Review

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Wolbachia Across Social Insects: Patterns and Implications

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Abstract

Wolbachia is one of the most common intracellular symbionts among insects, but the function of this infection and its effects on the host still remain largely unknown for many taxa. In the case of many social insects, living in colonies results in close interactions, facilitating the dispersion of the symbiont. However, having only one caste responsible for reproduction (queens) could restrict infections. Several groups of social insects have been reported to be highly infected with these symbionts. However, *Wolbachia* associations across social and closely related non-social insects in a comparative framework has not been examined to date, which may help us understand the role social behavior, geography, and ecology plays in structuring this association. Since 2006 the Multi-Locus Sequencing Type (MLST) approach has been shown to be appropriate for diversity studies, which consists of sequencing five genes spread across the *Wolbachia* genome. Over the past 15 yr, studies using this approach have been conducted worldwide and have generated large volumes of data. In this review article, we intend to produce a meta-analysis by combining MLST data from across social insects (ants, bees, termites, wasps) and beyond, together with their closest relatives to better elucidate the patterns behind the diversity of this symbiotic interaction. Our main findings reveal that the diversity of *Wolbachia* (supergroups) is correlated with the host insect taxonomy, biogeography, and social/solitary behavior. In addition, solitary insects such as bees and wasps can interact with a greater diversity of *Wolbachia* supergroup compared to their social representatives.

Key words: symbiosis, wasp, bee, ant, termite

Wolbachia is a bacterium of the Alphaproteobacteria class and Rickettsiales order, first identified in 1924 in the *Culex pipiens* mosquito (Hertig and Wolbach 1924). Despite being discovered practically one century ago, the knowledge surrounding its biology and effects on its hosts has only recently accelerated, mainly in the last 25 yr, facilitated by the advance and accessibility of molecular sequencing techniques (Werren et al. 1995, Bandi et al. 1998, Zhou et al. 1998). Currently, it is estimated that 52% of arthropods species around the world are infected with this bacterium (Weinert et al. 2015). It is estimated that over 65% of all insect species are susceptible to infection (Hilgenboecker et al. 2008). However, there is still much to discover about the biological and ecological circumstances that facilitate the acquisition of this bacterium, as well as the immense diversity behind the rate of infection. Beyond the limited understanding of infection mechanisms, there is still no consensus on *Wolbachia*'s role in many of its hosts (Russell 2012).

This bacterium can develop parasitic, commensalistic, and mutualistic relationships with their hosts, and many *Wolbachia* species have become closely intertwined with the reproductive histories of

their hosts as a result (Correa and Ballard 2016). *Wolbachia* frequently engages with host reproductive systems and behaviors to manipulate reproduction in a manner that is beneficial to itself, often employing some combination of cytoplasmic incompatibility, male feminization, male killing, and induced parthenogenesis (Hilgenboecker et al. 2008, Werren et al. 2008, Correa and Ballard 2016, Richardson et al. 2016). However, it is still unclear whether all these functions apply to a strain or supergroup of *Wolbachia*'s diversity, or whether it varies between different hosts (Russell et al. 2009). The effects of this bacterium-induced changes are as complex as their hosts are diverse.

Wolbachia's ability to manipulate a diverse range of hosts has allowed it to spread worldwide (Russell et al. 2009). *Wolbachia* has also been detected in different tissues of the insect's body and in different stages of development. For ants, *Wolbachia* has been detected in eggs, larvae, pupae, workers, queen, and more specifically in queens ovarioles (Wenseleers et al. 1998, Van Borm et al. 2008, Frost et al. 2010, Andersen et al. 2012, Ramalho et al. 2017, Ramalho et al. 2018) as well as in the head, mesosome, and gaster of

the workers (Ramalho et al. 2019) and different tissues (Frost et al. 2010). And the same widespread pattern appears to also occur in beetles with *Wolbachia* found across all developmental stages and body parts of *Brontispa longissimi*, and the density of infection appears to be independent of sex, but more related to the host's life stage (Ali, Muhammad, and Hou 2018).

Wolbachia Supergroups

Wolbachia pipientis is an incredibly diverse endosymbiont, and despite having received this name after the host on which it was first identified, it is not clear whether it is just one species. Therefore, the most appropriate way to designate the diversity of infections linked to these taxa is through phylogenetically determined supergroups. Prior to the widespread use of Multi-Locus Sequencing Type (MLST) methods, *wsp* gene sequences were used to determine phylogenetic relationships between *Wolbachia* strains. This gene, acquired from the surface proteins of the bacterium, proved highly variable between different strains. The variety between individual strains is substantial enough that the single *wsp* gene can help resolve otherwise unclear phylogenetic relationships (Zhou et al. 1998).

Using the *wsp* method, most arthropod-associated *Wolbachia* supergroups are defined as A and B, the most common among arthropods (Baldo et al. 2006a, Lo et al. 2007). Supergroups C and D are restricted to nematodes (Bandi et al. 1998). Supergroups E and F have been found in *Collembola* (Czarnetzki and Tebbe 2003), arthropods (Panaram and Marshall 2007), and nematodes (Fenn and Blaxter 2004). Supergroup G is restricted to spiders (Rowley et al. 2004) and supergroup H are recovered in association with dampwood termites (Bordenstein and Rosengaus 2005). However, dependence on *wsp* alone may not always provide accurate results. In Martins et al. (2012), a *wsp* analysis of the ant genus *Solenopsis* determined that several of the screened strains belonged to supergroup B. An MLST analysis of the same samples revealed that all the strains actually belonged to supergroup A (Martins and Bueno 2014). The difference between the results provided by the two methods is clearly significant, and it is believed that the MLST result is more credible, as *wsp* has since appeared to be more susceptible to both positive selection and recombination, likely resulting in less reliable indication of phylogenetic relationships (Baldo et al. 2005; Baldo et al. 2006a, b; Werren et al. 2008). This recombination and positive selection can interfere in the resolution and inference of clades during phylogenetic analysis of *Wolbachia* and result in the assignment of incorrect supergroups.

Multi-locus sequence typing, or MLST, reduces the possibility of false relationships being drawn based on a single gene. MLST for *Wolbachia* sequencing relies on five conserved genes: *gatB*, *coxA*, *hcpA*, *ftsZ*, and *fbpA*. These housekeeping genes were selected on the basis of being present and widely distributed throughout the sequenced genomes and having demonstrated strong stabilizing selection within genus *Wolbachia* (Baldo et al. 2006a). This method has been proven effective in detecting strain diversity within a single host species and in recognition of similar strains across multiple hosts (Maiden et al. 1998, Baldo et al. 2006a).

While MLST provides a more accurate representation of supergroup phylogenetics, defining taxa by supergroups still limits information to earlier evolutionary events, such as the divergence of supergroups A and B between 58 and 67 million years ago (Werren et al. 1995). Without information from the finer phylogenetic scale, the complex interactions between *Wolbachia* and their arthropod hosts cannot be properly understood (Russell et al. 2009, Russell 2012). Still, supergroup identification illustrates important

evolutionary divergences that are still essential to our understanding of *Wolbachia*'s history.

Consequences of Having *Wolbachia*

There are four main known negative reproductive effects of *Wolbachia* on its hosts: cytoplasmic incompatibility, male feminization, male killing, and induced parthenogenesis (Werren et al. 2008). But there is also evidence of other effects such as *Wolbachia* can increase fitness by increasing fecundity and relative fitness in infected females mosquitoes (Zélé et al. 2018), providing nutrients to bed bug hosts (Hosokawa et al. 2010, Nikoh et al. 2014), and by reducing the duration between generations in parasitoid wasps (Reumer et al. 2010). Infections can induce thelytokous parthenogenesis in parasitic wasps (Kremer et al. 2009) and cause populations to split, which could eventually result in speciation. *Wolbachia* infections have been speculated to be a major factor in insect diversification.

In ants (Family Formicidae), the hymenopteran sex determination and eusocial lifestyle present unique challenges and opportunities for the *Wolbachia* symbiont. Due to their haplodiploid sex determination, it is unlikely that feminization could occur (Russell 2012). Seven ant species that exhibit parthenogenesis have been screened for and tested negative for *Wolbachia* infections (Wenseleers and Billen 2000, Himler et al. 2009), so it is unlikely that *Wolbachia* is the cause of parthenogenesis in those ant taxa.

It is possible that the primary functions of *Wolbachia* in Formicidae are entirely novel. Since worker ants are sterile females and typically take no part in the reproduction of the colony, *Wolbachia* can become absent from the workers and yet still be passed down maternally through the queen line (Russell 2012). This would remove any health detriments *Wolbachia* might have on the workers while maintaining the vertical transmission within the species. In the widely distributed Palearctic species *Formica truncorum* (Fabricius 1804), workers can be 'cured' of *Wolbachia* as they age (Wenseleers et al. 2002). This curing process is so far unique to ants, as other insects can lose infections due to transmission failure or exposure to extreme conditions but never as a systematic response. Clearing *Wolbachia* infections may occur in other members of Formicidae, such as *Formica exsectoides* and members of the neotropical leaf-cutter ant genera *Atta* and *Acromyrmex* (Keller et al. 2001, Van Borm et al. 2008, Frost et al. 2010). Most notably the males and workers of *Acromyrmex echinator* and *A. octospinosus* had lower infection rates than the queens (Van Borm et al. 2008). These infection clearing colonies can be discovered by looking for polymorphic infections within workers in the same colony, or a higher rate of infection in the queens than the workers. The fire ant *Solenopsis invicta* has been shown to have a similar rate of infection between all castes, males that steadily survive infections, and near perfect vertical transmission of the bacterium (Bouwma and Shoemaker 2011), so in this case the ants do not appear to clear infections systematically. In the east Asian myrmicine species *Vollenhovia emeryi*, colonies produce either long or short-winged queens. *Wolbachia* has been lost in a group of Korean short-winged *V. emeryi* but is still found in all long-winged populations as well as the short-winged Japanese population (Noh et al. 2020). A number of confounding factors also make loss of infection difficult to determine. For example, in Rey et al. (2013) *Wasmannia auropunctata* ants displayed an apparent loss of *Wolbachia* infections, but the researchers attributed this to increased temperatures of the new habitat and relaxed selection under the conditions of the habitat shift.

Wolbachia in ants could provide their hosts with essential nutrients in some cases. It is known that ants that are at extremes

of the food diversity scale, i.e., herbivores and predators, generally have more endosymbionts than the more generalist species in the middle with more diversity to their diets (Moreau 2020a). However, this may seem to directly contrast the *Wolbachia* clearing *Atta* and *Acromyrmex*, which have limited diets based on fungal farms (De fine licht and Boomsma 2010). Wider taxon sampling is needed to determine whether there is any correlation between foraging diversity and *Wolbachia* function in ants.

In addition, the function of *Wolbachia* in Formicidae is almost entirely unknown. Out of the four main observed routes of *Wolbachia* activity, it is likely that only cytoplasmic incompatibility and male killing have a potential to occur in ants (Russell 2012). Cytoplasmic incompatibility is incredibly hard to test due to the difficulty in breeding many different ant colonies and arranging artificial nuptial flights. Male killing likely does not occur in *S. invicta* (Bouwma and Shoemaker 2011), and it seems unlikely that it occurs in most other ants since the majority of ant species readily produce males. Besides this, two recent studies have pioneered experiments in the laboratory and tested the effects of *Wolbachia* on ant colonies: the first was able to verify that *Wolbachia* accelerates the life cycle of a colony of *Monomorium pharaonis*, since it accelerates the production of new queens and colony growth (Singh and Linksvayer 2020), and the second concluded that *Wolbachia* plays a role as vitamin B supplement in the phantom ant, *Tapinoma melanocephalum* (Cheng et al. 2019). Further studies should focus on understanding the effects of the different strains of *Wolbachia*, and their mechanisms in which it acts in different host species.

Another possible advantage that *Wolbachia* infections may offer for ants is defense against internal parasites and viruses. Ants are under attack from parasitoids such as phorid flies and parasitoid wasps (Quevillon and Hughes 2018), RNA viruses, and many other natural enemies on a day to day basis (Valles and Hashimoto 2009, Valles et al. 2009, Yang et al. 2010). In mosquitos, they can reduce loads of malarial *Plasmodium* parasites and filarial nematodes by promoting the upregulation of immunity gene expression (Kambris et al. 2009, 2010). *Wolbachia* can also protect against RNA viruses, as is the case in *Drosophila* (Hedges et al. 2008, Teixeira et al. 2008). Other bacterial symbionts such as *Hamiltonella*, *Serratia*, and *Spiroplasma* also protect their insect hosts from a variety of threats (e.g., Jaenike et al. 2010, Xie et al. 2010).

One species that can serve as a good ant model for these systems is *S. invicta*, a Neotropical fire ant with a wide native and invasive range spanning four continents. Attracting a stupendous number of researchers since its introduction to the United States in the 1930s, it has become the world's most well-known ant. *Solenopsis invicta* has over two thousand references to its name since its redescription in 1972 by Buren ('Web of Science [v.5.35] - BIOSIS Previews Basic Search' 2020), around threefold more than any other ant species (Wild 2009). With its large geographic range, it offers many natural experiments in progress to sample the relationship between *Wolbachia* infection and parasitoid/RNA virus control. Preliminary studies have shown that both *Wolbachia* infection status and RNA viruses vary across different populations (Valles and Hashimoto 2009, Yang et al. 2010). In these studies, it was found that the Argentinian population with the lowest *Wolbachia* infections also had the highest RNA virus prevalence. Further work is needed, but this could suggest that *Wolbachia* can help defend ants against viruses and other internal attacks.

Termites form another major clade of independently evolved eusocial insects. Instead of the haplodiploidy seen in ants, they exhibit X-Y chromosome sex determination (Lacy 1980). *Wolbachia* infections in termites are dominated by supergroup F, which can cause cytoplasmic incompatibility. Supergroup F *Wolbachia* have also been found to be an essential symbiont that synthesizes B vitamins in bedbugs and important nutrients in filarial nematodes (Nikoh et al. 2014), but their function in termites is not fully understood. Like in ants, parthenogenetic lineages of termites often lack *Wolbachia* infections (Matsuura et al. 2004, Hellemans et al. 2019, Yashiro and Lo 2019), so it is unlikely that the infections induce parthenogenesis. Non-MLST methods have also found widely distributed A & B supergroup *Wolbachia* in some termites (Baldo et al. 2006a, Lo and Evans 2007, Roy and Harry 2007), as well as a novel supergroup H found within the western U.S. dampwood termite species *Zootermopsis angusticollis* and *Z. nevadensis* using the genes 16S rDNA, dnaA, gltA, groEL, and ftsZ (Bordenstein and Rosengaus 2005). *Zootermopsis angusticollis* is an invasive species in Hawaii (Haverty et al. 2000) and a hybrid form of two *Z. nevadensis* subspecies (*Z. n. nevadensis* × *nuttingi*) has become established in Japan (Yashiro et al. 2018), so these would be interesting candidates to monitor for the status of supergroup H *Wolbachia* within introduced populations. Although the effects of *Wolbachia* infections on termites is not well understood, current literature makes a similar skew towards studies on supergroup determination and strain diversity (Salunke et al. 2010, Hellemans et al. 2019).

Apart from the ants, the presence of *Wolbachia* have not been as intensely studied throughout the broader Hymenoptera. It is known that both solitary and social bees are shown to harbor *Wolbachia* infections (Gerth et al. 2011,2015; Saeed and White 2015), including commercial honeybees of the *Apis mellifera* species group (Pattabhiramaiah, Brückner and Reddy 2011). Commercial honeybees are extremely important to the agricultural industry, contributing an estimated 8.3 to 16.4 billion dollars in pollination services in the United States alone (Losey and Vaughan 2006). These honeybees have experienced incredible and concerning declines in recent years, and it is believed that a major contributor to these losses involves the interactions between different microorganisms the bees both encounter and harbor (Brown and Paxton 2009, Aebi and Neumann 2011). If the key to understanding colony collapse disorder is identifying the interplay between the microbiota of honeybees, including *Wolbachia*, a great deal of research is required to fully understand the mechanisms by which *Wolbachia* and other microorganisms infect and proliferate in honeybees. Besides honeybees, eusocial bumblebees have also been commercialized for pollination services, but there is even less research on the presence of *Wolbachia* in eusocial bumblebees (Velthuis and van Doorn 2006, Gerth et al. 2015). Generally, studies on these select groups of Hymenopterans (bees and wasps) have also only just begun to scratch the surface of *Wolbachia*'s effects on its host, focusing primarily on presence or absence of infections, supergroups of each infection, and the phylogenies of infections in relation to host phylogenies (Gerth et al. 2011, Ahmed et al. 2015, Klopfstein et al. 2016).

Much of what we know about *Wolbachia* in wasps is from agriculturally significant species that are easy to collect. *Wolbachia* in solitary wasps can induce thelytokous parthenogenesis (Kremer et al. 2009), protect against hyperparasitism (Duplouy et al. 2015), cause cytoplasmic incompatibility (Gueguen et al. 2012), and reduce the number of males in the population (Reumer et al. 2010). In the ichneumonid wasp genus *Diplazon*, *Wolbachia* has been found to distort DNA barcoding and make it less reliable than morphological characters for identification, since the DNA barcoding gene, mtDNA COI, is maternally inherited. Additionally, *Wolbachia* may be the cause of mtDNA divergence with very little nuclear and morphological divergence in fig wasps (Xiao et al. 2012). Adult apheline wasps (*Eretmocerus* sp.

nr. *furuhashii*) have also been found to be horizontal vectors for *Wolbachia* in whiteflies (Ahmed et al. 2015). Unfortunately, there is not much on MLST studies with *Wolbachia* in social wasps. Besides these studies highlighted above, yet we still do not know very much about the consequences of having *Wolbachia* for most groups of insect hosts.

Wolbachia Acquisition Route

Wolbachia is known to be vertically transmitted and is passed from mothers to offspring (Narita et al. 2009, Bouwma and Shoemaker 2011, Gerth et al. 2013, Duplouy et al. 2015, Ali, Muhammad, and Hou 2018, Ali et al. 2018, Ramalho et al. 2018). For this reason, *Wolbachia* phylogenies often track host mitochondrial DNA, as mitochondria are also transferred maternally (Werren et al. 2008). Significant mismatches in host mtDNA and *Wolbachia* strain phylogenies could show ancestral acquisitions and losses of *Wolbachia* (Xiao et al. 2012). Hymenoptera are known to have fast rates of mtDNA evolution, and particularly the parasitic Hymenoptera (Raychoudhury et al. 2009). Furthermore, it is possible that *Wolbachia* infections can directly influence mtDNA divergence in infected taxa and populations (Klopfstein et al. 2016).

There is mounting evidence that *Wolbachia* can exhibit horizontal transfer in different hosts (Raychoudhury et al. 2009; Frost et al. 2010; Salunke et al. 2010; Stahlhut et al. 2010; Gerth et al. 2011, 2013; Symula et al. 2013; Zhang et al. 2013; Bing et al. 2014; Boivin et al. 2014; Morrow et al. 2014; Ahmed et al. 2015, 2016; Jiang et al. 2018; Shaikevich et al. 2019; Tseng et al. 2019). Horizontal transmission can occur through contact between host species, through environmental contamination, or direct interactions such as parasitism. Kleptoparasites of ants have displayed identical *Wolbachia* infections to their hosts, suggesting horizontal transmission. In these cases, the ant cricket parasitoids (*Myrmecophilus americanus*) depend on *Paratrechina longicornis* ants exclusively, relying completely on trophallaxis with ants to acquire food (Tseng et al. 2019). This tissue-level interaction also occurs between several other parasitoid-host symbioses and fosters a viable opportunity for horizontal transmission (Heath et al. 1999, Kronauer and Pierce 2011, Correa and Ballard 2016, Tseng et al. 2019). Also, parasitoid ovipositors can transmit *Wolbachia* infections between different host individuals (Ahmed et al. 2015). However, there are also cases where *Wolbachia* has not been horizontally transmitted even with close contact between potential host species, such as in the case of the obligate socially parasitic *Acromyrmex insinuator* and its host *A. echinator* (Tolley et al. 2019).

Evidence for horizontal transmission within a species is scarce, but solid evidence has been found for *Wolbachia* transmission via parasitoid wasp attack in the whitefly species *Bemisia tabaci* (Ahmed et al. 2015). It is likely that whiteflies can also pick up *Wolbachia* B and O supergroups from plant surfaces (Bing et al. 2014). Horizontal transmission has also likely occurred at least once in tsetse flies (Symula et al. 2013). The lack of congruence between host phylogenies and that of the corresponding *Wolbachia* infections suggests that horizontal transmission must be at play between species (O'Neill et al. 1992, Cordaux et al. 2001, Ahmed et al. 2013a, Tolley et al. 2019). This trend of incongruence was also been observed between host mitochondrial DNA (mtDNA) and nuclear DNA (nuDNA) markers. This difference is indicative of different evolutionary events (likely horizontal transmission between species) occurring to alter mtDNA and nuDNA along different paths (Tseng et al. 2019). Apart from the apparent 'jumps' made by infections between more distantly related host taxa, little observational evidence

(such as observed trophallaxis between parasitoids and their hosts) exists to account for each and every instance of horizontal transmission (Russell 2012). Despite this, artificial transfers between different insect genera have proved successful in lab settings, suggesting that the infection of more evolutionarily distant species is plausible (Zabalou et al. 2004, Hoffmann et al. 2011). What is known are the minimum conditions required for horizontal transmission to occur, from Tolley et al. (2019): '1) there must be suitable environmental conditions (in the new host as well as the medium/environment the bacterium transitions through), 2) the bacterial strain must have the genetic potential for transfer, and 3) there must be a mechanism that will mediate the HT event'. Besides the evidence suggesting horizontal transmission between less-related species, several studies have also observed related *Wolbachia* infections inhabiting related hosts (Baldo and Werren 2007). However, cospeciation is incredibly rare, and the general incongruence between host and *Wolbachia* phylogenies still yields the idea that strict maternal transmission is not a realistic possibility (Russell 2012).

Furthermore, the rarity of cospeciation is also indicative of the possibility that most infections transmitted horizontally do not persist beyond an individual or a generation (Russell 2012). And beyond the loss and gain of infections on an individual level, strains of *Wolbachia* have been gained and lost multiple times within species. Tseng et al. (2019) hypothesize that this pattern, visible in *Paratrechina longicornis* ants with the *w*LonF strain, is possibly a result of *w*LonF having a commensalistic relationship with the ant. Because of the negligible effects of the symbiont, substantial selection would not be a present factor in fostering a closely developed symbiotic relationship. The loss of *w*LonF occurs at a very low rate similar to horizontal transmission of the strain, allowing it to persist in the host (Tseng et al. 2019). The multiple gains and losses of infections among individuals, generations, and entire populations complicate discovery of the true relationships between *Wolbachia* and its hosts.

Gaps in the Knowledge of *Wolbachia* and Social Insects

Wolbachia's effects are of unique interest within the eusocial insects. Ants and other eusocial insects with similar social structures develop female-dominated colonies to maintain caste ratios necessary for colony function. This specialized haplodiploidy presents a potential opportunity for *Wolbachia*, which proliferates well under female-skewed sex ratios (Russell 2012). MLST analyses of ants with single infections showed the vast majority belonging to supergroup A, and the remaining few belonging to supergroup B and F (Baldo et al. 2006a, Frost et al. 2010, Russell 2012, Ramalho et al. 2017, Kelly et al. 2019). This trend corroborated similar findings from analyses within a more broadly Hymenopteran clade, suggesting that the Hymenopterans in these studies were possibly predisposed to infections by supergroup A (Russell 2012). However, Russell (2012) also raised the possibility that one of the supergroup clades gained a superior ability to colonize Hymenopteran hosts, or that one of them lost a function that resulted in a significantly reduced frequency of infections.

Despite ants being the most thoroughly sampled invertebrate order, there are still knowledge gaps concerning the various consequences of *Wolbachia* infections within the clade. The wide sampling of ants allows broad study of the biology, ecology, and evolution of these insects, but lacks sufficient observations and data to reach a similar depth of knowledge with regards to understanding the interactions between *Wolbachia* and ants (Russell 2012).

Between social and solitary insects, there is a strong difference in diversity of ecological niches, and possibly a disparity between the amount of inter-species interactions individuals of different social-ites would encounter as a result. For example, within Hymenoptera, social bees have been found to perform more generalized foraging, while solitary bees fulfill more specialized niches (Biesmeijer et al. 2005). At the same time, the success of eusocial colonies has also resulted in great success and geographic diversity for *Cephalotes atratus* ants (Kelly et al. 2019). It is possible that such expansion of geographic range could expose a species to a greater opportunity for transmission. Other social taxa we incorporated into this study included solitary and social bees and wasps, and termites (eusocial). Many of these studies focusing on specific Hymenopteran genera give a glimpse of the depth of knowledge to be discovered by conducting MLST analyses across a variety of insect taxa.

Studying the finer-scale phylogenetic relationships of a specific group can reveal specific instances of horizontal transmission, codivergence, double infections, and induced parthenogenesis (Kremer et al. 2009, Raychoudhury et al. 2009, Reumer et al. 2010, Boivin et al. 2014, Ahmed et al. 2015, Saeed and White 2015). Similarly, horizontal transmission also appears to be a strong possibility within multiple termite genera, and certain infections have been shown to be completely pervasive throughout the individuals of a colony, regardless of caste, sex, or sexual versus parthenogenetic origin (Salunke et al. 2010, Hellemans et al. 2019b).

In this study, we reviewed trends of *Wolbachia* infections by focusing on social insects (ants, bees, termites, and wasps) and other insects (planthoppers, mosquitoes, beetles, etc.) that exhibit a wide range of varying social behaviors but are differentiable by the presence (eusocials) or absence (non-eusocials) of cooperative brood care, overlapping generations, and differentiated adult castes (Wilson and Hölldobler 2005). Our main goal was to search for manuscripts with *Wolbachia* screening by MLST approach that provides certain robustness to the data (since five different genes are analyzed) and a broader context through meta-analysis. This present study has compiled data since 2006 (15 yr of study), and our review pulled from over 60 manuscripts to collect information about the species with *Wolbachia* infections (more than 400 species were included, including more than 200 ants, bees, termites, and wasps harboring *Wolbachia* infections). We report supergroup, social or solitary status, and finally, the biogeography from which the infection was isolated. In addition to looking for trends and associations in *Wolbachia* infection patterns for social insects, our data also uncover the gaps where few hosts have been examined using the MLST technique. Therefore, our study also serves as a guide for future studies that should aim to try to recover important missing data to understand more about the complex association of this bacterium with host insects.

Methods

All our literature research has focused on compiling observations from insects that have been shown to be positively infected with *Wolbachia* and have been screened by the MLST method. Our data compiled data from about 60 manuscripts and more still unpublished available from the MLST *Wolbachia* database (https://pubmlst.org/Wolbachia/), accounting for about 700 observations of *Wolbachia* associated with insects. After this active search, we created a table with information about the insect's taxonomy, social/solitary behavior status, recovered *Wolbachia* supergroup, and biogeography. This table is available in Supp Material 1 (online only).

For the meta-analysis, we consider each *Wolbachia* observation present in different species, locations, and different supergroups as a unique observation. Also, as several studies we included can be based on the same observations of *Wolbachia*, a careful search for duplicates was carried out to avoid bias in our statistical analyzes. Our analysis of these data sought to apply Pearson's χ^2 test with simulated p-value by Monte Carlo simulation and based on 5,000 replicates to investigate the associations of the variables collected in this meta-analysis. All analyzes and figures were conducted in R (R Core Team 2020) using the dplyr package (Wickham et al. 2015).

Results and Discussion

Social and Solitary Insects

In this study, we evaluated more than 400 species of insects with distributions worldwide, with more than 200 species of social insects specifically (ants, bees, termites, and wasps) from studies that used the MLST methodology to screen for *Wolbachia* (positive), including the *gatB*, *coxA*, *hcpA*, *ftsZ*, and *fbpA* genes. Using these criteria, we obtained the following *Wolbachia* data: ants *n* = 82, bees *n* = 156, termites *n* = 8, and wasps *n* = 94 (Fig. 1A).

In general, our survey confirms that the most common supergroups among insects are A and B among social insects of the order Hymenoptera, as is the case of ants, bees and wasp (Fig. 1B) (Werren and Windsor 2000). Except for termites (Order Blattodea) that mainly are infected with strains belonging to supergroup F (Fig. 1B). Some ants, bees, and wasps were infected by supergroup F, but they are rare, according to our data (Fig. 1B). We also documented the geographic location of the host Families infected with *Wolbachia* (Fig. 1C). Efforts for future studies that seek to understand more about the *Wolbachia*-host relationship should use the present study as a guide to target new hosts and locations.

Our survey also recovered some less frequent supergroups among insects, such as supergroup O, in *Bemisia tabaci* (Hemiptera: Aleyrodidae), a solitary whitefly found in Anhui, China, and the authors believe it came from horizontal transmission from plants (Bing et al. 2014). Another less frequent supergroup was supergroup H, in *Zootermes angusticollis* (Blattodea, Archotermopsidae), a social termite found in the USA (Dr Laura Baldo depositor, from MLST database). Both supergroups are singletons therefore we excluded these two supergroups (O and H) from the subsequent statistical analyses.

Across all the species we studied we only found six species that were infected with two different *Wolbachia* supergroups. Five of them were wasps with solitary behavior and one species of ant, and therefore social (Supp Material 2 [online only]). Our data suggest that these species do not follow a biogeography pattern, for example the species *Nasonia vitripennis* had supergroups A and B, in the United States, Russia, and the Netherlands. In addition, the study reporting this observation suggested that the host acquired the different *Wolbachia* supergroups via horizontal transmission (Raychoudhury et al. 2009).

Below, we will focus our analyses on testing whether the different variables collected in this meta-analysis have a significant association, in an attempt to understand the trends and facilitators of *Wolbachia* diversity. Firstly, our analyses focused on analyzing ants, bees, termites, and wasps grouped to reveal the big picture of *Wolbachia* infection across these insects, mainly from the point of view of biogeography and social/solitary status behavior.

The results of the meta-analysis confirm that there are associations of the variables examined: 1) diversity of *Wolbachia* (supergroups) and the different social insects in the present study
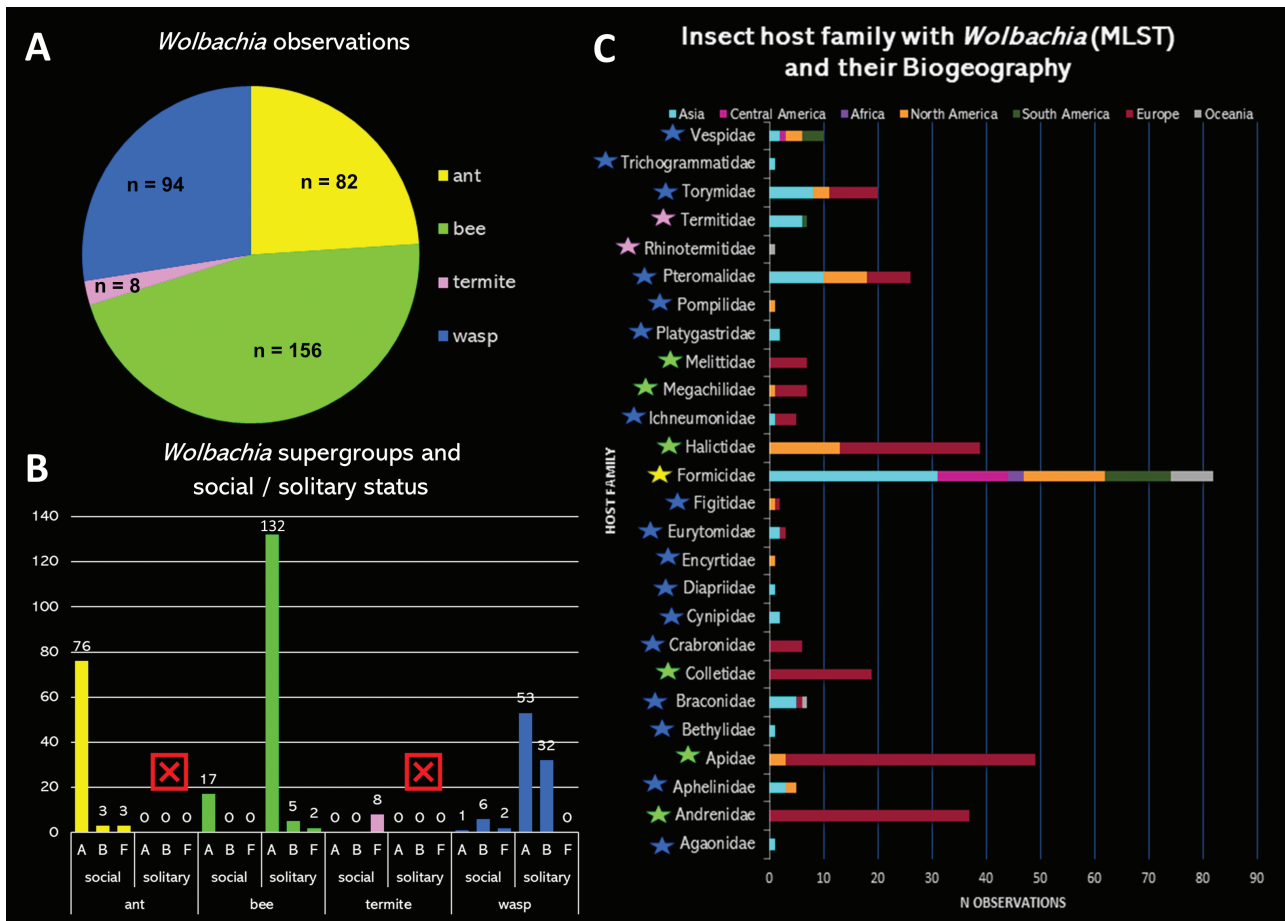
Fig. 1. *Wolbachia* observations recovered by the MLST method in this meta-analysis study. (A) *Wolbachia* observations in each insect group. (B) *Wolbachia* supergroups recover in social and solitary insects. The red X indicates that there are no ants and termites with solitary behaviors. (C) Observations of *Wolbachia* in the different host families. The highlighted stars indicate the group of insects the family belongs: yellow star indicates ant; green star indicates bee, pink star indicates termite, and blue star indicates wasp.

(χ^2 = 263.25, P = 0.0004, and Fig. 2A); 2) *Wolbachia* diversity (supergroups) and host biogeography (χ^2 = 41.492, P = 0.0016, and Fig. 2B) and 3) *Wolbachia* diversity (supergroups) and social / solitary behavior status (χ^2 = 23.086, P = 0.0004, and Fig. 2C).

To explore whether this finding for social insects is true for other orders of insects, we included just solitary insects: Coleoptera n = 48; Diptera n = 74; Hemiptera n = 20; Hymenoptera n = 250; and finally, Lepidoptera n = 186. The results for insect order (χ^2 = 235.47, P = 0.0004) and also biogeography (χ^2 = 170.98, P = 0.0004) are also associated with the diversity of *Wolbachia* supergroups, therefore, the same pattern explored for social insects is found across all insects we examined (Fig. 3).

Social and Solitary Status Affecting *Wolbachia* Diversity

As mentioned before, bees and wasps include solitary and social species (Gerth et al. 2015, Ahmed et al. 2016). Between the solitary and social bees, solitary bees fulfill a broader variety of niches, therefore experiencing different ecological interactions than their social counterparts (Biesmeijer et al. 2005). Because of the variation within these two insect groups, we believe that their inclusion in this study and future *Wolbachia*-based research is essential to understand the different consequences of *Wolbachia* infection between social and solitary insects.

Our data also show that solitary bees and wasps have been more frequently studied to understand the association of *Wolbachia* by the MLST method than that of individuals who exhibit social behavior and live in colonies (Saeed and White 2015). However, our compiled data shows that there is an association between the different *Wolbachia* supergroups and biogeography (χ^2 = 99.062, P = 0.0003) and also for *Wolbachia* supergroup and social/solitary status (χ^2 = 7.826, P = 0.028) (Table 1). In addition, solitary status showed greater diversity of *Wolbachia* (different supergroups) compared to social insects (Fig. 1B), which may suggest that solitary status may serve as a promoter of diversity in the acquisition of *Wolbachia*. To confirm this trend regarding the association of the insect's social and solitary behavior and its interaction with *Wolbachia*, new studies should focus on increasing social representatives of these insects.

We will now focus our study on looking for trends in *Wolbachia* infections within each of the insects analyzed separately.

Ants

Thirty-six ant genera were included in this study and the genus *Pheidole* having the most MLST data (n = 16) with representatives of species from Asia, Oceania, and North America. Of all the insects studied here, *Wolbachia* studies by the MLST method of ants have a largest sampled distribution (Asia n = 31, Central America n = 13, North America n = 15, South America n = 12, Africa n = 3, and
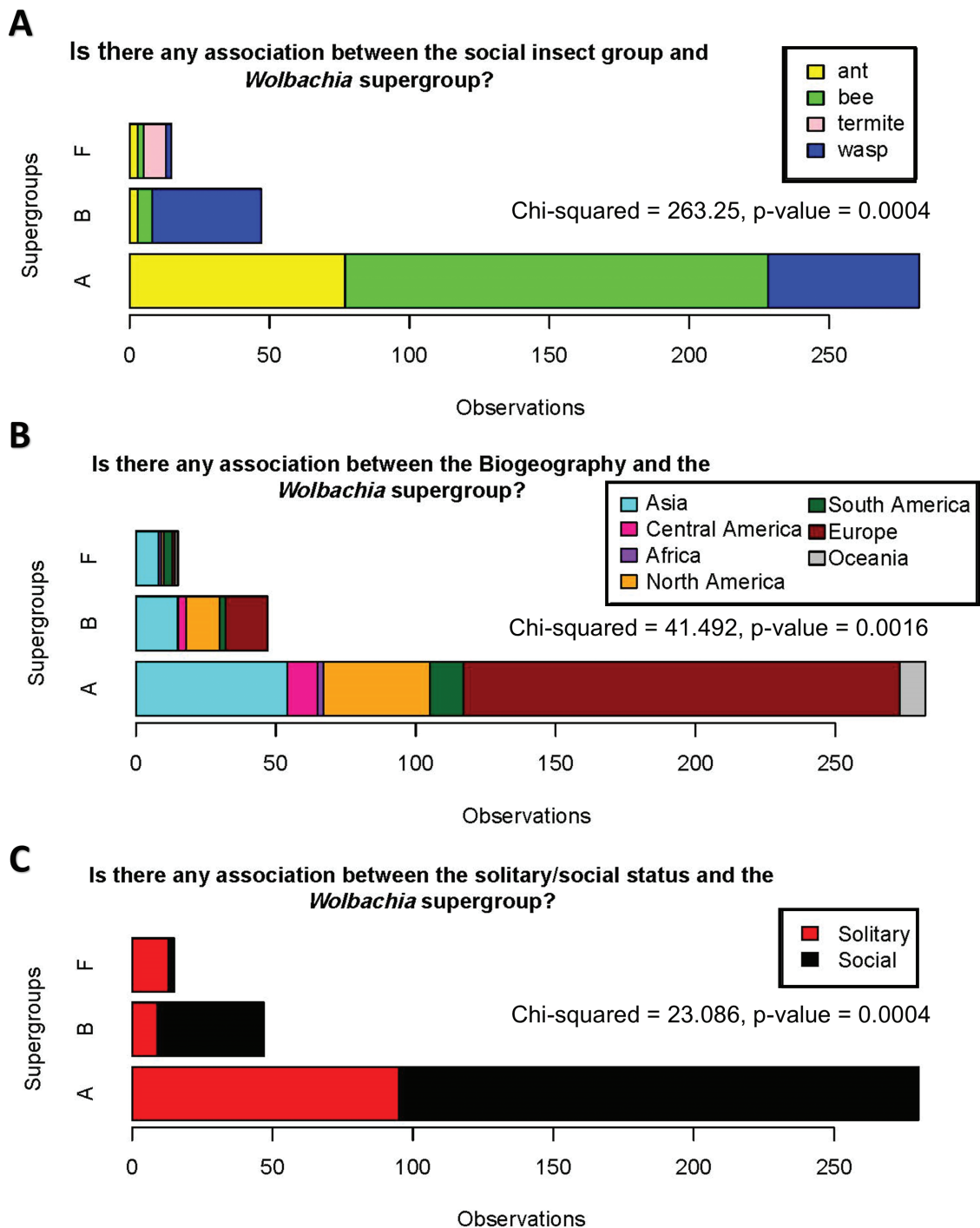
Fig. 2. Exploring the associations of the variables collected regarding *Wolbachia* Supergroups and hosts information in this meta-analysis study. (A) Association between *Wolbachia* supergroup and host insect group. (B) Association between *Wolbachia* supergroups and host biogeography. (C) Association between *Wolbachia* supergroup and social/solitary status host behavior.

Oceania *n* = 8) and still, there are few studies conducted with species from Africa and Oceania (Russell et al. 2009, Rey et al. 2013, Ahmed et al. 2016). Interestingly, there is no data for any *Wolbachia* (MLST) associated with ants from Europe (Fig. 4). Our data support other findings for ants, showing that supergroup A (*n* = 76) is the most common for the family, with only a few representatives of supergroup B (*n* = 3) and F (*n* = 3). Thus, our meta-analysis confirms that there is no association between *Wolbachia* supergroups and biogeography (χ^2 = 17.3, *P* = 0.078, and Table 2).

In the previous 2012 *Wolbachia* review by Russell, guiding questions were posed for future research on *Wolbachia* in ants. Below we will address these questions by adding updates since Russell (2012).

Does CI Occur in Ants?
The first recorded case of cytoplasmic incompatibility in ants was found with supergroup A *Wolbachia* in the global tramp ant *Cardiocondyla obscurior* (Ün et al. 2020). Originating from SE Asia,
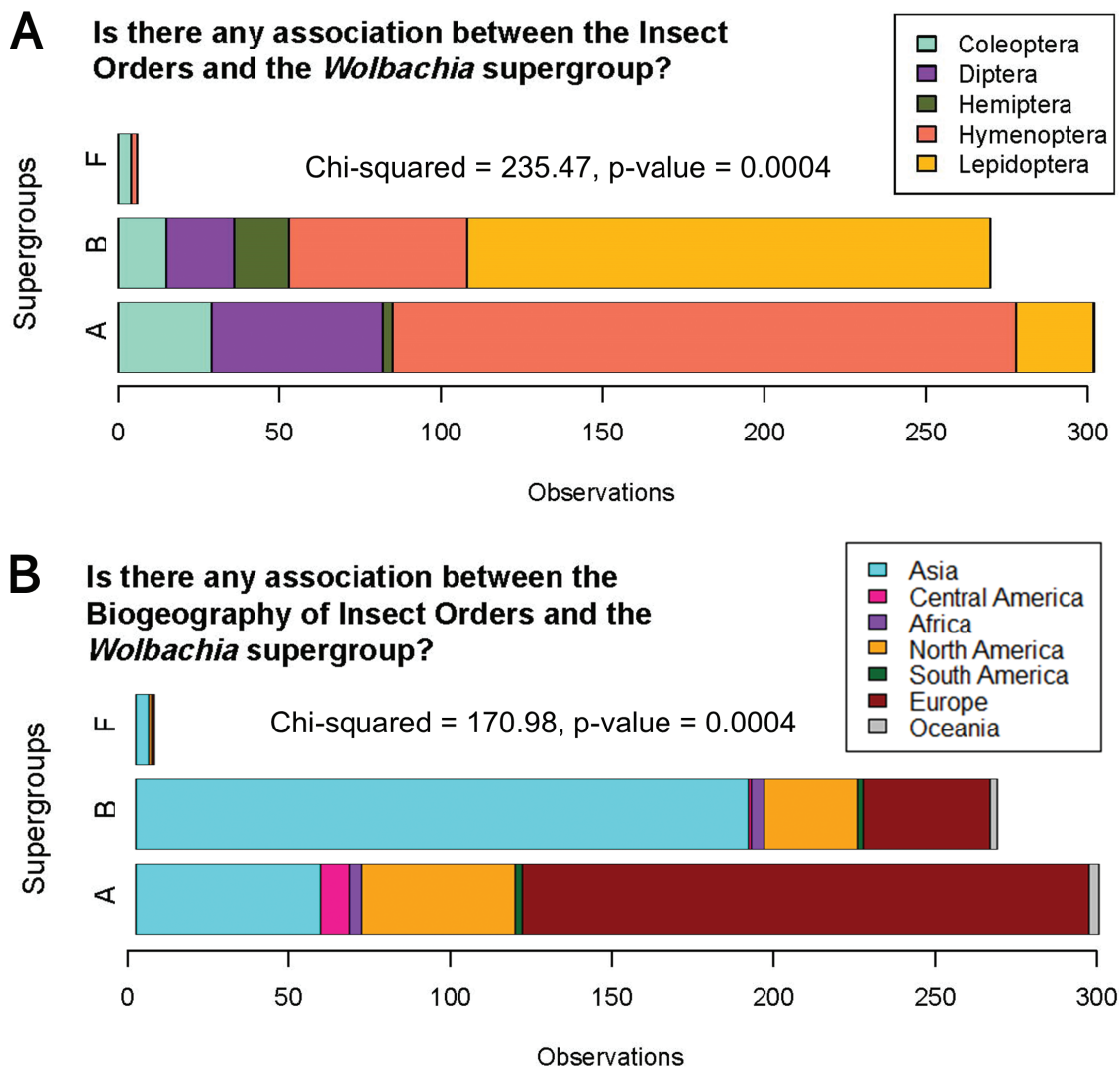
Fig. 3. Exploring the associations of the variables collected regarding *Wolbachia* supergroups and other insect hosts Orders with insects' information in this meta-analysis study. (A) Association between *Wolbachia* supergroup and host insect Order. (B) Association between *Wolbachia* supergroups and host biogeography.

Table 1. Meta-analysis applied to test the influence of social behavior/solitary status, biogeography, and different *Wolbachia* supergroups

Insects	*Wolbachia* supergroup and biogeography?	*Wolbachia* supergroup and social/solitary status?
Insects that have social and solitary behavior		
Bee and Wasp	$\chi^2 = 99.062$, $P = 0.0003$ ✓	$\chi^2 = 7.826$, $P = 0.028$ ✓
Insects that have just social behavior		
Ant and Termite	$\chi^2 = 16.179$, $P = 0.105$	N/A only social species

this inconspicuous myrmicine ant now has a vast introduced range across warmer areas of the world. This species is known to produce both winged and ergatoid males (Seifert 2003, Fuessl et al. 2018). Researchers found that introduced Japanese and Brazilian populations exhibited CI when crossed, but this could be cured when *Wolbachia* was removed (Ün et al. 2020).

Can *Wolbachia* Defend Ants Against Natural Enemies?

Ants are a valuable resource for many parasitoids and internal parasites and are constantly under attack. *Wolbachia* may perform the same functions as has been found in other insects (Hedges et al. 2008; Teixeira et al. 2008; Kambris et al. 2009, 2010), but there have not been significant studies on this effect of *Wolbachia* in ants.

Do Ants Serve as Melting-Pot Hosts?

This is still particularly difficult to test, especially with the *wsp* and MLST method. Often when multiple infections are detected they are only reported, but not explored (Russell et al. 2009, Ramalho et al. 2017, Kelly et al. 2019).

Is Loss From Workers a Common Phenomenon?

Wolbachia has been reported as being lost from workers of *Acromyrmex* and *Formica* (Keller et al. 2001, Wenseleers et al. 2002, Van Borm et al. 2008, Frost et al. 2010, Russell 2012). In addition, studies of *Camponotus textor* and *Cephalotes atratus* show different rates of *Wolbachia* infections within the same colony (Ramalho et al. 2017, Kelly et al. 2019, Reeves et al. 2020),
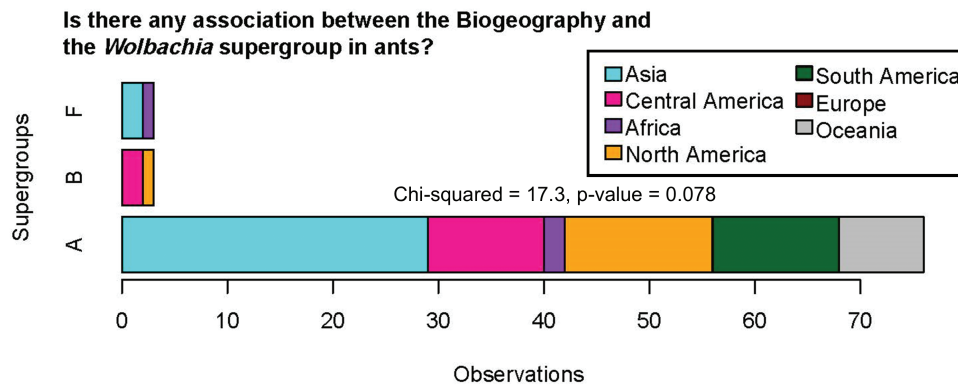
Fig. 4. *Wolbachia* supergroups associated with ants and their biogeography. Note the most common supergroup is A and there are no representatives from Europe.

Table 2. Meta-analysis applied to each group of insects analyzed separately

Insects	*Wolbachia* supergroup and biogeography?	*Wolbachia* supergroup and social/solitary status?
Ant	$\chi^2 = 17.3$, $P = 0.078$	N/A only social species
Bee	$\chi^2 = 3.814$, $P = 0.208$	$\chi^2 = 0.896$, $P = 0.723$
Termite	$\chi^2 = 6.250$, $P = 0.060$	N/A only social species
Wasp	$\chi^2 = 53.554$, $P = 0.007$ ✓	$\chi^2 = 24.304$, $P = 0.0004$ ✓

Note that only wasps seem to have associations between the *Wolbachia* supergroup and biogeography and social/solitary behavior status.

which could be explained by the fact that some workers lose the infection at a certain stage of development as has been observed for leaf-cutting ants (Van Borm et al. 2008). However, to understand the processes and mechanisms behind the loss of infection by *Wolbachia* in ant workers, it still needs to be better explored. In addition to the observed losses of infections for ants, in the termite, *Cubitermes* from Gabon were found to have different rates of *Wolbachia* infection amongst different castes. Alates had the most infections, while amongst non-alates the white workers (transitional stage before mature soldiers) exhibited higher infection rate compared to other workers (Roy et al. 2015), and this could be related to the loss of *Wolbachia* in workers or a higher fidelity of vertical transmission in the reproductive caste.

Does the Mode of Colony Founding Truly Correlate With *Wolbachia* Prevalence?

Russell (2012) noted that previous studies (Wenseleers et al. 1998) had shown ant species with parasitic, colony budding, or generally non-independent lifestyles had been observed to harbor *Wolbachia* more frequently. The authors did not find this trend with their entire data due to extensive sampling within the primarily independently founding genera of *Formica*, *Atta*, and *Acromyrmex* that may have skewed the analysis. When they removed these data, the trend became statistically significant. Our review did not test for *Wolbachia* prevalence due to mode of colony founding because of our incomplete knowledge of the life histories of most species.

Bees

Solitary bees are particularly prone to *Wolbachia* infections, and recent screenings suggest that they are even more susceptible than previously thought, as new MLST data reveals additional species that

were previously not known to carry infections (Gerth et al. 2015, Saeed and White 2015). With regards to transmission, a study suggested that *Wolbachia* seems to follow patterns of vertical transmission more dominantly than previously thought (Gerth et al. 2013). The activity of horizontal transmission is also supported by MLST analyses demonstrating bees from the same region can harbor identical *Wolbachia* strains at a rate higher than expected by chance (Gerth et al. 2015). Each of these studies contributes a notable piece of information to the larger picture of *Wolbachia* in bees, which are known to carry infections by supergroups A, B, and F (Gerth et al. 2011, Ahmed et al. 2013b, Gerth et al. 2013, Glowska et al. 2015, Saeed and White 2015, Ahmed et al. 2016, Sonet et al. 2018). Of these supergroups, supergroup A is most commonly found in bees and generally throughout studied Hymenoptera (Russell et al. 2009, Gerth et al. 2015).

Our study included 23 bee genera, and the best-studied genus using the MLST method was *Andrena* ($n = 38$). Our results show that most of the bees studied were sampled from Europe, and only a few individuals were from North America. All other locations (Asia, Africa, Central America, Oceania, and South America) have not been tested for *Wolbachia* using the MLST method, indicating that future work should prioritize the study of bees (both solitary and social) in these locations to inform a global understanding of the *Wolbachia* diversity associated with bees.

Wasps

Most MLST studies focused on solitary parasitoid wasps. According to Klopfstein et al. (2016), parasitoids may be especially susceptible to *Wolbachia* infections, and many of the wasps screened in this study had multiple (double and triple) infections as well. Parasitoid wasps have also been shown to act as phoretic vectors of *Wolbachia* between hosts (Ahmed et al. 2015). In this closely studied interaction, the parasitoids did not appear to be affected by the *Wolbachia* infection, and were thus labeled as phoretic vectors, or bodies of transmission in a commensalistic relationship with the infection. The researchers concluded that the wasps were able to transmit *Wolbachia* for up to 48 h after feeding or ovipositing on an infected whitefly (Ahmed et al. 2015). Another parasitoid wasp, *Leptopilina victoriae*, appears to be similarly unaffected in its role as a vector for *Wolbachia* into drosophilid hosts (Gueguen et al. 2012). In the two last-mentioned studies, the affected hosts (*Bemisia* whiteflies and *Drosophila* flies) interestingly benefited from the *Wolbachia* infections. Immatures developed at a faster rate, and adults survived in increased proportions (Gueguen et al. 2012, Ahmed et al. 2015).

In this present study, 39 wasp genera were included, and the genus best studied by the MLST method is *Nasonia* (*n* = 30). In wasps, our data suggest that there is an association between solitary behavior and specific *Wolbachia* supergroups (χ^2 = 24.304, *P* = 0.0004, and Table 2). To confirm whether the behavior of being solitary facilitates *Wolbachia* infection, further studies sampling a greater number of social wasps are necessary to provide comparative statistics. The biogeography of the host also seems to impact the diversity of *Wolbachia* (supergroups) for wasps (χ^2 = 53.554, *P* = 0.007, and Table 2). In addition, as is the case with *Wolbachia* MLST data for bees, the wasp data occupies a greater distribution throughout Asia, Europe, and North America. Other locations have few representative samples (Central America with *n* = 1, Oceania with *n* = 1, and South America with *n* = 4). Very little data is available for the social wasps beyond supergroup data, which falls in line with data for solitary counterparts: supergroup A is the most represented throughout the sampled individuals from the order (Russell et al. 2009, Gerth et al. 2011).

Termites

Termites are a special group of social cockroaches (Moreau 2020b, Inward, Beccaloni and Eggleton 2007, Pellens et al. 2007, Ware et al. 2008), and tracking *Wolbachia* supergroups and prevalence throughout *Blattodea* relating to termites will prove interesting. The termite MLST sequences available are all from the higher termites (Family *Termitidae*). Our review did not find any MLST data for solitary roaches or anything in between. Using only *wsp* sequencing, it has been shown that some lower termites harbor A supergroup *Wolbachia*, while a *Cubitermes* sp. *affinis subarquatus* harbors diverse strains from both A & B supergroups (Baldo et al. 2006a, Lo and Evans 2007, Roy and Harry 2007). To understand the diversity and infection rate by *Wolbachia* within this eusocial insect group, the east Asian and North American subsocial wood roaches in the genus *Cryptocercus* (Maekawa and Nalepa 2011) and the eight families of lower termites (Krishna et al. 2013) need to be screened for *Wolbachia*.

In search of more data in the database, we found a unique observation of *Wolbachia* in *Zootermopsis angusticollis* (dampwood termites, *Archotermopsidae* family) of the supergroup H in the United States (deposited by Laura Baldo) by the MLST methodology. This sample was excluded from the statistical analysis because it was a single observation; however, it will be important to assess whether there are differences between the termite strains belonging to the New and Old World. Outside of the MLST methodology, two termite species also have been found to harbor supergroup H *Wolbachia* (*Zootermopsis angusticollis* and *Z. nevadensis nevadensis* × *nuttingi*) and they have been inadvertently introduced to Hawaii and Japan respectively, and monitoring of these populations and applying the MLST methodology proposed by Baldo et al. (2006a) could help researchers understand the origin and dispersion of this supergroup among the termites (Bordenstein and Rosengaus 2005).

Three termite genera were included in this study and the genus most studied by the MLST method is *Odontotermes* (*n* = 6). Termites in general have few studies involving symbiosis with *Wolbachia* by the MLST method (Salunke et al. 2010). Our data are based on six observations from Asia (India), while South America (Brazil, French Guiana, and Trinidad) and Oceania (Australia) have only one study each. All other geographic regions do not have any MLST data from termites. Even so, our data reveal that all individuals have strains belonging to the supergroup F, confirming to be a standard for the group.

Our data indicate that more effort is needed to increase *Wolbachia* (MLST) sampling for termites, because only a few studies focus on understanding this symbiosis (data retrieved from MLST database from depositor Yogesh Shouche, Baldo and Werren 2007, Salunke et al. 2010, Hellemans et al. 2019a). Even so, these results from Australia, Brazil, French Guiana, India, and Trinidad corroborate that Supergroup F's *Wolbachia* is common among termites.

Conclusion

Combining the trends of *Wolbachia* infection with our knowledge of the biology of eusocial and solitary insects, can uncover the patterns that promote *Wolbachia* infection and its success among different groups of insect hosts around the world. Our data reveal that solitary insects such as bees and wasps can interact with a greater diversity of *Wolbachia* supergroup compared to social ants, bees, and wasps. Although the reason for this pattern is not clear two possible explanations for the lack of diversity in social compared to solitary insects are 1) solitary insects have more individual interactions with other taxa, and 2) social insects inhabit a smaller number of ecological niches than solitary species. In general, the supergroup diversity of *Wolbachia* is correlated with the host insect taxonomy, biogeography, and social/solitary behavior. We hope this study can serve as a guide for sampling to increase our understanding of the diversity of *Wolbachia*-host interactions across the insects.

Supplementary Material

Supplementary data are available at *Annals of the Entomological Society of America* online.
Supplementary material 1. Complete meta-analysis data used in this study.
Supplementary material 2. Host species that showed infection with two different *Wolbachia* supergroups and localities.

Acknowledgments

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