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DOI link to the version of record on the publisher’s site
MINOR REVIEW

Could bacterial associations determine the success of weevil species?

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Harper Adams University; Scottish Government's Rural and Environment Science and Analytical Services Division

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...
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 Acyrthosiphon 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 neaophidis (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 Seratia 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, Lefèvre, Delobel, Charles, & Heddi, 2002; Toju & Fukatsu, 2011). Importantly, Anbutsu et al. (2017) working on the black hard weevil Pachyrhynchus internalis (Fairmaire) showed that Ca. Nardonella is involved in insect cuticle formation by contributing to tyrosine synthesis. Suppressing Ca. Nardonella, by
| Bacterium                  | Weevil spp.                          | Symbiont function                              | Approach                                      | References                                                        |
|---------------------------|--------------------------------------|------------------------------------------------|-----------------------------------------------|------------------------------------------------------------------|
| *Candidatus Nardonella*   | *Pachyrhynchus infernalis*           | Tyrosine provisioning for cuticle synthesis    | Genomic, transcriptomic and functional analysis | Anbutsu et al. (2017) and Kuriwada et al. (2010)                  |
|                           | *Rhynchophorus ferrugineus*          |                                                 |                                               |                                                                  |
|                           | *Sipalinus gigas*                    |                                                 |                                               |                                                                  |
|                           | *Eusceps postfasciatus*              |                                                 |                                               |                                                                  |
| *Sodalis pierantonius*    | *Sitophilus* spp.                    | Tyrosine provisioning for cuticle synthesis    | Fitness and microscopy                         | Vigneron et al. (2014)                                           |
| (SPE)                     | *Sitophilus oryzae*                  | Synthesis of vitamins: pantothenic acid, biotin and riboflavin | Artificial diet with different compositions  | Wicker (1983)                                                    |
|                           |                                     | Bacteria provide mitochondria with vitamins   | Enzymatic assays                              | Heddi, Lefebvre, and Nardon (1993) and Heddi, Grenier, Khatchadourian, Charles, and Nardon (1999) |
|                           | *Sitophilus oryzae*                  |                                                 |                                               |                                                                  |
|                           |                                      | Methionine metabolism                          | Amino acid analysis                           | Grenier, Nardon, and Nardon (1986)                                |
|                           | *Sitophilus oryzae*                  | Reduced flight activity in insects deprived of bacteria | Artificial diet and behavioural assays       |                                                                  |
|                           | *Sitophilus zeamais*                 |                                                 |                                               |                                                                  |
| *Wolbachia*               | Tribe Naupactini                     | Induced parthenogenesis                         | Correlation between infection and parthenogenesis | Rodríguez, Confalonieri, Guedes, and Laneri (2010) and Elias-Costa, Confalonieri, Laneri, and Rodríguez (2019) |
| *Hypera postica*          |                                     | Induced cytoplasmic incompatibility            | Cross-mating, genetic analysis and microscopic localisation | Hsiao and Hsiao (1985a, 1985b) and Leu, Li, and Hsiao (1989)    |
| *Straphosoma melanogrammum* | Speciation induced through cytoplasmic incompatibility | Genetic analysis                              |                                               |                                                                  |
| *Straphosoma capitatum*   | Egg development                      | Egg hatching rate after antibiotic treatment   |                                               | Chen, Lu, Cheng, Jiang, and Way (2012) and Son, Luckhart, Zhang, Lieber, and Lewis (2008) |
| *Lissorhoptrus oryzophilus* | Cellulolytic activity to exploit their natural food source | Amplified ribosomal DNA restriction analysis |                                               |                                                                  |
| *Otiorhynchus suksatus*   |                                      |                                                 |                                               |                                                                  |
| *Gut and whole insect microbiota* | *Curculio* species               | Food source influences microbiota composition | Cloning                                       | Merville et al. (2013)                                           |
| *Hylobius abietis*        |                                      |                                                 |                                               |                                                                  |
| *Gut microbiota*          | *Dendroctonus armandi*               | Cellulolytic activity to exploit their natural food source | Amplified ribosomal DNA restriction analysis | Hu, Yu, Wang, and Chen (2014)                                    |
| *Rhynchophorus ferrugineus* |                                      |                                                 |                                               |                                                                  |
| *Gut microbiota*          | *Hypothenemus hampei*                | *Pseudomonas* bacteria detoxify caffeine       | Bacteria isolation and antibiotic treatment   | Ceja-Navarro et al. (2015)                                      |
| *Gut microbiota*          | *Curculio chinensis*                 | *Gut bacteria detoxify saponins*               | Illumina sequencing and fitness              | Zhang et al. (2020)                                              |
| *Whole insect microbiota* | *Dendroctonus ponderosae*            | *Microbiota is enriched with terpene degradation genes | Pyrosequencing                               | Adams et al. (2013)                                              |

(Continues)
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 *Eusceps 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; Lockey, 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.

| Table 1 | Bacterium | Weevil spp. | Symbiont function | Approach | References |
|---|---|---|---|---|---|
| Gut microbiota | Hylobius abietis | Gut bacteria degrade terpenes and contribute to host fitness | Illumina sequencing | Berasategui et al. (2017) |
| Gut and whole insect microbiota | Dendroctonus ponderosae | Geographic origin does not affect the microbiota composition | Pyrosequencing | Morera-Margarit et al. (2019) |
| Whole insect microbiota | Sitona obsoletus | Candidate bacteria involved in resistance to parasitic wasp used as a biological control | Pyrosequencing | White et al. (2015) |
| Gut and whole insect microbiota | Irenimus aequalis | Candidate bacteria involved in resistance to parasitic wasp used as a biological control | Pyrosequencing | White et al. (2015) |
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 coleoptericin 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 coleopterics family, named Coleoptericin-A, controls and restricts the population of its primary symbiont SPE inside the bacteriocytes (Login et al., 2011). This coleoptericin seems to be conserved as it interacts not only with SPE but also with Ca. Nardonella. Weevils treated with dsRNA targeting the coleoptericin-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 coleoptericin-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 Coleoptericin-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. ZEAMAIS 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 *Otiarhynchus 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 autoploidy. *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?

Rodriguero 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 melanogrammum* (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 co-inhabiting 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 (Chevolat) 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 Rahmella. 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 Moltyninae, 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 aethiopoides (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 nutritionally 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.

ACKNOWLEDGEMENTS

P.M.M. was funded by the James Hutton Institute and Harper Adams University through a joint PhD studentship. A.J.K. and C.M. were funded through the strategic research program funded by the Scottish Government’s Rural and Environment Science and Analytical Services Division. T.W.P. was supported by Harper Adams University. At the James Hutton Institute (Aberdeen, UK), we thank Dr Jenni Stockan for helpful comments on the manuscript. We thank two anonymous reviewers for their valuable comments and suggestions. We are grateful to Jaume Morera Margarit for creating the graphical illustration.

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How to cite this article: Morera-Margarit P, Pope TW, Mitchell C, Karley AJ. Could bacterial associations determine the success of weevil species? *Ann Appl Biol*. 2020;1–11. https://doi.org/10.1111/aab.12625