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**Harper Adams
University**

**MINOR REVIEW**

Could bacterial associations determine the success of weevil species?

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Abstract

The weevil superfamily Curculionoidea is the largest insect group and so the largest animal group on earth. This taxon includes species which represent an important threat to many economically important crops and, therefore, pose a risk to agriculture and food security. Insect–bacteria associations have been recognised to provide the insect host with many benefits, such as ensuring the acquisition of essential nutrients or protecting the host from natural enemies. The role of bacteria associations within the weevil superfamily remains nonetheless understudied in comparison with other insect taxa. This review draws together existing knowledge on the influence of bacteria associated with weevils known to be agricultural pest species. The implications of these weevil–bacterial associations in determining pest status and their relevance to targeted pest management interventions are discussed. Specific consideration is given to the role of bacteria in cuticle formation, flight activity, reproduction manipulation and adaptation to different environments and food sources.

KEYWORDS

Candidatus Nardonella, incompatible insect technique, microbiota, parthenogenesis, RNAi-biocide, *Sitophilus* primary endosymbiont, *Wolbachia*

1 | WEEVILS, AN UNDERSTUDIED BUT EXTENSIVE INSECT TAXON

The nature of the association between insects and bacteria has been a controversial point for decades. In the early 1900s, a few scientists started to hypothesise that the presence of bacteria in insects was not a random event as bacteria seemed to be involved in important insect processes (reviewed by Steinhaus, 1940). However, these studies were limited by the available techniques at that time, mainly microscopy and culturing, to study microorganism morphology and physiology (reviewed by Handelsman, 2004). In the late 1990s, bacterial studies experienced a paradigm shift when Carl Woese determined that the 16S rRNA gene sequence from the small prokaryotic

ribosome subunit could be considered a molecular chronometer which could be used as a taxonomy identifier (Woese, 1987). This new approach enabled the identification of prokaryotes taxonomically from complex samples in a culture-independent manner. At the same time, the techniques employed to sequence nucleotide molecules advanced rapidly. In 2005, technological advances allowed the automation of multiple sequencing reactions in parallel. This improvement created a platform for mass sequencing at an unprecedented time/cost efficiency, which represented the beginning of high-throughput sequencing (reviewed by Heather & Chain, 2016). Technological innovation, together with the use of the 16S rRNA gene to identify prokaryotes, have enabled remarkable progress in studies focused on insect–bacteria associations, although less effort has been invested in

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the weevil taxon. For instance Web of Science contains 1,512 results for the search term “aphid*” and “bacteria*” despite there being only 5,000 aphid species described (Blackman & Eastop, 2000). By contrast, Web of Science returns 335 results for the search term “weevil*” and “bacteria*” even though there are more than 60,000 described weevil species (Alonso-Zarazaga & Lyal, 2002; Web of Science database searched on April 6, 2020, UK).

To date, many studies have demonstrated the potential importance of the insect–bacteria partnerships for host fitness. Some insects with restricted diets rely on bacteria to compensate nutritional deficiencies. For instance, the pea aphid *Acyrtosiphon pisum* (Harris) is provided with essential amino acids and the vitamin riboflavin by its obligate endosymbiotic bacterium *Buchnera aphidicola* (Nakabachi & Ishikawa, 1999; reviewed by Douglas, 2016) and the tsetse fly *Glossina morsitans* (Westwood) is provided with essential vitamins by the obligate endosymbiotic bacterium *Wigglesworthia glossinidia* (Akman et al., 2002; Nogge, 1981). Importantly, certain bacteria have been shown to render their insect hosts less susceptible to predators and pathogens. This has been illustrated for the pea aphid, which is protected from parasitism by the parasitoid wasp *Aphidius ervi* (Haliday) when aphids are infected with the facultative bacterium *Hamiltonella defensa* (Oliver, Moran, & Hunter, 2005; Oliver, Russell, Moran, & Hunter, 2003) and from infection by the entomopathogenic fungus *Pandora neoaphidis* (Remaud & Hennebert) when aphids harbour the facultative bacterium *Regiella insecticola* (Scarborough, Ferrari, & Godfray, 2005). Bacteria can also influence host reproduction as in the case of members from the genus *Wolbachia*, broadly recognised as reproductive parasites, which for instance increase fecundity of the fruit fly *Drosophila simulans* (Sturtevant; Weeks, Turelli, Harcombe, Reynolds, & Hoffmann, 2007; reviewed by Werren, Baldo, & Clark, 2008).

The weevil superfamily Curculionoidea is the largest insect group (Alonso-Zarazaga & Lyal, 2002) and harbours agricultural pest species that are distributed around the world. For instance, the red palm weevil *Rhynchophorus ferrugineus* (Olivier) is an important pest of palm trees that causes considerable economic losses to crops grown in countries in the Gulf, Middle East and Europe (European Commission, 2011). The sibling weevil species *Sitophilus oryzae* (Linnaeus), the rice weevil, and *Sitophilus zeamais* (Motschulsky), the maize weevil, are important pests of stored grain, rice, maize, barley and wheat globally (Grahame, 2017), while the vine weevil *Otiorhynchus sulcatus* (Fabricius) damages a wide range of horticultural crops around the world (Moorhouse, Charnley, & Gillespie, 1992). Weevil species are, therefore, numerous and problematic for farmers globally. Studies focused on understanding weevil–bacteria associations are still scarce when compared with other insect families. Additionally, these are biased towards those weevil species that are relevant from an agricultural perspective. The present review has intended to conduct a comprehensive search of the literature but it is inevitably dominated by research focused on weevil agricultural pest species. In the following sections, research on weevil–bacteria associations is discussed and the implications of these associations for the biology of the weevil species are considered (Table 1). The intention here is also to assess the

importance of these associations for the pest status of weevils, underlining the existing knowledge gaps and identifying priorities for future investigations in this field. Advancements in this research area will ultimately contribute to the development of improved weevil pest control strategies.

2 | TOUGHER THAN TOUGH: CANDIDATUS NARDONELLA, AN IMPORTANT PLAYER IN CUTICLE FORMATION

Studies of weevil-associated bacteria have typically focused on the symbiotic association between the bacterium *Candidatus Nardonella* and different weevil species. Research started at the beginning of the 1900s with the observation of intracellular microorganisms confined to specialised cells, called bacteriocytes, in the rice weevil *S. oryzae* (Linnaeus), although it remained undetermined as to whether the observed bacteria constituted a “symbiotic organ” or were simply “accessory cells” (Mansour, 1927, 1930; Pierantoni, 1927). It was not until the beginning of the 21st century that Lefèvre et al. (2004), using a phylogenetic analysis of the 16S rRNA gene, identified this microorganism as a γ -proteobacterium and designated the new lineage *Candidatus Nardonella*. This lineage was subsequently found to be widespread throughout the weevil superfamily and it was estimated to have become associated with weevils 125 million years ago (Conord et al., 2008; Lefèvre et al., 2004). Nevertheless, some studies have found that *Ca. Nardonella* has been replaced in species of the genus *Curculio* and *Sitophilus*, highlighting the dynamic nature of insect–bacteria associations (Lefèvre et al., 2004; Toju et al., 2010; Toju, Tanabe, Notsu, Sota, & Fukatsu, 2013). Symbiont displacement may have occurred following genome degradation of the original symbiont and loss of function(s) essential for host survival, creating an opportunity for a facultative bacterium capable of substituting the function(s) to form a new obligate association (reviewed by Moya, Peretó, Gil, & Latorre, 2008; Moya, Gil, & Latorre, 2009; Sudakaran, Kost, & Kaltenpoth, 2017). There are well documented cases of this phenomenon for instance in aphids such as the cedar aphid *Cinara cedris* (Mimeur). The primary symbiont of this aphid, *B. aphidicola*, has undergone a process of genome degradation leading to the loss of functions essential for the aphid host. The abundant facultative symbiont *Serratia symbiotica* appears to fulfil the absent functions and is a putative candidate for substituting the primary symbiont (Pérez-Brocal et al., 2006).

Subsequent studies focused on identifying *Ca. Nardonella* in other weevil species and on studying other features of its biology, such as population dynamics during different insect life stages or the location of the *Ca. Nardonella* bacteriocytes in insect tissues (Hosokawa et al., 2015; Hosokawa & Fukatsu, 2010; Huang et al., 2016; Mansour, 1930; Nardon, Lefevre, Delobel, Charles, & Heddi, 2002; Toju & Fukatsu, 2011). Importantly, Anbutsu et al. (2017) working on the black hard weevil *Pachyrhynchus infernalis* (Fairmaire) showed that *Ca. Nardonella* is involved in insect cuticle formation by contributing to tyrosine synthesis. Suppressing *Ca. Nardonella*, by

TABLE 1 Symbiotic bacteria and microbiota for weevil species

Bacterium	Weevil spp.	Symbiont function	Approach	References
<i>Candidatus Nardonella</i>	<i>Pachyrhynchus infernalis</i> <i>Rhynchophorus ferrugineus</i> <i>Sipalinius gigas</i> <i>Euscepes postfasciatus</i>	Tyrosine provisioning for cuticle synthesis	Genomic, transcriptomic and functional analysis	Anbutso et al. (2017) and Kuriwada et al. (2010)
<i>Sodalis pierantonius</i> (SPE)	<i>Sitophilus</i> spp.	Tyrosine provisioning for cuticle synthesis	Fitness and microscopy	Vigneron et al. (2014)
	<i>Sitophilus oryzae</i>	Synthesis of vitamins: pantothenic acid, biotin and riboflavin	Amino acid analysis	Wicker and Nardon (1982)
	<i>Sitophilus oryzae</i>	Bacteria provide mitochondria with vitamins	Artificial diet with different compositions	Wicker (1983)
	<i>Sitophilus oryzae</i> and <i>Sitophilus zeamais</i>	Methionine metabolism	Enzymatic assays	Heddi, Lefebvre, and Nardon (1993) and Heddi, Grenier, Khatchadourian, Charles, and Nardon (1999)
<i>Wolbachia</i>	Tribe Naupactini	Reduced flight activity in insects deprived of bacteria	Artificial diet and behavioural assays	Gasnier-Fauchet, Gharib, and Nardon (1986)
	<i>Hypera postica</i>	Induced cytoplasmic incompatibility	Correlation between infection and parthenogenesis	Grenier, Nardon, and Nardon (1994)
	<i>Strophosoma melanogrammum</i> <i>Strophosoma capitatum</i>	Speciation induced through cytoplasmic incompatibility	Genetic analysis	Rodrigero, Confalonieri, Guedes, and Lanteri (2010) and Elias-Costa, Confalonieri, Lanteri, and Rodrigo (2019)
	<i>Lissorhoptrus oryzophilus</i> <i>Otiorynchus sulcatus</i>	Egg development	Cross-mating, genetic analysis and microscopic localisation	Hsiao and Hsiao (1985a, 1985b) and Leu, Li, and Hsiao (1989)
Gut and whole insect microbiota	<i>Curculio</i> species	Food source influences microbiota composition	Egg hatching rate after antibiotic treatment	Kotásková, Kolasa, and Kajtoch (2018)
	<i>Hylobius abietis</i>		Cloning	Chen, Lu, Cheng, Jiang, and Way (2012) and Son, Luckhart, Zhang, Lieber, and Lewis (2008)
Gut microbiota	<i>Dendroctonus armandi</i>	Cellulolytic activity to exploit their natural food source	Pyrosequencing	Merville et al. (2013)
	<i>Rhynchophorus ferrugineus</i>		Amplified ribosomal DNA restriction analysis	Berasategui et al. (2016)
Gut microbiota	<i>Hypothenemus hampei</i>	<i>Pseudomonas</i> bacteria detoxify caffeine	Pyrosequencing	Hu, Yu, Wang, and Chen (2014)
	<i>Curculio chinensis</i>	Gut bacteria detoxify saponins	Bacteria isolation and antibiotic treatment	Muhammad, Fang, Hou, and Shi (2017)
Whole insect microbiota	<i>Dendroctonus ponderosae</i>	Microbiota is enriched with terpene degradation genes	Pyrosequencing	Ceja-Navarro et al. (2015)
			Pyrosequencing	Zhang et al. (2020)
			Pyrosequencing	Adams et al. (2013)

(Continues)

TABLE 1 (Continued)

Bacterium	Weevil spp.	Symbiont function	Approach	References
Gut microbiota	<i>Hylobius abietis</i>	Gut bacteria degrade terpenes and contribute to host fitness	Illumina sequencing	Berasategui et al. (2017)
Gut and whole insect microbiota	<i>Dendroctonus valens</i> <i>Dendroctonus mexicanus</i>	Geographic origin does not affect the microbiota composition	Pyrosequencing	Hernández-García et al. (2018)
	<i>Otiorynchus sulcatus</i>		Illumina sequencing	Morera-Margarit et al. (2019)
	<i>Dendroctonus ponderosae</i>		Pyrosequencing	Adams et al. (2013)
Whole insect microbiota	<i>Sitona obsoletus</i> <i>Sitona discoideus</i> <i>Listronotus bonariensis</i> <i>Ireninus aequalis</i> <i>Steriphus variabilis</i>	Candidate bacteria involved in resistance to a parasitic wasp used as a biological control	Pyrosequencing	White et al. (2015)

administering antibiotics in the diet at a larval stage, resulted in adults with low tyrosine titres and reddish, crumpled and/or deformed elytra. Similarly, Kuriwada et al. (2010) eliminated *Ca. Nardonella* from the West Indian sweet potato weevil *Euscepes postfasciatus* (Fairmaire) larvae by antibiotic treatment, which resulted in smaller adults with reddish cuticles.

The insect cuticle represents the first level of interaction between the insect and its environment and has been shown to play an important role in intra- and interspecific communication, water loss reduction and protection against predators and pathogens (Gibbs, Mousseau, & Crowe, 1991; Hamilton & Bulmer, 2012; Hamilton, Lay, & Bulmer, 2011; Howard & Blomquist, 2005; Locky, 1988; Ortiz-Urquiza & Keyhani, 2013; Tseng, Lin, Hsu, Pike, & Huang, 2014; Turillazzi et al., 2000; Weissling & Giblin-Davis, 1993). Despite the importance of the cuticle, the presence of *Ca. Nardonella* in the black hard and sweet potato weevils seems not to be essential for survival in laboratory conditions (Anbutsu et al., 2017; Kuriwada et al., 2010). Nonetheless, in a more natural environment the developmental defects derived from the absence of this symbiont likely impair the fitness of the insect. The importance of the weevil cuticle is also revealed in *Sitophilus* weevils. In this weevil genus, *Ca. Nardonella* was substituted by another bacterial symbiont named *Sodalis pierantonius* or SPE. This symbiont, amongst other functions, provides the weevil host with tyrosine and phenylalanine, similarly to *Ca. Nardonella*. These amino acids are then used in the formation of the adult cuticle. In this way, symbionts ensure that adult insects form a thick protective cuticle in shorter time (Vigneron et al., 2014; Wicker & Nardon, 1982). This similar functionality in two separate symbiont species illustrates the importance of weevil cuticle.

Cuticle colouration was shown to be important in deterring predatory lizards in the weevils *Pachyrhynchus tobafolius* (Kano) and *Kashotonus multipunctatus* (Kôno) (Tseng et al., 2014). The cuticle was also shown to reduce water loss in the weevil species *Rhynchophorus cruentatus* (Fabricius) (Weissling & Giblin-Davis, 1993). Although not a weevil, the saw-toothed grain beetle *Oryzaephilus surinamensis* (Linnaeus) associated symbionts were also shown to be involved in cuticle melanisation and resistance to desiccation (Engl et al., 2018). The subterranean termites *Reticulitermes flavipes* (Kollar) and *Reticulitermes virginicus* (Banks) secrete β -1,3-glucanase onto the cuticle that prevents infection by the pathogenic fungus *Metarhizium brunneum* (Petch) (Hamilton et al., 2011). This fungus is commonly used in pest control strategies targeting a wide variety of arthropods, including weevils. Although few studies have investigated the role of weevil cuticle in protection against pathogens, it has been suggested that a thick cuticle could improve the mechanical defence against predators (reviewed by Lemoine, Engl, & Kaltenpoth, 2020). It would therefore be interesting to test if the physical properties of the cuticle as well as cuticular secretions protect weevils from such pathogens.

Given the importance of the insect cuticle, further studies on the interaction between *Ca. Nardonella* and its hosts could provide insights into pest control strategies targeting weevil species known to carry this bacterium. RNAi-based biocides offer innovative approaches to target agricultural pests such as the brown planthopper

Nilaparvata lugens (Stål), the Asian corn borer *Ostrinia furnacalis* (Guenée), spotted wing drosophila *Drosophila suzukii* (Matsumura) and western flower thrips *Frankliniella occidentalis* (Pergande) (Li, Guan, Guo, & Miao, 2015; Murphy, Tabuloc, Cervantes, & Chiu, 2016; Whitten et al., 2016). Could RNAi based-biocides be designed to target weevil symbionts?

Antimicrobial peptides from the coleopterucin family are important in the weevil immune system. These peptides are also known to be involved in regulating symbiosis in some weevil species. In the maize weevil, a member of the coleopterucins family, named Coleopterucin-A, controls and restricts the population of its primary symbiont SPE inside the bacteriocytes (Login et al., 2011). This coleopterucin seems to be conserved as it interacts not only with SPE but also with *Ca. Nardonella*. Weevils treated with dsRNA targeting the *coleopterucin-A* gene showed reduced transcription levels of this gene and, as a consequence, bacterial symbionts were observed outside the bacteriocytes. "Symbiont escape" from the bacteriocytes did not affect weevil mortality under laboratory conditions. However, Anselme et al. (2008) observed that symbionts outside the bacteriocytes are recognised by the insect immune system as pathogenic elements and an immune response is mounted upon detection. RNAi-based biocides targeting the *coleopterucin-A* gene could therefore promote symbiont escape. This would in turn activate the insect immune reaction possibly rendering insects more susceptible to other pest control treatments. If this biocide were to be applied at a developmental stage for which the symbiosis is crucial, insects would also harbour detrimental developmental defects. Additionally, targeting specifically this gene would make it a safer pesticide by reducing negative effects on nontarget organisms. Thus, based on the function of Coleopterucin-A for weevil symbiosis, it would be interesting to explore the application of RNAi-based biocides targeting this antimicrobial peptide.

3 | TO FLY OR NOT TO FLY: *S. ORYZAE* AND *S. ZEAMAI*S BACTERIA AFFECT FLIGHT ACTIVITY

The possibility of bacteria compensating for amino acid and vitamin deficiencies in the plant-based diet of the insect was investigated for the rice weevil and the maize weevil by comparison of untreated insects with insects that were bacteria-free as a result of a heat treatment. In this way, Wicker and Nardon (1982) and Wicker (1983) showed that bacteria in these weevils are involved in the metabolic route that provides the insect host with phenylalanine and tyrosine from stored forms, and in the synthesis of the vitamins pantothenic acid, biotin and riboflavin. A subsequent study by Gasnier-Fauchet et al. (1986) also suggested that bacteria were involved in the metabolism of the amino acid methionine and its derivatives sarcosine and methionine sulfoxide.

Several studies have shown that the influence of bacteria on host fitness goes beyond compensating for poor diets. Heddi et al. (1993) observed in rice weevils that mitochondrial activity was higher in

weevils with bacteria compared with weevils without bacteria, although mitochondrial enzymatic activities were absent in bacteria isolated from these weevils. Hence, based on earlier discoveries that the bacteria were involved in amino acid and vitamin metabolism, it was suggested that these bacteria could be providing mitochondria with intermediary metabolites to maintain normal activity. Later studies confirmed that the intracellular symbiont of *Sitophilus* weevils SPE interacts with mitochondria by providing this organelle with pantothenic acid and riboflavin (Heddi et al., 1999; Heddi, Charles, Khatchadourian, Bonnot, & Nardon, 1998). Grenier et al. (1994) reported reduced or null flight activity in both the rice weevil and the maize weevil when individuals were deprived of bacteria by heat treatment, and that this effect could in some cases be partially restored by enriching diets with vitamins. Flight requires a large quantity of adenosine triphosphate (ATP) which is provided from cellular respiration via the mitochondrial Krebs cycle. Pantothenic acid and riboflavin are coenzymes needed for the correct progression of the enzymatic reactions occurring within this metabolic pathway. Thus, *Sitophilus* symbiont SPE in these two species of weevils is indirectly involved in flight activity by providing mitochondria with necessary components to allow the production of sufficient energy to fly. The importance of bacteria for flight activity in *S. oryzae* and *S. zeamais* raises an interesting question: could bacterial infection of insects be manipulated to reduce weevil dispersion? By doing this, neighbouring areas would be protected from the insect improving efficiency of integrated pest management (IPM) strategies targeting these pests.

4 | THE COMPLICATED CASE OF WEEVIL-ASSOCIATED *WOLBACHIA*: CAN *WOLBACHIA* MANIPULATE WEEVIL REPRODUCTION?

Bacteria of the genus *Wolbachia* are intracellular α -proteobacteria initially observed by Hertig and Wolbach (1924) in the ovaries of the mosquito *Culex pipiens* (Linnaeus). Currently, *Wolbachia* is considered to be widely spread amongst arthropods and it has been estimated to infect 66% of species within this phylum (Hilgenboecker, Hammerstein, Schlattmann, Telschow, & Werren, 2008). This bacterium may inhabit host ovaries and testes, but it can also inhabit somatic tissues such as the brain, muscles, the midgut or the salivary glands (Dobson et al., 1999). It is transmitted to the progeny vertically through the germ line, although host-bacterium phylogenetic incongruences have revealed that *Wolbachia* can also be transmitted horizontally (O'Neill, Giordano, Colbert, Karr, & Robertson, 1992; Werren, Zhang, & Guo, 1995). The combination of these two transmission routes has enabled bacteria from this genus to spread intra- and interspecifically between arthropods coinhabiting the same environment. For example, *Wolbachia* is transmitted to a parasitic wasp *Leptopilina boulardi* (Barbotin, Carton and Keiner-Pillault) horizontally from its infected fruit fly host *D. simulans* and successively transmitted to the wasp offspring for at least three generations (Heath, Butcher, Whitfield, & Hubbard, 1999).

The success of *Wolbachia* lies in its ability to manipulate host reproduction to increase its frequency within the host population. The most common reproductive manipulation is cytoplasmic incompatibility which occurs when two individuals with different *Wolbachia* cytoplasmic load, that is, with or without *Wolbachia* or with different *Wolbachia* strains, mate. In the case of mating between *Wolbachia* positive females and *Wolbachia* negative males, paternally inherited chromosomes will not be transferred to the offspring. This reproductive incompatibility favours, as a result, the fixation of *Wolbachia* infection within the host population. *Wolbachia* can also induce asexual reproduction or parthenogenesis by aborting the first mitosis that yields the haploid female gamete. This leads to the production of diploid egg cells that can eventually originate a *Wolbachia* infected clonal female lineage (Stouthamer, Breeuwer, & Hurst, 1999).

The weevil superfamily is known for harbouring many polyploid species that reproduce by thelytokous apomictic parthenogenesis in which egg cells are generated by mitosis and develop into female offspring (Saura, Lokki, & Suomalainen, 1993; Suomalainen, 1962; Suomalainen, Saura, & Lokki, 1987). It was initially proposed that parthenogenesis in weevils emerged as a result of a two-step stochastic process. At the first step, insects hybridise with a closely related species generating a new lineage with higher genetic diversity. This step is then followed by the fusion of unreduced gametes generated by meiotic errors, consequently increasing the chromosomal load of the lineage. This is thought to promote the appearance of apomictic parthenogenesis as the most effective reproduction strategy because of meiotic problems caused by the increased chromosomal load (Saura et al., 1993). Polyploid parthenogenetic forms have, for example, been seen in *Otiorhynchus scaber* (Linnaeus) following hybridisation with *O. nodosus* (Robinson) and subsequent fusion of gametes that had not undergone meiotic chromosome reduction (Stenberg & Lundmark, 2004; Stenberg, Terhivuo, Lokki, & Saura, 2000). Nonetheless, hybridisation is not the only mechanism by which asexual reproduction can arise in species of weevil, as for instance the parthenogenetic triploid vine weevil is of non-hybrid origin (Lundmark, 2010). The polyploid lineage of this species of weevil originated from fusion of unreduced gametes within the same species or autopolyploidy. *Wolbachia* induces asexual reproduction in a variety of arthropod species, such as the thelytokous parthenogenetic spider mite species from the genus *Bryobia* (Weeks & Breeuwer, 2001). Could *Wolbachia* also be involved in weevil parthenogenesis?

Rodrigo et al. (2010) and Elias-Costa et al. (2019) found a strong correlation between the presence of *Wolbachia* and parthenogenesis in weevils of the tribe Naupactini. However, the majority of studies seemed to indicate a lack of involvement of *Wolbachia* in weevil parthenogenesis. The influence of *Wolbachia* on reproduction in the rice water weevil *Lissorhoptrus oryzophilus* (Kuschel) was investigated by comparing invasive populations in China, which reproduce asexually, with a native population from the United States, which reproduces sexually. *Wolbachia* was present in weevils from all locations regardless of the reproduction strategy, implying it is not involved in promoting parthenogenesis (Huang et al., 2016). Stenberg and Lundmark (2004) recorded *Wolbachia* almost exclusively in sexual

forms of *O. scaber* rather than in asexual forms meaning that this bacterium was not behind the origin of parthenogenesis, at least for this species of weevil. Similarly, Lachowska, Rożek, and Holecová (2008) suggested that weevil parthenogenesis originated from meiotic chromosome reduction failure in a sexual ancestor in weevils from the subfamily Entiminae. In this study, vestiges of meiosis were observed in developing eggs, indicating that apomictic parthenogenesis evolved from automictic parthenogenesis, as was previously proposed by Saura et al. (1993). Mazur et al. (2016) studied the genetic variability of the nuclear, mitochondrial and *Wolbachia* DNA in various populations of the parthenogenetic weevil *Eusomus ovulum* (Germar). The same *Wolbachia* strain was shared by all weevil populations studied. However, this *Wolbachia* strain was present also in other parthenogenetic weevil species cohabiting the same area. This suggests that *Wolbachia* has been acquired from the environment. This reproductive parasite then, seems to be benefiting from a higher transmission rate in asexual insects, rather than being the origin of parthenogenesis in this weevil species.

Research into the influence of *Wolbachia* on weevil reproduction has not yet reached a final conclusion. Alternatively, other hypotheses to explain the prevalence of *Wolbachia* in parthenogenetic weevils have been proposed. Early studies of *Wolbachia* in weevils proposed that this proteobacterium causes cytoplasmic incompatibility in different populations of invasive alfalfa weevils *Hypera postica* (Gyllenhal) in the United States (Hsiao & Hsiao, 1985a, 1985b; Leu et al., 1989). The findings of Kotásková et al. (2018) also suggested that *Wolbachia* may have induced speciation in an ancestor of the weevils *Strophosoma melanogrammmum* (Forster) and *Strophosoma capitatum* (De Geer) by imposing a reproductive barrier. These two sibling weevil species are good models to study the influence of *Wolbachia* on the reproduction strategy as the first species is a parthenogenetic triploid species while the second is a sexual diploid species. Both weevil species carried *Wolbachia*, although the parthenogenetic species harboured only one *Wolbachia* strain whereas the sexual species carried three strains that differed from the strain found in the asexual counterpart. Thus, it was suggested that instead of inducing parthenogenesis, *Wolbachia* may have induced speciation through cytoplasmic incompatibility. Lachowska, Kajtoch, and Knutelski (2010) investigated the presence of *Wolbachia* in 40 European weevil species, from four subfamilies within the family Curculionidae, and related the infection status to adaptation and reproduction. In this study, *Wolbachia* was recorded almost twice as frequently in parthenogenetic weevils compared to sexual weevils. However, it was proposed that rather than inducing parthenogenesis, *Wolbachia* might have been again benefiting from infecting these weevils to increase its chances of transmission. *Wolbachia* could also increase its presence within a weevil population by being essential for the normal development of eggs. In the rice water weevil and the vine weevil for instance, reducing *Wolbachia* titre by antibiotic treatment in eggs decreased egg hatching rate (Chen et al., 2012; Son et al., 2008). The function of *Wolbachia* for weevil biology and fitness to date harbours more questions than answers. Nonetheless, we expect that future research will bring a more comprehensive understanding of the influence of *Wolbachia* on development and fitness for this large insect group.

From an agricultural perspective, it would be interesting to test the possibility of exploiting *Wolbachia* induced cytoplasmic incompatibility for weevil control applying the incompatible insect technique (IIT). This technique uses males of the target pest which are artificially inoculated with a *Wolbachia* strain that creates a reproductive barrier with females of the target pest by cytoplasmic incompatibility. Mass release of infected (sterile) males that mate with wild females leads to decline in the pest population (Brelsfoard & Dobson, 2009). To date, the use of this technique to control populations of weevil pests has been limited because of poor prediction of the spread of *Wolbachia* but also because of lack of understanding of the role of this bacterium for the weevil biology. *Wolbachia* can be horizontally transferred within and between species as was seen between the rice weevil and the maize weevil, and between the maize weevil and its parasitoid wasp *Theocolax elegans* (Westwood) (Carvalho, Corrêa, de Oliveira, & Guedes, 2014). Hence, the newly introduced *Wolbachia* could ultimately spread to target as well as to non-target organisms within the same habitat. Although it remains unclear what role this bacterium plays in parthenogenesis in weevils, the application of IIT could inadvertently give rise to an asexual strain that may be better able to spread, as has been seen for a naturally occurring asexual strain of *O. scaber* (Stenberg & Lundmark, 2004). Further research is, therefore, needed to clarify if *Wolbachia* can be used as a safe IPM strategy, such as IIT.

5 | A FAST-MOVING FIELD: HIGH-THROUGHPUT SEQUENCING IN THE STUDY OF WEEVIL MICROBIOTA INVOLVED IN DIGESTION AND DETOXIFICATION

The 21st century has seen a large number of studies investigating insect bacterial communities applying high-throughput sequencing. This has been due largely to recent improvements in the available sequencing technology for metagenomic research. These studies have taxonomically characterised bacteria inhabiting a great variety of insect species. Gut microbiota has received special attention because of its importance in shaping insect–plant interactions (Frago, Dicke, & Godfray, 2012). Colman, Toolson, and Takacs-Vesbach (2012) for instance compared the gut bacterial communities of 62 insect species and showed that taxonomy as well as diet are important in determining the composition of gut bacterial microbiota. Amongst weevils, increasing numbers of studies have applied high-throughput sequencing to determine how the weevil's microbiota enables insect adaptation to different food sources and toxic plant metabolites.

Diet has been shown to be a major factor in shaping the bacterial community for different weevil species in agreement with Colman et al. (2012). Merville et al. (2013) showed that four *Curculio* species coinhabiting oak trees had a very similar whole-body bacterial community composition despite being separate species, indicating that the food source may exert an important influence on the microbiota composition. Likewise, Berasategui et al. (2016) observed that the gut bacterial community of the pine weevil *Hylobius abietis* (Linnaeus) was

closer in composition to bark beetles from different locations with a similar diet than to other weevil species feeding on non-conifer food sources. Changes in the diet in an experimental setup were also found to alter the bacterial community in the red palm weevil when considering the entire insect (Montagna et al., 2015). Similar results were found for the cotton boll weevil *Anthonomus grandis* (Boheman) when only considering the gut (Ben Guerrero et al., 2016). Cellulolytic activity has also been found in the bacterial microbiota of the Chinese white pine beetle larval gut *Dendroctonus armandi* (Tsai and Li) (Hu et al., 2014) and the gut of red palm weevil larvae (Muhammad et al., 2017), which is probably required to exploit their natural food sources.

The bacterial microbiota in some weevil species is also involved in detoxifying plant secondary metabolites produced to deter herbivorous insects. Caffeine is a naturally occurring plant secondary metabolite toxic to insects and abundant in coffee beans (Nathanson, 1984). However, the coffee berry borer *Hypothenemus hampei* (Ferrari) is able to complete its life cycle entirely on caffeine-rich green coffee beans. A study by Ceja-Navarro et al. (2015) demonstrated that this is possible because of the association with *Pseudomonas* bacteria that detoxify caffeine by expressing caffeine demethylase genes. Saponins are another type of plant secondary metabolite with insecticidal activity (De Geyter, Lambert, Geelen, & Smagghe, 2007). Plants from the genus *Camellia*, the leaves of which may be consumed as tea, contain high levels of saponins both the leaves and the seeds. Despite this, the *Camellia* weevil *Curculio chinensis* (Chevrolat) is able to feed and complete its life cycle entirely in *Camellia* seeds. A study by Zhang et al. (2020) showed that two bacterial members of this weevil's microbiota, *Acinetobacter calcoaceticus* and *Acinetobacter oleivorans*, enzymatically break down saponins. Thus, it has been hypothesised that *Camellia* weevils are able to resist saponin toxicity because of their associated bacteria.

Terpenes are a set of toxic secondary metabolites abundant in conifer trees. Bark beetles from the weevil subfamily Scolytinae, however, are able to feed and complete their life cycle on terpenoid-rich conifer trees (reviewed by Six, 2013). Adams et al. (2013) studied the whole body bacterial microbiota harboured by the conifer feeding mountain pine beetle *Dendroctonus ponderosae* (Hopkins). This study showed that the weevil microbiota was enriched with bacterial genes involved in terpene degradation, mainly belonging to bacteria in the genera *Pseudomonas* and *Rahnella*. It was then proposed that bacterial members of the mountain pine beetle microbiota are actively involved in detoxifying these plant metabolites. Berasategui et al. (2017) studied the gut microbiota of a conifer-feeding weevil from the subfamily Molytinae, the pine weevil *H. abietis*. Similar to the mountain pine beetle, this weevil's microbiota harboured terpenoid degrading genes. At a fitness level, fecundity was negatively affected when weevils were deprived of bacteria following antibiotic treatment. However, survival was not significantly affected. Based on these results, Berasategui et al. (2017) suggested that rather than detoxifying terpenes, the pine weevil gut microbiota seems to be degrading terpenoids to provide the weevil host with an additional source of nutrients. Given the role that plant toxins exert in deterring

herbivorous pests, the role of bacteria in degrading these toxins merits further attention. Future research should, however, aim at considering not solely microbiota analysis but also include insect fitness measurements.

Various studies have revealed the presence of a core microbiota shared by populations of the same weevil species found at separate geographic locations. This is the case for the gut microbiota of the bark beetles *Dendroctonus valens* (Le Conte) and *Dendroctonus mexicanus* (Hopkins) in Mexico (Hernández-García et al., 2018) and for the whole body microbiota of the vine weevil in the United Kingdom (Morera-Margarit et al., 2019). For example, mountain pine beetles collected at separate locations shared a similar bacterial community. These beetles inhabit tree galleries where larvae grow and adults reproduce. The bacteria identified from tree galleries were not significantly different to the bacteria harboured by the insects (Adams et al., 2013). It is not known if this “core microbiota” pattern extends to other weevil species or if the bacteria shared by weevils at different locations are involved in key functions for the survival of the insect host such as in exploiting food sources, reproduction or defence against pathogens. Further investigations could highlight opportunities for exploring pest control strategies targeting bacteria essential for the insect’s fitness as well as physiological evidence of the bacterial function.

While still a developing field, studies of bacterial communities will advance through combining knowledge of ecology, physiology, genetics and evolution (Christian, Whitaker, & Clay, 2015; Douglas & Werren, 2016). In terms of pest control, there is scant research focused on applying knowledge gleaned through microbiota studies to design pest control strategies. For instance, the characterisation of the bacterial community of various native and invasive weevil species in New Zealand identified candidate bacteria involved in resistance to the parasitic wasp *Microctonus aethioides* (Loan), used as a biological control against these species of weevil (White et al., 2015). As this is a fast-moving area of research, it is likely that discoveries in the near future will begin to find their way into IPM programmes targeting weevils that are agricultural pests around the world.

6 | THE ROSETTA STONE: TRANSLATING MICROBIOTA ANALYSIS INTO ECOLOGICAL INSIGHTS

In this review we have shown how weevil-bacteria associations are relevant for weevil adaptation and evolution, but also for determining the detrimental effect as agricultural pest species. However, additional research is still needed to deepen our understanding of weevil-bacteria associations. For instance, the symbionts *Ca. Nardonella* and *SPE* share a common function in providing the host with tyrosine, which is required in cuticle formation (Anbutsu et al., 2017; Kuriwada et al., 2010; Vigneron et al., 2014). It is possible that the extra tyrosine produced by weevils carrying symbionts provided an evolutionary advantage by ensuring more rapid polymerisation of the exoskeleton even on nutritiously poor diets. Did these associations enable weevils

to broaden their range of host species? Or, what is the mechanism used by *Wolbachia* to manipulate egg development in the rice and vine weevils (Chen et al., 2012; Son et al., 2008)? The technological innovations developed through studies of other groups of organisms should be applied to study bacteria in weevils.

In the “era of omics,” the application of metagenomics and metatranscriptomics could provide valuable information to understand the function of the associated bacteria for the weevil host. This could allow the identification of candidate bacteria influencing the development and/or adaptation of the weevil host, which could be confirmed with functional analyses. Weevil phenotypes derived from the manipulation of these candidate bacteria, for example by selective removal or introduction, could reveal meaningful associations. Ultimately, this could allow knowledge acquired from characterising weevil microbiota to be translated into an understanding of the role of bacteria in weevil ecology and provide valuable information to design more efficient and sustainable pest control strategies.

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REFERENCES

- Adams, A. S., Aylward, F. O., Adams, S. M., Erbilgin, N., Aukema, B. H., Currie, C. R., ... Raffa, K. F. (2013). Mountain pine beetles colonizing historical and naïve host trees are associated with a bacterial community highly enriched in genes contributing to terpene metabolism. *Applied and Environmental Microbiology*, 79, 3468–3475.
- Akman, L., Yamashita, A., Watanabe, H., Oshima, K., Shiba, T., Hattori, M., & Aksoy, S. (2002). Genome sequence of the endocellular obligate symbiont of tsetse flies, *Wigglesworthia glossinidia*. *Nature Genetics*, 32, 402–407.
- Alonso-Zaragoza, M. A., & Lyal, C. H. C. (2002). Addenda and corrigenda to “a world catalogue of families and genera of Curculionioidea (Insecta: Coleoptera)”. *Zootaxa*, 63, 1.
- Anbutsu, H., Moriyama, M., Nikoh, N., Hosokawa, T., Futahashi, R., Tanahashi, M., ... Fukatsu, T. (2017). Small genome symbiont underlies cuticle hardness in beetles. *Proceedings of the National Academy of Sciences of the United States of America*, 114, E8382–E8391.
- Anselme, C., Pérez-Brocal, V., Vallier, A., Vincent-Monegat, C., Charif, D., Latorre, A., ... Heddi, A. (2008). Identification of the weevil immune genes and their expression in the bacteriome tissue. *BMC Biology*, 6, 43.

- Ben Guerrero, E., Soria, M., Salvador, R., Ceja-Navarro, J. A., Campos, E., Brodie, E. L., & Talia, P. (2016). Effect of different lignocellulosic diets on bacterial microbiota and hydrolytic enzyme activities in the gut of the cotton boll weevil (*Anthonomus grandis*). *Frontiers in Microbiology*, 7, 2093.
- Berasategui, A., Axelsson, K., Nordlander, G., Schmidt, A., Borg-Karlson, A.-K., Gershenson, J., ... Kaltenpoth, M. (2016). The gut microbiota of the pine weevil is similar across Europe and resembles that of other conifer-feeding beetles. *Molecular Ecology*, 25, 4014–4031.
- Berasategui, A., Salem, H., Paetz, C., Santoro, M., Gershenson, J., Kaltenpoth, M., & Schmidt, A. (2017). Gut microbiota of the pine weevil degrades conifer diterpenes and increases insect fitness. *Molecular Ecology*, 26, 4099–4110.
- Blackman, R. L., & Eastop, V. F. (2000). *Aphids on the world's crops: An identification and information guide* (2nd ed.). New York, NY: John Wiley.
- Brelsfoard, C. L., & Dobson, S. L. (2009). *Wolbachia*-based strategies to control insect pests and disease vectors. *Asia Pacific Journal of Molecular Biology & Biotechnology*, 17, 55–63.
- Carvalho, G. A., Corrêa, A. S., de Oliveira, L. O., & Guedes, R. N. C. (2014). Evidence of horizontal transmission of primary and secondary endosymbionts between maize and rice weevils (*Sitophilus zeamais* and *Sitophilus oryzae*) and the parasitoid *Theocolax elegans*. *Journal of Stored Products Research*, 59, 61–65.
- Ceja-Navarro, J. A., Vega, F. E., Karaoz, U., Hao, Z., Jenkins, S., Lim, H. C., ... Brodie, E. L. (2015). Gut microbiota mediate caffeine detoxification in the primary insect pest of coffee. *Nature Communications*, 6, 7618.
- Chen, S.-J., Lu, F., Cheng, J.-A., Jiang, M.-X., & Way, M. O. (2012). Identification and biological role of the endosymbionts *Wolbachia* in rice water weevil (Coleoptera: Curculionidae). *Environmental Entomology*, 41, 469–477.
- Christian, N., Whitaker, B. K., & Clay, K. (2015). Microbiomes: Unifying animal and plant systems through the lens of community ecology theory. *Frontiers in Microbiology*, 6, 869.
- Colman, D. R., Toolson, E. C., & Takacs-Vesbach, C. D. (2012). Do diet and taxonomy influence insect gut bacterial communities? *Molecular Ecology*, 21, 5124–5137.
- Conord, C., Despres, L., Vallier, A., Balmant, S., Miquel, C., Zundel, S., ... Heddi, A. (2008). Long-term evolutionary stability of bacterial endosymbiosis in Curculionoidea: Additional evidence of symbiont replacement in the Dryophthoridae family. *Molecular Biology and Evolution*, 25, 859–868.
- De Geyter, E., Lambert, E., Geelen, D., & Smagghe, G. (2007). Novel advances with plant saponins as natural insecticides to control pest insects. *Pest Technology*, 1, 96–105.
- Dobson, S. L., Bourtzis, K., Braig, H. R., Jones, B. F., Zhou, W., Rousset, F., & O'Neill, S. L. (1999). *Wolbachia* infections are distributed throughout insect somatic and germ line tissues. *Insect Biochemistry and Molecular Biology*, 29, 153–160.
- Douglas, A. E. (2016). How multi-partner endosymbioses function. *Nature Reviews Microbiology*, 14, 731–743.
- Douglas, A. E., & Werren, J. H. (2016). Holes in the hologenome: Why host-microbe symbioses are not holobionts. *mBio*, 7, e02099.
- Elias-Costa, A. J., Confalonieri, V. A., Lanteri, A. A., & Rodriguez, M. S. (2019). Game of clones: Is *Wolbachia* inducing speciation in a weevil with a mixed reproductive mode? *Molecular Phylogenetics and Evolution*, 133, 42–53.
- Engl, T., Eberl, N., Gorse, C., Krüger, T., Schmidt, T. H. P., Plarre, R., ... Kaltenpoth, M. (2018). Ancient symbiosis confers desiccation resistance to stored grain pest beetles. *Molecular Ecology*, 27, 2095–2108.
- European Commission. (2011). *The insect killing our palm trees. EU efforts to stop the red palm weevil*. Luxembourg: Office for Official Publications of the European Communities.
- Frago, E., Dicke, M., & Godfray, H. C. J. (2012). Insect symbionts as hidden players in insect-plant interactions. *Trends in Ecology & Evolution*, 27, 705–711.
- Gasnier-Fauchet, F., Gharib, A., & Nardon, P. (1986). Comparison of methionine metabolism in symbiotic and aposymbiotic larvae of *Sitophilus oryzae* L. (Coleoptera: Curculionidae)—I. Evidence for a glycine N-methyltransferase-like activity in the aposymbiotic larvae. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, 85, 245–250.
- Gibbs, A., Mousseau, T. A., & Crowe, J. H. (1991). Genetic and acclimatory variation in biophysical properties of insect cuticle lipids. *Proceedings of the National Academy of Sciences of the United States of America*, 88, 7257–7260.
- Grahame, J. (2017). Maize (greater grain) weevil (339). Pacific pests and pathogens. https://www.pestnet.org/fact_sheets/maize_greater_grain_weevil_339.htm
- Grenier, A. M., Nardon, C., & Nardon, P. (1994). The role of symbiotes in flight activity of *Sitophilus* weevils. *Entomologia Experimentalis et Applicata*, 70, 201–208.
- Hamilton, C., & Bulmer, M. S. (2012). Molecular antifungal defenses in subterranean termites: RNA interference reveals in vivo roles of terminins and GNBPs against a naturally encountered pathogen. *Developmental & Comparative Immunology*, 36, 372–377.
- Hamilton, C., Lay, F., & Bulmer, M. S. (2011). Subterranean termite prophylactic secretions and external antifungal defenses. *Journal of Insect Physiology*, 57, 1259–1266.
- Handelsman, J. (2004). Metagenomics: Application of genomics to uncultured microorganisms. *Microbiological & Molecular Biological Reviews*, 68, 669–685.
- Heath, B. D., Butcher, R. D. J., Whitfield, W. G. F., & Hubbard, S. F. (1999). Horizontal transfer of *Wolbachia* between phylogenetically distant insect species by a naturally occurring mechanism. *Current Biology*, 9, 313–316.
- Heather, J. M., & Chain, B. (2016). The sequence of sequencers: The history of sequencing DNA. *Genomics*, 107, 1–8.
- Heddi, A., Charles, H., Khatchadourian, C., Bonnot, G., & Nardon, P. (1998). Molecular characterization of the principal symbiotic bacteria of the weevil *Sitophilus oryzae*: A peculiar G + C content of an endocytobiotic DNA. *Journal of Molecular Evolution*, 47, 52–61.
- Heddi, A., Grenier, A.-M., Khatchadourian, C., Charles, H., & Nardon, P. (1999). Four intracellular genomes direct weevil biology: Nuclear, mitochondrial, principal endosymbiont, and *Wolbachia*. *Proceedings of the National Academy of Sciences of United States of America*, 96, 6814–6819.
- Heddi, A., Lefebvre, F., & Nardon, P. (1993). Effect of endocytobiotic bacteria on mitochondrial enzymatic activities in the weevil *Sitophilus oryzae* (Coleoptera: Curculionidae). *Insect Biochemistry and Molecular Biology*, 23, 403–411.
- Hernández-García, J. A., Gonzalez-Escobedo, R., Briones-Roblero, C. I., Cano-Ramírez, C., Rivera-Orduña, F. N., & Zúñiga, G. (2018). Gut bacterial communities of *Dendroctonus valens* and *D. mexicanus* (Curculionidae: Scolytinae): A metagenomic analysis across different geographical locations in Mexico. *International Journal of Molecular Sciences*, 19, 2578.
- Hertig, M., & Wolbach, S. B. (1924). Studies on Rickettsia-like microorganisms in insects. *Journal of Medical Research*, 44, 329–374.
- Hilgenboecker, K., Hammerstein, P., Schlattmann, P., Telschow, A., & Werren, J. H. (2008). How many species are infected with *Wolbachia*?—A statistical analysis of current data. *FEMS Microbiology Letters*, 281, 215–220.
- Hosokawa, T., & Fukatsu, T. (2010). *Nardonella* endosymbiont in the West Indian sweet potato weevil *Euscepes postfasciatus* (Coleoptera: Curculionidae). *Applied Entomology and Zoology*, 45, 115–120.
- Hosokawa, T., Koga, R., Tanaka, K., Moriyama, M., Anbutsu, H., & Fukatsu, T. (2015). *Nardonella* endosymbionts of Japanese pest and non-pest weevils (Coleoptera: Curculionidae). *Applied Entomology and Zoology*, 50, 223–229.
- Howard, R. W., & Blomquist, G. J. (2005). Ecological, behavioral, and biochemical aspects of insect hydrocarbons. *Annual Review of Entomology*, 50, 371–393.

- Hsiao, C., & Hsiao, T. H. (1985a). Rickettsia as the cause of cytoplasmic incompatibility in the alfalfa weevil, *Hypera postica*. *Journal of Invertebrate Pathology*, 45, 244–246.
- Hsiao, T. H., & Hsiao, C. (1985b). Hybridization and cytoplasmic incompatibility among alfalfa weevil strains. *Entomologia Experimentalis et Applicata*, 37, 155–159.
- Hu, X., Yu, J., Wang, C., & Chen, H. (2014). Cellulolytic bacteria associated with the gut of *Dendroctonus armandi* larvae (Coleoptera: Curculionidae: Scolytinae). *Forests*, 5, 455–465.
- Huang, X., Huang, Y., Zhang, J., Lu, F., Wei, J., & Jiang, M. (2016). The symbiotic bacteria *Nardonella* in rice water weevil (Coleoptera: Curculionidae): Diversity, density, and associations with host reproduction. *Annals of the Entomological Society of America*, 109, 415–423.
- Kotásková, N., Kolasa, M., & Kajtoch, Ł. (2018). Contrasting patterns of molecular diversity and *Wolbachia* infection in bisexual and parthenogenetic *Strophosoma* weevils (Coleoptera: Curculionidae). *Entomological Science*, 21, 385–395.
- Kuriwada, T., Hosokawa, T., Kumano, N., Shiromoto, K., Haraguchi, D., & Fukatsu, T. (2010). Biological role of *Nardonella* endosymbiont in its weevil host. *PLoS One*, 5, e13101.
- Lachowska, D., Kajtoch, Ł., & Knutelski, S. (2010). Occurrence of *Wolbachia* in central European weevils: Correlations with host systematics, ecology, and biology. *Entomologia Experimentalis et Applicata*, 135, 105–118.
- Lachowska, D., Rožek, M., & Holecová, M. (2008). New data on the cytology of parthenogenetic weevils (Coleoptera, Curculionidae). *Genetica*, 134, 235–242.
- Lefèvre, C., Charles, H., Vallier, A., Delobel, B., Farrell, B., & Heddi, A. (2004). Endosymbiont phylogenesis in the Dryophthoridae weevils: Evidence for bacterial replacement. *Molecular Biology and Evolution*, 21, 965–973.
- Lemoine, M. M., Engl, T., & Kaltenpoth, M. (2020). Microbial symbionts expanding or constraining abiotic niche space in insects. *Current Opinion in Insect Science*, 39, 14–20.
- Leu, S.-J. C., Li, J. K.-K., & Hsiao, T. H. (1989). Characterization of *Wolbachia postica*, the cause of reproductive incompatibility among alfalfa weevil strains. *Journal of Invertebrate Pathology*, 54, 248–259.
- Li, H., Guan, R., Guo, H., & Miao, X. (2015). New insights into an RNAi approach for plant defence against piercing-sucking and stem-borer insect pests. *Plant, Cell & Environment*, 38, 2277–2285.
- Lockey, K. H. (1988). Lipids of the insect cuticle: Origin, composition and function. *Comparative Biochemistry and Physiology—Part B: Biochemistry*, 89, 595–645.
- Login, F. H., Balmand, S., Vallier, A., Vincent-Monégat, C., Vigneron, A., Weiss-Gayet, M., ... Heddi, A. (2011). Antimicrobial peptides keep insect endosymbionts under control. *Science*, 334, 362–365.
- Lundmark, M. (2010). *Otiorhynchus sulcatus*, an autopolyploid general-purpose genotype species? *Hereditas*, 147, 278–282.
- Mansour, K. (1927). Memoirs: The development of the larval and adult mid-gut of *Calandra oryzae* (Linn.): The rice weevil. *Journal of Cell Science*, s2-71, 313–352.
- Mansour, K. (1930). Memoirs: Preliminary studies on the bacterial cell-mass (accessory cell-mass) of *Calandra oryzae* (Linn.): The rice weevil. *Journal of Cell Science*, s2-73, 421–435.
- Mazur, M. A., Holecová, M., Lachowska-Cierlik, D., Lis, A., Kubisz, D., & Kajtoch, Ł. (2016). Selective sweep of *Wolbachia* and parthenogenetic host genomes—The example of the weevil *Eusomus ovulum*. *Insect Molecular Biology*, 25, 701–711.
- Merville, A., Venner, S., Henri, H., Vallier, A., Menu, F., Vavre, F., ... Bel-Venner, M.-C. (2013). Endosymbiont diversity among sibling weevil species competing for the same resource. *BMC Evolutionary Biology*, 13, 28.
- Montagna, M., Chouaia, B., Mazza, G., Prosdoci, E. M., Crotti, E., Mereghetti, V., ... Daffonchio, D. (2015). Effects of the diet on the microbiota of the red palm weevil (Coleoptera: Dryophthoridae). *PLoS One*, 10, e0117439.
- Moorhouse, E. R., Charnley, A. K., & Gillespie, A. T. (1992). A review of the biology and control of the vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae). *Annals of Applied Biology*, 121, 431–454.
- Morera-Margarit, P., Bulgarelli, D., Pope, T. W., Graham, R. I., Mitchell, C., & Karley, A. J. (2019). The bacterial community associated with adult vine weevil (*Otiorhynchus sulcatus*) in UK populations growing on strawberry is dominated by *Candidatus Nardonella*. *Entomologia Experimentalis et Applicata*, 167, 186–196.
- Moya, A., Gil, R., & Latorre, A. (2009). The evolutionary history of symbiotic associations among bacteria and their animal hosts: A model. *Clinical Microbiology and Infection*, 15, 11–13.
- Moya, A., Peretó, J., Gil, R., & Latorre, A. (2008). Learning how to live together: Genomic insights into prokaryote–animal symbioses. *Nature Reviews Genetics*, 9, 218–229.
- Muhammad, A., Fang, Y., Hou, Y., & Shi, Z. (2017). The gut entomotype of red palm weevil *Rhynchophorus ferrugineus* Olivier (Coleoptera: Dryophthoridae) and their effect on host nutrition metabolism. *Frontiers in Microbiology*, 8, 2291.
- Murphy, K. A., Tabuloc, C. A., Cervantes, K. R., & Chiu, J. C. (2016). Ingestion of genetically modified yeast symbiont reduces fitness of an insect pest via RNA interference. *Scientific Reports*, 6, 22587.
- Nakabachi, A., & Ishikawa, H. (1999). Provision of riboflavin to the host aphid, *Acyrtosiphon pisum*, by endosymbiotic bacteria, *Buchnera*. *Journal of Insect Physiology*, 45, 1–6.
- Nardon, P., Lefevre, C., Delobel, B., Charles, H., & Heddi, A. (2002). Occurrence of endosymbiosis in Dryophthoridae weevils: Cytological insights into bacterial symbiotic structures. *Symbiosis*, 33, 227–241.
- Nathanson, J. A. (1984). Caffeine and related methylxanthines: Possible naturally occurring pesticides. *Science*, 226, 184–187.
- Nogge, G. (1981). Significance of symbionts for the maintenance of an optimal nutritional state for successful reproduction in hematophagous arthropods. In *Parasitology* (pp. 101–104). New York, NY: Cambridge University Press.
- O'Neill, S. L., Giordano, R., Colbert, A. M., Karr, T. L., & Robertson, H. M. (1992). 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. *Proceedings of the National Academy of Sciences of the United States of America*, 89, 2699–2702.
- Oliver, K. M., Moran, N. A., & Hunter, M. S. (2005). Variation in resistance to parasitism in aphids is due to symbionts not host genotype. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 12795–12800.
- Oliver, K. M., Russell, J. A., Moran, N. A., & Hunter, M. S. (2003). Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 1803–1807.
- Ortiz-Urquiza, A., & Keyhani, N. (2013). Action on the surface: Entomopathogenic fungi versus the insect cuticle. *Insects*, 4, 357–374.
- Pérez-Brocal, V., Gil, R., Ramos, S., Lamelas, A., Postigo, M., Michelena, J. M., ... Latorre, A. (2006). A small microbial genome: The end of a long symbiotic relationship? *Science*, 314, 312–313.
- Pierantoni, U. (1927). L'organo simbiotico nello sviluppo di *Calandra oryzae*. *Rendiconti della Reale Accademia delle Scienze Fisiche e Matematiche di Napoli*, 35, 244–250.
- Rodríguez, M. S., Confalonieri, V. A., Guedes, J. V. C., & Lanteri, A. A. (2010). *Wolbachia* infection in the tribe Naupactini (Coleoptera, Curculionidae): Association between thelytokous parthenogenesis and infection status. *Insect Molecular Biology*, 19, 631–640.
- Saura, A., Lokki, J., & Suomalainen, E. (1993). Origin of polyploidy in parthenogenetic weevils. *Journal of Theoretical Biology*, 163, 449–456.
- Scarborough, C. L., Ferrari, J., & Godfray, H. C. J. (2005). Aphid protected from pathogen by endosymbiont. *Science*, 310, 1781–1781.

- Six, D. L. (2013). The bark beetle *Holobiont*: Why microbes matter. *Journal of Chemical Ecology*, 39, 989–1002.
- Son, Y., Luckhart, S., Zhang, X., Lieber, M. J., & Lewis, E. E. (2008). Effects and implications of antibiotic treatment on *Wolbachia*-infected vine weevil (Coleoptera: Curculionidae). *Agricultural and Forest Entomology*, 10, 147–155.
- Steinhaus, E. A. (1940). The microbiology of insects. *Bacteriological Reviews*, 4, 17–57.
- Stenberg, P., & Lundmark, M. (2004). Distribution, mechanisms and evolutionary significance of clonality and polyploidy in weevils. *Agricultural and Forest Entomology*, 6, 259–266.
- Stenberg, P., Terhivuo, J., Lokki, J., & Saura, A. (2000). Clone diversity in the polyploid weevil *Otiiorhynchus scaber*. *Hereditas*, 132, 137–142.
- Stouthamer, R., Breeuwer, J. A., & Hurst, G. D. (1999). *Wolbachia pipientis*: Microbial manipulator of arthropod reproduction. *Annual Reviews in Microbiology*, 53, 71–102.
- Sudakaran, S., Kost, C., & Kaltenpoth, M. (2017). Symbiont acquisition and replacement as a source of ecological innovation. *Trends in Microbiology*, 25, 375–390.
- Suomalainen, E. (1962). Significance of parthenogenesis in the evolution of insects. *Annual Review of Entomology*, 7, 349–366.
- Suomalainen, E., Saura, A., & Lokki, J. (1987). *Cytology and evolution in parthenogenesis*, Boca Raton, Florida, USA: CRC Press.
- Toju, H., & Fukatsu, T. (2011). Diversity and infection prevalence of endosymbionts in natural populations of the chestnut weevil: Relevance of local climate and host plants. *Molecular Ecology*, 20, 853–868.
- Toju, H., Hosokawa, T., Koga, R., Nikoh, N., Meng, X. Y., Kimura, N., & Fukatsu, T. (2010). "*Candidatus* Curculioniphilus buchneri," a novel clade of bacterial endocellular symbionts from weevils of the genus *Curculio*. *Applied and Environmental Microbiology*, 76, 275–282.
- Toju, H., Tanabe, A. S., Notsu, Y., Sota, T., & Fukatsu, T. (2013). Diversification of endosymbiosis: Replacements, co-speciation and promiscuity of bacteriocyte symbionts in weevils. *ISME Journal*, 7, 1378–1390.
- Tseng, H.-Y., Lin, C.-P., Hsu, J.-Y., Pike, D. A., & Huang, W.-S. (2014). The functional significance of aposematic signals: Geographic variation in the responses of widespread lizard predators to colourful invertebrate prey. *PLoS One*, 9, e91777.
- Turillazzi, S., Sledge, M. F., Dani, F. R., Cervo, R., Massolo, A., & Fondelli, L. (2000). Social hackers: Integration in the host chemical recognition system by a paper wasp social parasite. *Naturwissenschaften*, 87, 172–176.
- Vigneron, A., Masson, F., Vallier, A., Balmand, S., Rey, M., Vincent-Monégat, C., ... Heddi, A. (2014). Insects recycle endosymbionts when the benefit is over. *Current Biology*, 24, 2267–2273.
- Weeks, A. R., & Breeuwer, J. A. J. (2001). *Wolbachia*-induced parthenogenesis in a genus of phytophagous mites. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, 268, 2245–2251.
- Weeks, A. R., Turelli, M., Harcombe, W. R., Reynolds, K. T., & Hoffmann, A. A. (2007). From parasite to mutualist: Rapid evolution of *Wolbachia* in natural populations of *Drosophila*. *PLoS Biology*, 5, e114.
- Weissling, T. J., & Giblin-Davis, R. M. (1993). Water loss dynamics and humidity preference of *Rhynchophorus cruentatus* (Coleoptera: Curculionidae) adults. *Environmental Entomology*, 22, 93–98.
- Werren, J. H., Baldo, L., & Clark, M. E. (2008). *Wolbachia*: Master manipulators of invertebrate biology. *Nature Reviews Microbiology*, 6, 741–751.
- Werren, J. H., Zhang, W., & Guo, L. R. (1995). Evolution and phylogeny of *Wolbachia*: Reproductive parasites of arthropods. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, 261, 55–63.
- White, J. A., Richards, N. K., Laugraud, A., Saeed, A., Curry, M. M., & McNeill, M. R. (2015). Endosymbiotic candidates for parasitoid defense in exotic and native New Zealand weevils. *Microbial Ecology*, 70, 274–286.
- Whitten, M. M. A., Facey, P. D., Del Sol, R., Fernández-Martínez, L. T., Evans, M. C., Mitchell, J. J., ... Dyson, P. J. (2016). Symbiont-mediated RNA interference in insects. *Proceedings of the Royal Society B: Biological Sciences*, 283, 20160042.
- Wicker, C. (1983). Differential vitamin and choline requirements of symbiotic and aposymbiotic *S. oryzae* (Coleoptera: Curculionidae). *Comparative Biochemistry and Physiology Part A: Physiology*, 76, 177–182.
- Wicker, C., & Nardon, P. (1982). Development responses of symbiotic and aposymbiotic weevils *Sitophilus oryzae* L. (Coleoptera, Curculionidae) to a diet supplemented with aromatic amino acids. *Journal of Insect Physiology*, 28, 1021–1024.
- Woese, C. R. (1987). Bacterial evolution. *Microbiological Reviews*, 51, 221–271.
- Zhang, S., Shu, J., Xue, H., Zhang, W., Zhang, Y., Liu, Y., ... Wang, H. (2020). The gut microbiota in camellia weevils are influenced by plant secondary metabolites and contribute to Saponin degradation. *mSystems*, 5, e00692-19. Retrieved from <https://msystems.asm.org/content/5/2/e00692-19>

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