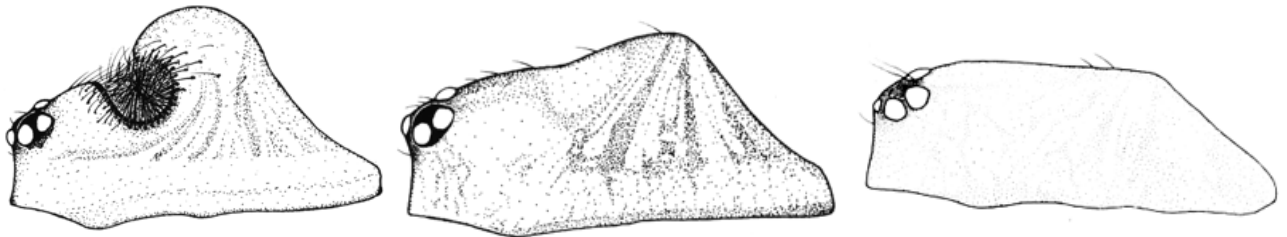

Fig 8a-8c: Sexual dimorphism and phenotype variability in males of *Oedothorax gibbosus*. 8a – prosoma of male form *gibbosus;* 8b – prosoma of male form *tuberosus*; 8c – prosoma of female (Author: Roberts, 1987; Růžička, 1978)

Sheetweb spider *Pityohyphantes phrygianus* [C. L. Koch, 1836] is common resident of spruce forests across Holarctic region (Kůrka et al., 2015). Altogether with *O. gibbosus* and *Erigone atra* [Blackwall, 1833] belong to spider model organisms in the studies focused on reproductive manipulator-host interactions. The female-based SR was previously attributed to different post-copulatory position. Specific shape of inner genitalia (spermatheca) suggested that during different abdominal position the sperm is stored in different spermatheca (2 paired types). This distribution can be controlled directly by the female and correlates with the degree of SR bias of the offspring (Gunnarsson et al., 2004). Later studies revealed, that *Wolbachia* infection play a crucial role in these observations (GUNNARSSON et al., 2009).

4.3.2 Parthenogenesis

Parthenogenesis, i.e., the formation of an embryo from an egg cell without fertilization is, in some cases, crucial benefit in extreme habitats or the ultimate mode of reproductive parasites to transfer to the next generation successfully vertically through the maternal line (Stouthamer, 1997). Its induction in insects is traditionally known in *Hymenoptera* caused by *Wolbachia*, but also by *Cardinium* or *Rickettsia* (Hagimori et al., 2006; Kremer et al., 2009; Weeks and Breeuwer, 2001; Zchori-Fein et al., 2001). Here, in haplo-diploid species, endosymbiont-driven parthenogenesis is hypothesized to be triggered by chromosomal duplication in unfertilized eggs, resulting in female embryos solely (Goodacre, 2011).

In spiders, parthenogenesis has been found for example in the small, leaf litter ochyroceratid *Theotima minutissimus* [Petrunkevitch, 1929] (Edwards et al., 2003) or the oonopid *Triaeris stenaspis*

[Simon, 1891] introduced to European greenhouses. Here, the parthenogenesis is not cause by endo-symbionts like *Wolbachia* or *Cardinium* (Korenko et al., 2009). Although the Wolbachia strain has been identified in the haplogyne spider *Dysdera erythrina* [Walckenaer, 1802], its presence has not been confirmed in parthenogenetic *Dysdera hungarica* [Kulczyński, 1897] (Cordaux, 2001; Rezáč et al., un-bublished data). This absence of a reproductive parasite may signify that petrogenesis in *D. hungarica* is caused by another bacterial species or that it is not caused by endosymbionts at all.

Although the impact of bacteria is not negligible, parthenogenesis is commonly induced among troglobiont species spontaneously. In caves, the population densities are low as well as probability of finding a compatible partner (Mammola and Isaia, 2017). Also, the conditions are very stable and pre-dictable thus the lack of genetic variability is not a such problem as in the surface. This reproduction is among spiders expected in a troglobiontic celotine *Coelotes troglocaecus* (family Agelenidae) from Okinawa Island (Shimojana and Nishihira, 2000) and *Anapistula ataecina* (family Symphytognathidae) from caves in Portugal (Cardoso and Scharff, 2009). These spiders are not expected to be parthenoge-netic due to bacterial infection, but it has never been directly studied in these species.

Overall, there is very little evidence to suggest that parthenogenesis in spiders is caused by par-asitic bacteria. It is upon the further research to test this hypothesis in more detail on a larger range of potentially suitable model species like those mentioned above.

4.3.3 Cytoplasmatic incompatibility

Cytoplasmatic incompatibility (CI) is a conditional sterility trait in which crossings between infected males and uninfected females result in offspring death or preventing uninfected progeny from being produced. Created eggs are fertilized, which rules out the explanation that incompatible crosses originate from fertilization failure. CI is considered to occur in diploid organisms because the DNA of infected males' sperm is removed from growing zygotes until rescued by eggs infected with a suitable bacterial strain. Uninfected eggs are unable of rescuing embryos, and male and female chromosomes are unable to unite, resulting in embryo death (Goodacre, 2011). Over time, CI increases the proportion of infected females in the host population (Gilbert et al., 2005; Rosenwald et al., 2020; Yen and Barr, 1971).

Today it is known that CI in some arthropods is caused by endosymbiotic bacteria of the genus *Wolbachia, Cardinium, Rickettsiella* and an unnamed strain of alphaproteobacterial *Rickettsiales* in the invasive coconut palm pest *Brontispa longissimi (Coleoptera:Chrysomelidae)* (Gotoh et al., 2007; Rosenwald et al., 2020; Sinkins, 2004; Takano et al., 2017). Spiders like the salticid *Habronattus pugillis* [Griswold, 1987] had postzygotic isolation reflected into lower survivability of progeny from between-population crossings, which might suggest CI (Goodacre et al., 2006; Masta and Maddison, 2002). Re-cently, first evidence of *Rickettsiella* causing CI in agricultural linyphiid spider *Mermessus fradeorum* [Berland, 1932] was revealed. However, the mechanism is unknown.

It might be possible that the lower fertility of distant between-population crosses compared to closed ones (as shown in Masta and Maddison (2002)) is caused by endosymbiotic bacteria with a reproductive manipulator strategy. In this case, the reproductive barrier could also be facilitated by the ability of bacterial manipulators to affect capacity of spiders to spread over long distances.

4.4   Dispersal behaviour

Spiders are known for their ability to spread to new suitable habitats. Effective dispersion allows them to provide ecosystem services consistently. This capability is crucial in disturbed or fragmented habitats. Individuals spread mainly by wind in the juvenile stages via behaviour called ballooning (Foelix, 2010).

Family *Linyphiidae* contains the smallest spider species in Europe (Kůrka et al., 2015). The size of only 2 mm predisposes them to be efficient aeronauts[8]. For example, money spider *Erigone atra* can disperse by ballooning throughout the year at any phenological stage (Weyman et al., 2002). It was found that dispersal behaviour can be influenced by endosymbiotic bacteria, among other factors. The presence of *Rickettsia* significantly eliminates the tendency to spread over long distances in *E. atra* compared to the antibiotic-treated group of spiders. This dispersal barrier is thought to help endosymbionts spread across the host population in particular area. Microbial community affects more females than males, and thus changes in long distance-dispersion could locally affect the sex ratio (Goodacre et al., 2009). This could lead to cocoon density increase and thus higher possibilities for the parasitoids like wasp *Gelis festinans* (reviewed in Goodacre, 2011).

The effect of endosymbionts on dispersion is obvious, but experimentally under-investigated. Most research targeting the influence of endosymbionts on host behaviour are focused on reproductive parasitism (Goodacre, 2011; Goodacre et al., 2006). Yet, studies assessing the impact of other behavioural aspects are equally important and call for more attention.

4.5   Dealing with stress

The stress-compensation benefit is a fascinating discovery in the endosymbiont-host relationship. The best-known studies probably come from aphidologists. Here, the Pea aphid *Acyrthosiphon pisum* is model organism for study of this phenomenon. The effect of the facultative endosymbionts on the reproduction under exposure to heat stress was revealed in this species. The combination of heat stress and absence of these bacteria lead to reproduction unsuccess and significant reduction of obligate endosymbionts (Montllor et al., 2002). Similar stress can be compensated by *Cardinium* in Silverleaf

---

[8] Individuals spreading by wind

whitefly *Bemisia tabaci*, [Gennadius 1889]. Furthermore, Pea aphid heat-tolerance secondary symbiont (PASS) is more frequent in aphids during summer heat stress compared to other seasons in California (Montllor et al., 2002).

Non-flying aphids are closely tied to the nourishing plant and thus are directly exposed to stress. In this situation they find it very difficult to escape. Therefore, it is not surprising that they have developed this endosymbiont-host-specific guardianship (Montllor et al., 2002). Spiders often have a wider ecological valence and are more resistant to extreme temperatures or starvation than other predators (Cramer and Maywright, 2008). Here, it is interesting that such bonds are known and studied in spiders as well. The role of bacterial entities in their bodies in their ecological performance is unknown so far.

<p style="text-align:center">*</p>

Spiders are an overall unique model for heavy metal research. Cadmium, for example, is one of the most serious agricultural pollutants showing high toxicity. It enters the organism very easily through the food chain (Liu 2009). Yang et al. (2018) metatranscriptomally studied the response of endosymbionts of the agroecosystem wolf spider *Pardosa pseudoannulata* [Bösenberg & Strand, 1906] to cadmium-induced stress. The hypothesis was that this stress may cause changes in the metabolic functions of the intestinal microbial community. Cadmium has a significant effect on basic metabolism and nutrient distribution, as well as on energy metabolism and antioxidant functions of the spider. These may be mechanisms to combat heavy metal toxicity (Yang et al., 2018). It will be important to test it experimentally in the future.

Heavy metal toxicity is escapable stress factor under certain ecological and behavioural conditions. Not every stress can be escaped, like the factors that cause or contribute significantly to global warming[9]. These experiments used 2 categories of carbon dioxide concentrations on the small linyphiid spider *Hylyphantes graminicola* and its symbiont *Wolbachia*. The "low" concentration of 400ppm is similar amount of carbon dioxide as it was recorded in the atmosphere in 2015. The "more stressful" concentration was double dosage of low one. It was revealed that increased carbon dioxide as well as *Wolbachia* infection significantly affect metabolic processes, signalling or catalytic activity in linyphiid spiders (Su et al., 2020). From the results it is likely that *H. graminicola* better resists the stress conditions when infected with *Wolbachia*, which increases enzyme and nutrient activity. This was reflected in the size of the carapace, faster development, levels of protein, amino acid content, and the activity of peroxidase and amylase (Su et al., 2019, 2020). These studies are pioneering, studying the impact of endosymbionts on hosts contextually.

---

[9] Such a factor is the growing concentration of carbon dioxide, which has increased by almost half since the industrial revolution in 1700s (US Department of Commerce n.d., 2022).

4.6   Predation behaviour

Predatory activity is an essential mechanism of spiders providing ecosystem services. The spiders have developed various hunting strategies to inhabit different niches. Hunting using silken webs is a hallmark of the family *Araneidae*, while active foraging is inherent in families such as *Lycosidae*, *Salticidae* or *Philodromidae* (Foelix, 2010).

In a study by Hu et al. (2019) spiders using different hunting strategies have been found to differ significantly in intestinal microbial community, but no specific strain has been found to correlate with these strategies. It should be noted that the authors analysed endosymbionts in only 3 spider species specialized in two strategies: web-builders and active hunters. For comparison, there was no resultant difference in the gut microbiota of the host spiders in the myrmecophagous spider *Campanicola campanulata* [Chen, 1993] (Theridiidae), which was artificially fed on two ant species that were significantly different in both microbiome and nutritional composition (Zhang et al., 2021). Studies in this area of research on the impact of endosymbionts on behaviour are scarce. It is possible that bacteria influence other areas of predation behaviour such as capacity, overkilling or indirectly through the impact on web design.

4.7   Anti-predation behaviour

There is an import trade-off to have an effective hunting strategy, but not to increase the chances of being eaten by another predator, for every predator in the middle of the food chain. Coloration may be effective way how to do that, although there are diversified adaptations from morphological, physiological to behavioural modifications. Bright colour can attract prey on one side, and being aposematic, thus protect from predation on the other side.

The striped coloration of Wasp orb-weaver spider *Argiope bruennichi* composed of yellow carotenoids could work in both directions. Both attracting prey and creating aposematic colouring against predators (Bush et al., 2008; Hsiung et al., 2019). However, the question remains how spiders form these difficult-to-synthetize substances. For example, aphids have been shown to form carotenoids, but this ability has been taken over by lateral gene transfer from fungi (Moran and Jarvik, 2010).

It is well known that carotenoids are an important part of sexual selection because most animals cannot synthesize them *de novo* and depone them to its tissues from the prey. This is a sign to females that a colour-above-average male can efficiently search for abundant food sources (von Lintig et al., 2005). The colour of the predator probably depends on the amount of prey consumed. Individuals of wasp spider species have very low variability in pattern and yellow shade. Here, it can be hypothetically endosymbiotic bacteria which may produce carotenoids as protection for itself against oxidative stress in metabolically active host's tissues. Such relationship was discovered by Daniel Sloan and Nancy

Moran (2012) who found out, that obligate endosymbiotic bacteria *Portiera* can biosynthesise carotenoid to its host – silverleaf whitefly *Bemisia tabaci* (*Hemiptera: Aleyrodidae*). This bacterium has homologous genes to those responsible for synthesis of carothenoids in aphids and originates in fungi. There was discovered unique microbial community with novel dominant bacterial symbiont in the wasp spider (Sheffer et al., 2020). This symbiont could thus, for example, offer new undiscovered ways of host-endosymbiont interactions.

5   Obstacles and problems of the topic

Despite the unusually interesting topic, the endosymbiotic-manipulation biology is very broad to review properly. Within the spider group alone, it has been studied on variety model organisms that differ in their evolutionary history and ecological requirements (e.g., *Hylyphantes graminicola, Argiope bruenichi, Erigone atra, Stegodyphus dumicola, etc.*). Furthermore, different hypothesis usually requires different methods. These results are therefore hardly comparable.

For example, studies from the second half of the last century using the Standard PCR method to detect endosymbiotic strains standardly produced an excess of false negative results. This was probably reflected in the (Werren et al., 1995) study, in which authors investigated the presence of two *Wolbachia* supergroups A and B in neotropics invertebrates, including spiders, using the Standard PCR method with *Wolbachia*-specific primers for the ftsZ gene. He confirmed the infection in only 16.9% of insect species (26 out of 154) and did not confirm it in any species of spiders from the families *Salticidae* or *Araneidae*, which are nowadays well-known *Wolbachia*-hosts (Goodacre et al., 2006; Oh et al., 2000).

The use of the Long PCR method, in contrast to the Standard PCR, has been shown to be approximately 6 orders of magnitude more sensitive when amplifying plasmid DNA into insect genomic DNA. Using this method, up to 76% of the 62 insect species tested positive for *Wolbachia* infection (Jeyaprakash and Hoy, 2000).

Furthermore, studies that focused on microbial diversity of spiders noted differences between bacterial 16S rRNA regions used. Using longer Sanger sequencing method which includes the V2 and V3 regions in addition to the V4 region, 2 new *Rickettsia* strains were detected. Based on the V4 region alone, three from four bacterial strains were not distinguishable (White et al., 2020b). It is therefore very difficult to form conclusions about the overall microbial diversity in spiders.

6   Conclusion

This thesis aimed to summarize the current knowledge on the impact of the endosymbiotic bacteria on spiders in the context of providing ecosystem services. Primarily scientific studies from journals listed on the Web of Science were included. Most of the studies focused on the interaction of facultative reproductive manipulators such as *Wolbachia, Cardium, Rickettsia* or *Spiroplasma* with the host. Only in recent years the influence of these manipulators on the previously observed female-biased sex ratio distortion and cytoplasmic incompatibility has been experimentally confirmed. In addition to affecting reproduction and after-mating behaviour, endosymbionts influence on the predatory and potentially anti-predatory behaviour, long-distance dispersal, and resistance to different stress variants. Based on this review, several behavioural aspects seem to be significantly subject to manipulation by endosymbionts. However, there remain many open questions and unresolved issues that need to be addressed to form a holistic view of the relationship between symbionts and spiders.

Fig 9: A scheme visualizing the reviewed areas of this thesis and ideas for future studies – in colour boxes.
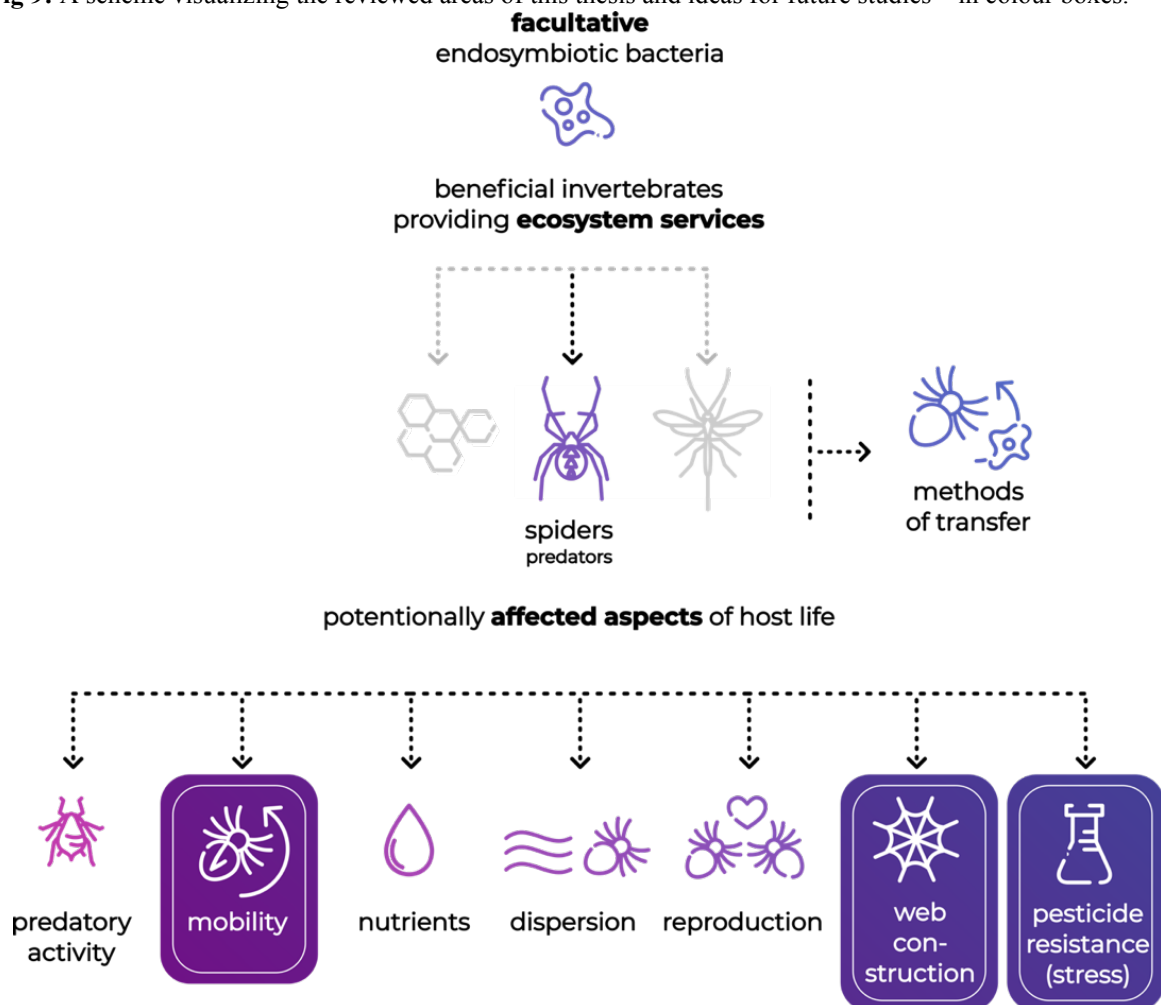
In the future, I would like to focus on the influence of facultative endosymbiotic bacteria on 1) mobility and locomotion of spiders, 2) web construction in orb-weavers from the family *Araneidae*, and

3) potential resistance to pesticide stress (Fig. 9). Pilot studies suggest that the presence of some endo-symbiotic strains such as *Rickettsia* could modify the susceptibility of spiders to pesticides commonly used in agroecosystems (Goodacre, 2011; unpublished data). Such findings would shed new light on the role of spiders in the provision of ecosystem services.

7   Abbrevitations

Abbrevitation	Explanation
CI	cytoplasmatic incompatibility
DCC	dosage compensation complex
FS	facultative symbiont(s)
HT	horizontal transfer
MŘ	Milan Řezáč (supervisor)
NG	Nela Gloríková
OS	obligate symbiont(s)
SD	sex determination
SRD	sex ratio distortion
VB	vitelline body
VG	vitellogenin
VT	vertical transfer

Tab. 1: Abbrevitations from the main text

Abbrevitation	Explanation
FMu	facultative mutualistic symbiont
FKom	facultative comensalistic symbiont
FReMa	facultative reproductive manipulator
BaOS	bacteriocyte-associated obligate symbiont(s)

Tab. 2: Abbrevitations from the supplementary material

Supplementary material

Phylum	Class	Order	Bacteria species	Strategy	Host species	Tissue	Behaviour modification	citation
Pseudomonadota	*Gamma-proteobacteria*	*Enterobacterales*	*Hamiltonella defensa* (Moran et al., 2005) Similar and *Serratia symbiotica*	FMu	Sporadically in whiteflies (*Bemisia tabac*), aphids (*Acyrthosiphon pisum*), psyllids	Intracellular and extracellular	Protection against **parasitoid wasps** in aphids by blocking their larval stages (*Aphidius ervi/eadyi*)	Moran et al. (2005); Degnan et al. (2009)
Pseudomonadota	*Gamma-proteobacteria*	*Enterobacterales*	*Regiella insecticola* (Moran et al., 2005)	FMu	Aphids (*Acyrthosiphon pisum, Myzus persicae*)	Intracellular and extracellular	Protection against **fungal pathogens,** certain strain protects against parasitoid *Aphidius colemani*	Moran et al. (2005); Vorburger et al. (2009)
Pseudomonadota	*Gamma-proteobacteria*	*Enterobacterales*	*Sodalis glossinidius* (Dale and Maudlin 1999)	FMu/FKom	Tsetse fly	Occasionally in midgut or haemolyph, salivary and milk glands, also in bacteriome with OS	Probably increasing **susceptibility to infection** by *Trypanosoma brucei*	Toh et al. (2006); Aksoy et al. (1997)
Bacteroidota	*Sphingobacteriia*	*Sphingobacteriales*	*Candidatus Cardinium hertigii* (Zchori-Fein et al. 2004)	FReMa	Arthropods (insects, arachnids), nematodes	Various tissues, intracellular (within cytoplasm of host cell)	**Manipulation:** female-biased sex-ratio distortion, cytoplasmatic incompatibility	Doremus et al. (2020); Gotoh et al. (2007)
Mycoplasmatota	*Mollicutes*	*Mycoplasmatales*	*Spiroplasma* sp.	FReMa	Many arthropods including *Hemiptera, Hymenoptera, Lepidoptera*; some strains are prevalent in plants	Various tissues, gut, haemolymph	Reproductive manipulators, plant-disease agents, protection against parasitic nematodes and wasps (*S. poulsonii* and *Drosophila*), male-killing	Veneti et al. (2005); Jaenike (2007); Yokomi et al. (2008)

Phylum	Class	Order	Bacteria species	Strategy	Host species	Tissue	Behaviour modification	citation
Pseudomonadota	*Alpha-proteobacteria*	*Rickettsiales*	Wolbachia sp.	FReMa, FMu	Various insects and arachnids and some nematodes	Various tissues, intracellular (within cytoplasm of host cell)	**Manipulation:** Male killing, feminization, parthenogenesis, cytoplasmatic incompatibility, **Advantages:** RNA-virus resistance, insecticide resistance, iron metabolism	Bagheri et al. (2019); Berticat et al. (2002); Fialho and Stevens (2000); Teixeira et al. (2008)
Pseudomonadota	*Gamma-proteobacteria*	*Enterobacterales*	*Buchnera aphidicola* (Munson et al. 1991)	BaOS	Aphids, studied in pea aphid *Acyrthosiphon pisum*	Bilobed bacteriome with 60-80 bacteriocytes, haemolymph during the transfer to egg cell	Synthesis of **tryptophan**, phenylalanine, vitamin **riboflavin (B2)**	Nakabachi and Ishikawa (1999); reviewed in Douglas (1998)
Pseudomonadota	*Gamma-proteobacteria*	*Enterobacterales*	Wigglesworthia glossinidia (Aksoy, 1995)	BaOS	Tsetse fly	Bacteriome in anterior midgut	Synthesis of **B-complex vitamins**	Akman et al. (2002); Aksoy et al. (1997)
Pseudomonadota	*Gamma-proteobacteria*	*Enterobacterales*	*Blochmannia* sp.	BaOS	Carpenter ant	Ovaries and midgut	Synthesis of essential and non-essential amino acids like tyrosine, helping hosts processing nitrogen, improving of the **overall health of the colony**	*B. floridanus:* Zientz et al. (2006) Feldhaar et al. (2007); Sauer et al. (2002)
Pseudomonadota	*Gamma-proteobacteria*	*Enterobacterales*	Candidatus *Baumannia cicadellinicola* (Moran et al., 2003)	BaOS	Leafhoppers (*Cicadellinae*), *Homalodisca coagulata*	Red-pigmented bacteriomes	Biosynthesis of **cofactors and vitamins**	Moran et al. (2003); Cottret et al. (2010)

Tab. 3: Short summary of the influence of the endosymbiotic bacteria (obligate, facultative manipulator and facultative mutualist) on the insect host.

Literature

* Secondary sources

Akman, L., Yamashita, A., Watanabe, H., Oshima, K., Shiba, T., Hattori, M., and Aksoy, S. (2002). Genome sequence of the endocellular obligate symbiont of tsetse flies, *Wigglesworthia glossinidia*. Nature Genetics *32*, 402–407. https://doi.org/10.1038/ng986.

Aksoy, S., Chen, X., and Hypsa, V. (1997). Phylogeny and potential transmission routes of midgut-associated endosymbionts of tsetse (*Diptera: Glossinidae*). Insect Molecular Biology *6*, 183–190. https://doi.org/10.1111/j.1365-2583.1997.tb00086.x.

Alicea-Serrano, A.M., Bender, K., and Jurestovsky, D. (2020). Not all spider silks are antimicrobial. The Journal of Arachnology *48*, 84–89. https://doi.org/10.1636/0161-8202-48.1.84.

Andersson, S.G., Zomorodipour, A., Andersson, J.O., Sicheritz-Pontén, T., Alsmark, U.C.M., Podowski, R.M., Näslund, A.K., Eriksson, A.-S., Winkler, H.H., and Kurland, C.G. (1998). The genome sequence of *Rickettsia prowazekii* and the origin of mitochondria. Nature *396*, 133–140. https://doi.org/10.1038/24094.

André, J., and Rouiller, C. (1957). The ultrastructure of the vitelline body in the oocyte of the spider *Tegenaria parietina*. The Journal of Biophysical and Biochemical Cytology *3*, 977. https://doi.org/10.1083/jcb.3.6.977.

Avilés, L. (1986). Sex-ratio bias and possible group selection in the social spider *Anelosimus eximius*. The American Naturalist *128*, 1–12. https://doi.org/10.1086/284535.

Avilés, L., and Maddison, W. (1991). When Is the Sex Ratio Biased in Social Spiders?: Chromosome Studies of Embryos and Male Meiosis in *Anelosimus* Species (*Araneae, Theridiidae*). The Journal of Arachnology *19*, 126–135.

Avilés, L., Mc Cormack, J., Cutter, A., and Bukowski, T. (2000). Precise, highly female–biased sex ratios in a social spider. Proceedings of the Royal Society of London. Series B: Biological Sciences *267*, 1445–1449. https://doi.org/10.1098/rspb.2000.1162.

Babczyńska, A., Sułowicz, S., Talik, E., Hermyt, M., Bednarek, A., Sawadro, M., and Molenda, A. (2019). Sterile capsule–egg cocoon covering constitutes an antibacterial barrier for spider *Parasteatoda tepidariorum* embryos. Physiological and Biochemical Zoology *92*, 115–124. https://doi.org/10.1086/701390.

Bäckhed, F., Ley, R.E., Sonnenburg, J.L., Peterson, D.A., and Gordon, J.I. (2005). Host-Bacterial Mutualism in the Human Intestine. Science *307*, 1915–1920. https://doi.org/10.1126/science.1104816.

* Bachmann, M. (1976). Das Gift der orthognathen Spinne Pterinochilus spec. Isolierung und teilweise biochemische und biologische Charakterisierung eines Neurotoxins und einer Hyaluronidase (Doctoral thesis, University of Basel) in Foelix, R.F. (2010). Biology of Spiders (Third) (New York, Oxford).

Bagheri, Z., Talebi, A.A., Asgari, S., and Mehrabadi, M. (2019). *Wolbachia* induce cytoplasmic incompatibility and affect mate preference in *Habrobracon hebetor* to increase the chance of its transmission to the next generation. Journal of Invertebrate Pathology 163, 1–7. https://doi.org/10.1016/j.jip.2019.02.005.

Baldo, L., Bordenstein, S., Wernegreen, J.J., and Werren, J.H. (2006). Widespread recombination throughout *Wolbachia* genomes. Molecular Biology and Evolution *23*, 437–449. https://doi.org/10.1093/molbev/msj049.

Baldo, L., Ayoub, N.A., Hayashi, C.Y., Russell, J.A., Stahlhut, J.K., and Werren, J.H. (2008). Insight into the routes of *Wolbachia* invasion: high levels of horizontal transfer in the spider genus *Agelenopsis* revealed by *Wolbachia* strain and mitochondrial DNA diversity. Molecular Ecology *17*, 557–569. https://doi.org/10.1111/j.1365-294X.2007.03608.x.

* Bartels, M. (1930). Uber den Fressmechanismus und chemischen Sinn einiger Netzspinnen. Rev. Suisse Zool *37*, 1-41 in Foelix, R.F. (2010). Biology of Spiders (Third) (New York, Oxford).

Baumann, P. (2005). Biology of Bacteriocyte-Associated Endosymbionts of Plant Sap-Sucking Insects. Annual Review of Microbiology *59*, 155–189. https://doi.org/10.1146/annurev.micro.59.030804.121041. **[Review].**

Berticat, C., Rousset, F., Raymond, M., Berthomieu, A., and Weill, M. (2002). High *Wolbachia* density in insecticide-resistant mosquitoes. Proc Biol Sci *269*, 1413–1416. https://doi.org/10.1098/rspb.2002.2022.

Bigliardi, E., Selmi, M.G., Corona, S., Bandi, C.A., and Sacchi, L. (1995). Membrane systems in endocytobiosis III. Ultrastructural features of symbionts and vacuolar membrane in bacteriocytes of the wood-eating cockroach *Cryptocercus punctulatus* (*Dictyoptera, Cryptocercidae*). Bollettino Di Zoologia *62*, 235–238. https://doi.org/10.1080/11250009509356070.

Birkhofer, K., Rusch, A., Andersson, G.K.S., Bommarco, R., Dänhardt, J., Ekbom, B., Jönsson, A., Lindborg, R., Olsson, O., Rader, R., et al. (2018). A framework to identify indicator species for ecosystem services in agricultural landscapes. Ecological Indicators *91*, 278–286. https://doi.org/10.1016/j.ecolind.2018.04.018.

Bright, M., and Bulgheresi, S. (2010). A complex journey: transmission of microbial symbionts. Nature Reviews Microbiology *8*, 218–230. https://doi.org/10.1038/nrmicro2262. **[Review].**

* Buchner, P. (1965). Endosymbiosis of animals with plant microorganisms. (Interscience, New York) in book review by Barta, L.R. (1968). https://doi.org/10.2307/3757184

Busck, M.M., Settepani, V., Bechsgaard, J., Lund, M.B., Bilde, T., and Schramm, A. (2020). Microbiomes and Specific Symbionts of Social Spiders: Compositional Patterns in Host Species, Populations, and Nests. Frontiers in Microbiology *11*. https://doi.org/10.3389/fmicb.2020.01845.

Bush, A.A., Yu, D.W., and Herberstein, M.E. (2008). Function of bright coloration in the wasp spider *Argiope bruennichi* (*Araneae: Araneidae*). Proceedings of the Royal Society B: Biological Sciences *275*, 1337–1342. https://doi.org/10.1098/rspb.2008.0062.

Cardoso, P., and Scharff, N. (2009). First record of the spider family *Symphytognathidae* in Europe and description of *Anapistula ataecina* sp. n. (*Araneae*). Zootaxa *2246*, 45–57.

Ceccarelli, F.S., Haddad, C.R., and Ramírez, M.J. (2016). Endosymbiotic *Rickettsiales* (*Alphaproteobacteria*) from the spider genus *Amaurobioides* (*Araneae: Anyphaenidae*). The Journal of Arachnology *44*, 251–253.

Charlat, S., Hurst, G.D.D., and Merçot, H. (2003). Evolutionary consequences of *Wolbachia* infections. Trends in Genetics *19*, 217–223. https://doi.org/10.1016/S0168-9525(03)00024-6. **[Review].**

Chouvenc, T., Elliott, M.L., Šobotník, J., Efstathion, C.A., and Su, N.-Y. (2018). The termite fecal nest: a framework for the opportunistic acquisition of beneficial soil *Streptomyces* (*Actinomycetales: Streptomycetaceae*). Environmental Entomology *47*, 1431–1439. https://doi.org/10.1093/ee/nvy152.

Cordaux, R., Michel-Salzat, A., and Bouchon, D. (2001). *Wolbachia* infection in crustaceans: novel hosts and potential routes for horizontal transmission. Journal of Evolutionary Biology *14(2)*, 237-243. https://doi.org/10.1046/j.1420-9101.2001.00279.x

Cottret, L., Milreu, P.V., Acuña, V., Marchetti-Spaccamela, A., Stougie, L., Charles, H., and Sagot, M.-F. (2010). Graph-Based Analysis of the Metabolic Exchanges between Two Co-Resident Intracellular Symbionts, Baumannia cicadellinicola and Sulcia muelleri, with Their Insect Host, Homalodisca coagulata. PLOS Computational Biology 6, e1000904. https://doi.org/10.1371/journal.pcbi.1000904.

Cramer, K.L., and Maywright, A.V. (2008). Cold temperature tolerance and distribution of the brown recluse spider *Loxosceles reclusa* (*Araneae, Sicariidae*) in Illinois. Journal of Arachnology 136–139.

Currie, C.R., Poulsen, M., Mendenhall, J., Boomsma, J.J., and Billen, J. (2006). Coevolved crypts and exocrine glands support mutualistic bacteria in fungus-growing ants. Science *311*, 81–83. DOI: 10.1126/science.1119744.

Dale, C., and Moran, N.A. (2006). Molecular Interactions between Bacterial Symbionts and Their Hosts. Cell *126*, 453–465. https://doi.org/10.1016/j.cell.2006.07.014. **[Review].**

Degnan, P.H., Yu, Y., Sisneros, N., Wing, R.A., and Moran, N.A. (2009). *Hamiltonella defensa*, genome evolution of protective bacterial endosymbiont from pathogenic ancestors. Proceedings of the National Academy of Sciences *106*, 9063–9068. https://doi.org/10.1073/pnas.0900194106.

Doremus, M.R., Stouthamer, C.M., Kelly, S.E., Schmitz-Esser, S., and Hunter, M.S. (2020). *Cardinium* localization during its parasitoid wasp host's development provides insights into cytoplasmic incompatibility. Frontiers in Microbiology *11*, 606399. https://doi.org/10.3389/fmicb.2020.606399.

Douglas, A.E. (1998). Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria *Buchnera*. Annual Review of Entomology *43*, 17–37. https://doi.org/10.1146/annurev.ento.43.1.17. **[Review].**

Durkin, E.S., Cassidy, S.T., Gilbert, R., Richardson, E.A., Roth, A.M., Shablin, S., and Keiser, C.N. (2021). Parasites of spiders: Their impacts on host behavior and ecology. The Journal of Arachnology *49*, 281–298. https://doi.org/10.1636/JoA-S-20-087. **[Review].**

Duron, O., Hurst, G.D.D., Hornett, E.A., Josling, J.A., and Engelstaedter, J. (2008). High incidence of the maternally inherited bacterium *Cardinium* in spiders. Molecular Ecology *17*, 1427–1437. https://doi.org/10.1111/j.1365-294X.2008.03689.x.

Edwards, R.L., Edwards, E.H., and Edwards, A.D. (2003). Observations of *Theotima minutissimus* (*Araneae, Ochyroceratidae*), a parthenogenetic spider. The Journal of Arachnology *31*, 274–277. https://doi.org/10.1636/0161-8202(2003)031[0274:OOTMAO]2.0.CO;2

Engel, P., Martinson, V.G., and Moran, N.A. (2012). Functional diversity within the simple gut microbiota of the honey bee. Proceedings of the National Academy of Sciences *109*, 11002–11007. https://doi.org/10.1073/pnas.1202970109.

Evans, S.C., Shaw, E.M., and Rypstra, A.L. (2010). Exposure to a glyphosate-based herbicide affects agrobiont predatory arthropod behaviour and long-term survival. Ecotoxicology *19*, 1249–1257. https://doi.org/10.1007/s10646-010-0509-9.

Feldhaar, H., Straka, J., Krischke, M., Berthold, K., Stoll, S., Mueller, M.J., and Gross, R. (2007). Nutritional upgrading for omnivorous carpenter ants by the endosymbiont *Blochmannia*. BMC Biology *5*, 48. https://doi.org/10.1186/1741-7007-5-48.

Fialho, R.F., and Stevens, L. (2000). Male-killing *Wolbachia* in a flour beetle. Proceedings of the Royal Society of London. Series B: Biological Sciences *267*, 1469–1473. https://doi.org/10.1098/rspb.2000.1166.

* Fisher, R.A. (1958). The genetical theory of natural selection (Рипол Классик).

Foelix, R.F. (2010). Biology of Spiders (Third) (New York, Oxford).

Fukatsu, T., and Nikoh, N. (1998). Two Intracellular Symbiotic Bacteria from the Mulberry Psyllid *Anomoneura mori* (*Insecta, Homoptera*). Applied and Environmental Microbiology *64*, 3599–3606. https://doi.org/10.1128/AEM.64.10.3599-3606.1998.

Gilbert, L.I., Iatrou, K., and Gill, S.S. (2005). Comprehensive molecular insect science (Elsevier).

Gloríková, N., and Řezáč, M. (2022). Lethal concentrations of glyphosate-based herbicide on nymphs of agroecosystem spider predator *Phylloneta impressa* L. Koch 1881. Chilean Journal of Agricultural Research *82*. [Accepted].

Goodacre, S.L. (2011). Endosymbiont Infections in Spiders. In Advances in Insect Physiology, Vol 40: Spider Physiology and Behaviour - Physiology, J. Casas, ed. pp. 137–153. https://doi.org/10.1016/B978-0-12-387668-3.00003-9. **[Review].**

Goodacre, S.L., Martin, O.Y., Thomas, C.F.G., and Hewitt, G.M. (2006). *Wolbachia* and other endosymbiont infections in spiders. Molecular Ecology *15*, 517–527. https://doi.org/10.1111/j.1365-294X.2005.02802.x.

Goodacre, S.L., Martin, O.Y., Bonte, D., Hutchings, L., Woolley, C., Ibrahim, K., George Thomas, C.F., and Hewitt, G.M. (2009). Microbial modification of host long-distance dispersal capacity. Bmc Biology *7*, 1–8. https://doi.org/10.1186/1741-7007-7-32.

Gotoh, T., Noda, H., and Ito, S. (2007). *Cardinium* symbionts cause cytoplasmic incompatibility in spider mites. Heredity *98*, 13–20. https://doi.org/10.1038/sj.hdy.6800881.

Gunnarsson, B., Uhl, G., and Wallin, K. (2004). Variable Female Mating Positions and Offspring Sex Ratio in the Spider *Pityohyphantes phrygianus* (*Araneae: Linyphiidae*). Journal of Insect Behavior *17*, 129–144. https://doi.org/10.1023/B:JOIR.0000025137.59613.71.

Gunnarsson, B., Goodacre, S.L., and Hewitt, G.M. (2009). Sex ratio, mating behaviour and *Wolbachia* infections in a sheetweb spider. Biological Journal of the Linnean Society *98*, 181–186. https://doi.org/10.1111/j.1095-8312.2009.01247.x.

Guo, Y., Hoffmann, A.A., Xu, X.-Q., Mo, P.-W., Huang, H.-J., Gong, J.-T., Ju, J.-F., and Hong, X.-Y. (2018). Vertical transmission of *Wolbachia* is associated with host vitellogenin in *Laodelphax striatellus*. Frontiers in Microbiology *9*. https://doi.org/10.3389/fmicb.2018.02016.

Hagimori, T., Abe, Y., and Miura, K. (2006). The first finding of a *Rickettsia* bacterium associated with parthenogenesis induction among insects. Current Microbiology *52*, 97–101. https://doi.org/10.1007/s00284-005-0092-0.

Harwood, J.D., Sunderland, K.D., and Symondson, W.O. (2004). Prey selection by linyphiid spiders: molecular tracking of the effects of alternative prey on rates of aphid consumption in the field. Molecular Ecology *13*, 3549–3560. https://doi.org/10.1111/j.1365-294X.2004.02331.x

Harwood, J.D., Sunderland, K.D., and Symondson, W.O.C. (2005). Monoclonal antibodies reveal the potential of the tetragnathid spider *Pachygnatha degeeri* (*Araneae: Tetragnathidae*) as an aphid predator. Bulletin of Entomological Research *95*, 161–167. https://doi.org/10.1079/BER2004346.

Hongoh, Y., Sharma, V.K., Prakash, T., Noda, S., Toh, H., Taylor, T.D., Kudo, T., Sakaki, Y., Toyoda, A., Hattori, M., et al. (2008). Genome of an endosymbiont coupling N2 fixation to cellulolysis within protist cells in termite gut. Science *322*, 1108–1109. https://doi.org/10.1126/science.1165578.

Hosokawa, T., Ishii, Y., Nikoh, N., Fujie, M., Satoh, N., and Fukatsu, T. (2016). Obligate bacterial mutualists evolving from environmental bacteria in natural insect populations. Nature Microbiology *1*, 15011. https://doi.org/10.1038/nmicrobiol.2015.11.

Hsiung, B.-K., Shawkey, M.D., and Blackledge, T.A. (2019). Color production mechanisms in spiders. The Journal of Arachnology *47*, 165–180. https://doi.org/10.1636/JoA-S-18-022. **[Review].**

Hu, G., Zhang, L., Yun, Y., and Peng, Y. (2019). Taking insight into the gut microbiota of three spider species: No characteristic symbiont was found corresponding to the special feeding style of spiders. Ecology and Evolution *9*, 8146–8156. https://doi.org/10.1002/ece3.5382.

Hurst, G.D., and Jiggins, F.M. (2000). Male-killing bacteria in insects: mechanisms, incidence, and implications. Emerging Infectious Diseases *6*, 329–336. doi: 10.3201/eid0604.000402. **[Review].**

Hurst, L.D., and Vollrath, F. (1992). Sex-ratio adjustment in solitary and social spiders. Trends in Ecology & Evolution *7*, 326–327. https://doi.org/10.1016/0169-5347(92)90122-R. **[Review].**

Husnik, F., and Keeling, P.J. (2019). The fate of obligate endosymbionts: reduction, integration, or extinction. Current Opinion in Genetics & Development *58–59*, 1–8. https://doi.org/10.1016/j.gde.2019.07.014. **[Review].**

Jaenike, J. (2007). Spontaneous emergence of a new *Wolbachia* phenotype. Evolution *61*, 2244–2252. https://doi.org/10.1111/j.1558-5646.2007.00180.x.

Jeyaprakash, A., and Hoy, M.A. (2000). Long PCR improves *Wolbachia* DNA amplification: wsp sequences found in 76% of sixty-three arthropod species. Insect Molecular Biology *9*, 393–405. https://doi.org/10.1046/j.1365-2583.2000.00203.x.

Kennedy, S.R., Tsau, S., Gillespie, R., and Krehenwinkel, H. (2020). Are you what you eat? A highly transient and prey-influenced gut microbiome in the grey house spider *Badumna longinqua*. Molecular Ecology *29*, 1001–1015. https://doi.org/10.1111/mec.15370.

Kitajima, E. W., Groot, T. V., Novelli, V. M., Freitas-Astúa, J., Alberti, G. and de Moraes, G. J. (2007). In situ observation of the *Cardinium* symbionts of *Brevipalpus* (*Acari: Tenuipalpidae*) by electron microscopy. Experimental and Applied Acarology *42*, 263-271. https://doi.org/10.1007/s10493-007-9090-1.

Koch, H., and Schmid-Hempel, P. (2011). Socially transmitted gut microbiota protect bumble bees against an intestinal parasite. Proceedings of the National Academy of Sciences *108*, 19288–19292. https://doi.org/10.1073/pnas.1110474108.

Koga, R., Meng, X.-Y., Tsuchida, T., and Fukatsu, T. (2012). Cellular mechanism for selective vertical transmission of an obligate insect symbiont at the bacteriocyte–embryo interface. Proceedings of the National Academy of Sciences *109*, E1230–E1237. https://doi.org/10.1073/pnas.1119212109.

Korenko, S., Šmerda, J., and Pekár, S. (2009). Life-history of the parthenogenetic oonopid spider, *Triaeris stenaspis* (*Araneae: Oonopidae*). European Journal of Entomology *106*, 217–223. https://doi.org/10.14411/eje.2009.028.

Kremer, N., Charif, D., Henri, H., Bataille, M., Prevost, G., Kraaijeveld, K., and Vavre, F. (2009). A new case of *Wolbachia* dependence in the genus *Asobara*: evidence for parthenogenesis induction in *Asobara japonica*. Heredity *103*, 248–256. https://doi.org/10.1038/hdy.2009.63.

Kuhn-Nentwig, L. (2003). Antimicrobial and cytolytic peptides of venomous arthropods. Cellular and Molecular Life Sciences CMLS *60*, 2651–2668. https://doi.org/10.1007/s00018-003-3106-8.

Kůrka, A., Řezáč, M., Macek, R., and Dolanskỳ, J. (2015). Pavouci České republiky (Academia).

Lacava, M., García, L.F., Viera, C., and Michalko, R. (2021). The pest-specific effects of glyphosate on functional response of a wolf spider. Chemosphere *262*, 127785. https://doi.org/10.1016/j.chemosphere.2020.127785.

Li, C., He, M., Yun, Y., and Peng, Y. (2020). Co-infection with *Wolbachia* and *Cardinium* may promote the synthesis of fat and free amino acids in a small spider, *Hylyphantes graminicola*. Journal of Invertebrate Pathology *169*, 107307. https://doi.org/10.1016/j.jip.2019.107307.

von Lintig, J., Hessel, S., Isken, A., Kiefer, C., Lampert, J.M., Voolstra, O., and Vogt, K. (2005). Towards a better understanding of carotenoid metabolism in animals. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease *1740*, 122–131. https://doi.org/10.1016/j.bbadis.2004.11.010.

Mammola, S., and Isaia, M. (2017). Spiders in caves. Proceedings of the Royal Society B: Biological Sciences *284*, 20170193. https://doi.org/10.1098/rspb.2017.0193. **[Review].**

Masta, S.E., and Maddison, W.P. (2002). Sexual selection driving diversification in jumping spiders. Proceedings of the National Academy of Sciences *99*, 4442–4447. https://doi.org/10.1073/pnas.072493099.

Mestre, L., Piñol, J., Barrientos, J.A., Espadaler, X., Brewitt, K., Werner, C., and Platner, C. (2013). Trophic structure of the spider community of a Mediterranean citrus grove: a stable isotope analysis. Basic and Applied Ecology *14*, 413–422. https://doi.org/10.1016/j.baae.2013.05.001.

Michalko, R., Pekár, S., and Entling, M.H. (2019). An updated perspective on spiders as generalist predators in biological control. Oecologia *189*, 21–36. https://doi.org/10.1007/s00442-018-4313-1. **[Review].**

Montllor, C.B., Maxmen, A., and Purcell, A.H. (2002). Facultative bacterial endosymbionts benefit pea aphids *Acyrthosiphon pisum* under heat stress. Ecological Entomology *27*, 189–195. https://doi.org/10.1046/j.1365-2311.2002.00393.x.

Moran, N.A., and Jarvik, T. (2010). Lateral transfer of genes from fungi underlies carotenoid production in aphids. Science *328*, 624–627. doi: 10.1126/science.1187113.

Moran, N.A., Dale, C., Dunbar, H., Smith, W.A., and Ochman, H. (2003). Intracellular symbionts of sharpshooters (*Insecta: Hemiptera: Cicadellinae*) form a distinct clade with a small genome. Environmental Microbiology *5*, 116–126. https://doi.org/10.1046/j.1462-2920.2003.00391.x.

Moran, N.A., Russell, J.A., Koga, R., and Fukatsu, T. (2005). Evolutionary relationships of three new species of *Enterobacteriaceae* living as symbionts of aphids and other insects. Applied and Environmental Microbiology *71*, 3302–3310. https://doi.org/10.1128/AEM.71.6.3302-3310.2005.

Moran, N.A., McCutcheon, J.P., and Nakabachi, A. (2008). Genomics and Evolution of Heritable Bacterial Symbionts. Annual Review of Genetics *42*, 165–190. https://doi.org/10.1146/annurev.genet.41.110306.130119. **[Review].**

Motta, E.V., Raymann, K., and Moran, N.A. (2018). Glyphosate perturbs the gut microbiota of honey bees. Proceedings of the National Academy of Sciences *115*, 10305–10310. https://doi.org/10.1073/pnas.1803880115.

Murray, R.G.E., and Schleifer, K.H. (1994). Taxonomic notes: a proposal for recording the properties of putative taxa of procaryotes. International Journal of Systematic and Evolutionary Microbiology *44*, 174–176.

Nakabachi, A., and Ishikawa, H. (1999). Provision of riboflavin to the host aphid, *Acyrthosiphon pisum,* by endosymbiotic bacteria, *Buchnera*. Journal of Insect Physiology *45*, 1–6. https://doi.org/10.1016/S0022-1910(98)00104-8.

Nazipi, S., Elberg, C.L., Busck, M.M., Lund, M.B., Bilde, T., and Schramm, A. (2021a). The bacterial and fungal nest microbiomes in populations of the social spider *Stegodyphus dumicola*. Systematic and Applied Microbiology *44*, 126222. https://doi.org/10.1016/j.syapm.2021.126222.

Nazipi, S., Vangkilde-Pedersen, S.G., Busck, M.M., Lund, D.K., Marshall, I.P.G., Bilde, T., Lund, M.B., and Schramm, A. (2021b). An antimicrobial *Staphylococcus sciuri* with broad temperature and salt spectrum isolated fromthe surface of the African social spider, *Stegodyphus dumicola*. Antonie van Leeuwenhoek *114*, 325–335. https://doi.org/10.1007/s10482-021-01526-6.

Nyffeler, M. (1999). Prey selection of spiders in the field. Journal of Arachnology 317–324. .

Oh, H.W., Kim, M.I.G., Shin, S.W., Bae, K.S., Ahn, Y.J., and Park, H.Y. (2000). Ultrastructural and molecular identification of a *Wolbachia* endosymbiont in a spider, *Nephila clavata*. Insect Molecular Biology *9*, 539–543. https://doi.org/10.1046/j.1365-2583.2000.00218.x.

Pekár, S. (2012). Spiders (Araneae) in the pesticide world: an ecotoxicological review. Pest Management Science *68*, 1438–1446. https://doi.org/10.1002/ps.3397. **[Review].**

Petcharad, B., Košulič, O., and Michalko, R. (2018). Insecticides alter prey choice of potential biocontrol agent *Philodromus cespitum* (*Araneae, Philodromidae*). Chemosphere *202*, 491–497. https://doi.org/10.1016/j.chemosphere.2018.03.134.

Rendon, D., Whitehouse, M.E., and Taylor, P.W. (2016). Consumptive and non-consumptive effects of wolf spiders on cotton bollworms. Entomologia Experimentalis et Applicata *158*, 170–183. https://doi.org/10.1111/eea.12390.

Řezáč, M., Gloríková, N., Wilder, S.M., and Heneberg, P. (2021). The sublethal effects of neonicotinoids on spiders are independent of their nutritional status. Scientific Reports *11*, 1–11. https://doi.org/10.1038/s41598-021-87935-z.

Riechert, S.E. (1999). The hows and whys of successful pest suppression by spiders: insights from case studies. Journal of Arachnology 387–396. **[Review].**

* Reimers-Fadhlaoui, K. (1995) Untersuchen uber die Struktur und die Bildung des Dotterkerns in Oocyten von Spinnen (Thesis, Ruhr University, Bochum) in Foelix, R.F. (2010). Biology of Spiders (Third) (New York, Oxford).

Rosenwald, L.C., Sitvarin, M.I., and White, J.A. (2020). Endosymbiotic *Rickettsiella* causes cytoplasmic incompatibility in a spider host. Proceedings of the Royal Society B-Biological Sciences *287*, 20201107. https://doi.org/10.1098/rspb.2020.1107.

Rowell, D.M., and Main, B.Y. (1992). Sex ratio in the social spider *Diaea socialis* (*Araneae: Thomisidae*). Journal of Arachnology 200–206.

Ruokolainen, L., Ikonen, S., Makkonen, H., and Hanski, I. (2016). Larval growth rate is associated with the composition of the gut microbiota in the *Glanville fritillary* butterfly. Oecologia *181*, 895–903. https://doi.org/10.1007/s00442-016-3603-8.

Sacchi, L., Nalepa, C.A., Bigliardi, E., Corona, S., Grigolo, A., Laudani, U., and Bandi, C. (1998). Ultrastructural studies of the fat body and bacterial endosymbionts of *Cryptocercus punctulatus* Scudder (*Blattaria: Cryptocercidae*). Symbiosis.

Sanders, D., Vogel, E., and Knop, E. (2015). Individual and species-specific traits explain niche size and functional role in spiders as generalist predators. Journal of Animal Ecology *84*, 134–142. https://doi.org/10.1111/1365-2656.12271.

Sapp, J. (2002). Paul Buchner (1886–1978) and hereditary symbiosis in insects. International Microbiology : The Official Journal of the Spanish Society for Microbiology *5*, 145–150. https://doi.org/10.1007/s10123-002-0079-7.

Sauer, C., Dudaczek, D., Hölldobler, B., and Gross, R. (2002). Tissue Localization of the Endosymbiotic Bacterium "*Candidatus Blochmannia floridanus*" in Adults and Larvae of the Carpenter Ant *Camponotus floridanus*. Appl. Environ. Microbiol. *68*, 4187–4193. https://doi.org/10.1128/AEM.68.9.4187-4193.2002.

Schmidt-Entling, M.H., and Siegenthaler, E. (2009). Herbivore release through cascading risk effects. Biology Letters *5*, 773–776. https://doi.org/10.1098/rsbl.2009.0436.

Settepani, V., Schou, M.F., Greve, M., Grinsted, L., Bechsgaard, J., and Bilde, T. (2017). Evolution of sociality in spiders leads to depleted genomic diversity at both population and species levels. Molecular Ecology *26*, 4197–4210. https://doi.org/10.1111/mec.14196.

Sharma, S. (2014). Investigation of antimicrobial properties of spider silk. University of Akron.

Sheffer, M.M., Uhl, G., Prost, S., Lueders, T., Urich, T., and Bengtsson, M.M. (2020). Tissue- and Population-Level Microbiome Analysis of the Wasp Spider *Argiope bruennichi* Identified a Novel Dominant Bacterial Symbiont. Microorganisms *8*, 8. https://doi.org/10.3390/microorganisms8010008.

Shimojana, M., and Nishihira, M. (2000). A New Cave-Dwelling Eyeless Spider of the Genus *Coelotes* (*Araneae: Amaurobiidae*) from Okinawa Island, the Ryukyu Islands, Japan, with Notes on Possible Parthenogenesis. Acta Arachnologica *49*, 29–40. https://doi.org/10.2476/asjaa.49.29.

Sinkins, S.P. (2004). *Wolbachia* and cytoplasmic incompatibility in mosquitoes. Insect Biochemistry and Molecular Biology *34*, 723–729. https://doi.org/10.1016/j.ibmb.2004.03.025. **[Review].**

Sotelo, J.R., and Trujillo-Cenóz, O. (1957). Electron microscope study of the vitelline body of some spider oocytes. The Journal of Biophysical and Biochemical Cytology *3*, 301–310. https://doi.org/10.1083/jcb.3.2.301.

* Stouthamer, R. (1997). *Wolbachia*-induced parthenogenesis. Inherited Microb-Organisms and Arthropod Reproduction, Influential Passengers 102–124.

Su, Q., Wang, X., Ilyas, N., Zhang, F., Yun, Y., Jian, C., and Peng, Y. (2019). Combined effects of elevated CO2 concentration and *Wolbachia* on *Hylyphantes graminicola* (*Araneae: Linyphiidae*). Ecology and Evolution *9*, 7112–7121. https://doi.org/10.1002/ece3.5276.

Su, Q.-C., Wang, X., Deng, C., Yun, Y.-L., Zhao, Y., and Peng, Y. (2020). Transcriptome responses to elevated CO2 level and *Wolbachia*-infection stress in *Hylyphantes graminicola* (*Araneae: Linyphiidae*). Insect Science *27*, 908–920. https://doi.org/10.1111/1744-7917.12701.

* Szathmáry, E., and Smith, J.M. (1995). The major transitions in evolution. (Oxford, UK: WH Freeman Spektrum) in Werren, J.H., and O'Neill, S.L. (1997). The evolution of heritable symbionts. Influential Passengers: Inherited Microorganisms and Arthropod Reproduction 1–41. **[Review].**

Takano, S., Tuda, M., Takasu, K., Furuya, N., Imamura, Y., Kim, S., Tashiro, K., Iiyama, K., Tavares, M., and Amaral, A.C. (2017). Unique clade of alphaproteobacterial endosymbionts induces complete cytoplasmic incompatibility in the coconut beetle. Proceedings of the National Academy of Sciences *114*, 6110–6115. https://doi.org/10.1073/pnas.1618094114.

Taylor, M.J., Bordenstein, S.R., and Slatko, B. (2018). Microbe Profile: *Wolbachia*: a sex selector, a viral protector and a target to treat filarial nematodes. Microbiology (Reading) *164*, 1345–1347. https://doi.org/10.1099/mic.0.000724. **[Review].**

Teixeira, L., Ferreira, Á., and Ashburner, M. (2008). The bacterial symbiont *wolbachia* induces resistance to rna viral infections in *drosophila melanogaster*. PLoS Biology *6*, e1000002. https://doi.org/10.1371/journal.pbio.1000002.

Toh, H., Weiss, B.L., Perkin, S.A.H., Yamashita, A., Oshima, K., Hattori, M., and Aksoy, S. (2006). Massive genome erosion and functional adaptations provide insights into the symbiotic lifestyle of *Sodalis glossinidius* in the tsetse host. Genome Research *16*, 149–156. https://doi.org/10.1101/gr.4106106.

Tyagi, K., Tyagi, I., and Kumar, V. (2021). Insights into the gut bacterial communities of spider from wild with no evidence of phylosymbiosis. Saudi Journal of Biological Sciences *28*, 5913–5924. https://doi.org/10.1016/j.sjbs.2021.06.059.

US Department of Commerce, N. Global Monitoring Laboratory - Carbon Cycle Greenhouse Gases.

Vanthournout, B., Swaegers, J., and Hendrickx, F. (2011). Spiders do not escape reproductive manipulations by *Wolbachia*. BMC Evolutionary Biology *11*, 15. https://doi.org/10.1186/1471-2148-11-15.

Veneti, Z., Bentley, J.K., Koana, T., Braig, H.R., and Hurst, G.D.D. (2005). A Functional Dosage Compensation Complex Required for Male Killing in Drosophila. Science *307*, 1461–1463. https://doi.org/10.1126/science.1107182.

* Vollrath, F. (1992). Spider webs and silk. Scientific American *266*, 70-76 in Foelix, R.F. (2010). Biology of Spiders (Third) (New York, Oxford).

Vorburger, C., Sandrock, C., Gouskov, A., Castañeda, L.E., and Ferrari, J. (2009). Genotypic variation and the role of defensive endosymbionts in an all-parthenogenetic host–parasitoid interaction. Evolution *63*, 1439–1450. https://doi.org/10.1111/j.1558-5646.2009.00660.x.

Weeks, A.R., and 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. https://doi.org/10.1098/rspb.2001.1797.

Werren, J.H., Windsor, D., and Guo, L.R. (1995). Distribution of *Wolbachia* among neotropical arthropods. Proceedings of the Royal Society of London. Series B: Biological Sciences *262*, 197–204. https://doi.org/10.1098/rspb.1995.0196.

Weyman, G.S., Sunderland, K.D., and Jepson, P.C. (2002). A review of the evolution and mechanisms of ballooning by spiders inhabiting arable farmland. Ethology Ecology & Evolution *14*, 307–326. https://doi.org/10.1080/08927014.2002.9522733. **[Review].**

White, J.A., Styer, A., Rosenwald, L.C., Curry, M.M., Welch, K.D., Athey, K.J., and Chapman, E.G. (2020). Endosymbiotic Bacteria Are Prevalent and Diverse in Agricultural Spiders. Microb Ecol *79*, 472–481. https://doi.org/10.1007/s00248-019-01411-w.

Wilson, E.O. (2000). Sociobiology: The new synthesis (Harvard University Press).

Wilson, A.C., Ashton, P.D., Calevro, F., Charles, H., Colella, S., Febvay, G., Jander, G., Kushlan, P.F., Macdonald, S.J., and Schwartz, J.F. (2010). Genomic insight into the amino acid relations of the pea aphid, *Acyrthosiphon pisum*, with its symbiotic bacterium *Buchnera aphidicola*. Insect Molecular Biology *19*, 249–258. https://doi.org/10.1111/j.1365-2583.2009.00942.x.

World Spider Catalog (2021). World Spider Catalog (Natural History Museum Bern).

Wright, S., and Goodacre, S.L. (2012). Evidence for antimicrobial activity associated with common house spider silk. BMC Research Notes *5*, 1–6. https://doi.org/10.1186/1756-0500-5-326.

Wu, M., Sun, L.V., Vamathevan, J., Riegler, M., Deboy, R., Brownlie, J.C., McGraw, E.A., Martin, W., Esser, C., and Ahmadinejad, N. (2004). Phylogenomics of the reproductive parasite *Wolbachia pipientis* wMel: a streamlined genome overrun by mobile genetic elements. PLoS Biology *2*, e69. https://doi.org/10.1371/journal.pbio.0020069.

Yang, H., Wang, J., Lv, Z., Tian, J., Peng, Y., Peng, X., Xu, X., Song, Q., Lv, B., Chen, Z., et al. (2018). Metatranscriptome analysis of the intestinal microorganisms in *Pardosa pseudoannulata* in response to cadmium stress. Ecotoxicology and Environmental Safety *159*, 1–9. https://doi.org/10.1016/j.ecoenv.2018.04.053.

Yen, J.H., and Barr, A.R. (1971). New hypothesis of the cause of cytoplasmic incompatibility in Culex pipiens L. Nature *232*, 657–658. https://doi.org/10.1038/232657a0.

Yokomi, R.K., Mello, A.F.S., Saponari, M., and Fletcher, J. (2008). polymerase chain reaction-based detection of *Spiroplasma citri* associated with citrus stubborn disease. Plant Disease *92*, 253–260. https://doi.org/10.1094/PDIS-92-2-0253.

Zchori-Fein, E., and Perlman, S.J. (2004). Distribution of the bacterial symbiont *Cardinium* in arthropods. Molecular Ecology *13*, 2009–2016. https://doi.org/10.1111/j.1365-294X.2004.02203.x.

Zchori-Fein, E., Gottlieb, Y., Kelly, S.E., Brown, J.K., Wilson, J.M., Karr, T.L., and Hunter, M.S. (2001). A newly discovered bacterium associated with parthenogenesis and a change in host selection behavior in parasitoid wasps. Proceedings of the National Academy of Sciences *98*, 12555–12560. https://doi.org/10.1073/pnas.221467498.

Zhang, L., Yun, Y., Hu, G., and Peng, Y. (2018). Insights into the bacterial symbiont diversity in spiders. Ecology and Evolution *8*, 4899–4906. https://doi.org/10.1002/ece3.4051.

Zhang, W., Liu, F., Zhu, Y., Han, R., Xu, L., and Liu, J. (2021). Differing dietary nutrients and diet-associated bacteria has limited impact on spider gut microbiota composition. Microorganisms *9*, 2358. https://doi.org/10.3390/microorganisms9112358.

Zientz, E., Beyaert, I., Gross, R., and Feldhaar, H. (2006). Relevance of the Endosymbiosis of *Blochmannia floridanus* and Carpenter Ants at Different Stages of the Life Cycle of the Host. Applied Environmental Microbiology *72*, 6027–6033. https://doi.org/10.1128/AEM.00933-06.