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Occurrence of three intracellular symbionts (*Wolbachia*, *Arsenophonus*, *Cardinium*) among ants in southern China

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ABSTRACT

Many ants are commonly infected by maternally inherited endosymbionts. We examined the prevalence of three bacterial endosymbionts (*Wolbachia*, *Arsenophonus*, and *Cardinium*) in ant populations in southern China. The results showed that the proportion of ant samples infected by *Wolbachia* and *Arsenophonus* was 30.20% and 18.79%, respectively. *Cardinium* was not found in any of our samples. Our results also indicated that more than 50% of the ant species (30 of 56) tested were infected by maternally inherited bacteria. Of the 56 ant species, 35.71% were infected by *Wolbachia* and 37.50% were positive for *Arsenophonus*. Co-occurrence of *Arsenophonus* and *Wolbachia* was found in the same colony in 7 of 56 ant species. This study suggests that *Wolbachia* and *Arsenophonus* are the main inherited bacteria in ants in southern China. These endosymbionts may have strong impacts on ant biology and can be manipulated for pest management.

1. Introduction

Microorganisms infect many arthropods and shape the ecology and evolution of their hosts (Weeks et al., 2003; Russell et al., 2009; Russell et al., 2013). Many endosymbionts have positive effects on their hosts. For instance, endosymbiotic *Blochmannia* bacteria live in insect internal organs and support the nutrition of the host (Feldhaar et al., 2007). Several endosymbionts, including the genera *Wolbachia*, *Cardinium*, *Rickettsia*, *Spiroplasma*, *Flavobacteria*, and *Arsenophonus*, cause a variety of reproductive alterations in their hosts, including cytoplasmic incompatibility (CI), male killing (MK), parthenogenesis induction (PI), and feminization of genetic males (Koivisto and Braig, 2003; Bonte et al., 2008; Cordaux et al., 2011; Gotoh et al., 2007).

As an important inherited symbionts of arthropods, *Arsenophonus* (Gammaproteobacteria: Enterobacteriales) was found to infect approximately 5% of species, including arachnids, ticks, cockroaches, hemipterans, hymenopterans, lice, flies, and coleopterans (Wilkes et al., 2011), and was present throughout host tissues as a result of widely disseminated infection (Huger et al., 1985). The intracellular bacterium *Wolbachia* (Alphaproteobacteria: Rickettsiales), the most widespread symbiont, has been detected in between 16% and 76% of arthropod samples (Werren et al., 1995; Jeyaprakash and Hoy, 2000) and is found in reproductive tissues of arthropods and nematodes (Werren, 1997). *Wolbachia* can manipulate the reproduction of their hosts and cause an impressive range of effects in their hosts (Charlat et al., 2003). *Cardinium* (Bacteroidetes: Bacteroidales), recently described bacteria, are vertically transmitted and cause multiple reproductive anomalies in their arthropod hosts (Zchori-Fein et al., 2001). Previous work showed that large numbers of *Cardinium* bacteria were found in the nurse cells and oocytes of their hosts (Zchori-Fein et al., 2001, 2004a). Zchori-Fein and Perlman (2004b) found that 6% of 99 screened insects and mites tested positive for *Cardinium*.

Ants provide a variety of crucial ecosystem services in many terrestrial ecosystems (Del Toro et al., 2012). Ants employ numerous microorganisms, and the interactions between ants and these microorganisms play key roles at the individual and colony levels of ant species (Boursaux-Eude and Gross, 2000; Zientz et al., 2005). Reproduction manipulation by endosymbionts, including *Arsenophonus*, *Wolbachia*, and *Cardinium*, may be important in the evolution of ant species (Sirviö and Pamilo, 2010; Keller et al., 2001), as sex ratio evolution and reproductive conflicts in ant societies are crucial issues in these species (Wenseleers et al., 1998). One survey showed that more than 50% of 50 Indo-Australian ant species tested positive for *Wolbachia* (Wenseleers et al., 1998). Sirviö and Pamilo (2010) found that 19% of *Formica cinerea* were infected by *Wolbachia* and 3.8% were infected by *Cardinium*. The prevalence of *Wolbachia* among ants of the genus *Solenopsis* is greater than 50% in Brazil (Martins et al., 2012). Studies from yellow crazy ant (*Anoplolepis gracilipes*) populations in the Pacific region showed infection rates of *Arsenophonus* ranging from 4.5% to 50.8%, while *Wolbachia* infected between 31.8% and 100% (Sebastien et al., 2012). Neither *Wolbachia* nor *Cardinium* is involved in the sex determination of the harvester ant species *Messor barbarus* and *M. capitatus* (Martínez-Rodríguez et al., 2013). Bouwma and Shoemaker (2011) indicated that *Wolbachia* likely do not play a role in cytoplasmic incompatibility, significant fitness effects, or male killing in the fire ant

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Solenopsis invicta. However, Van Borm et al. (2001) showed that *Wolbachia* may induce male killing or incompatible matings in leafcutter ant species of the genera *Atta* and *Acromyrmex*. Therefore, the phenotypic effects of these reproductive endosymbionts are still unclear in ants and require further exploration.

China has an incredible diversity of ecosystems and species, with more than 939 valid named ant species and subspecies (Hymenoptera: Formicidae) within 103 genera (Guénard and Dunn, 2012). However, the incidence of inherited bacteria in ants has received less attention in China. The diversity and distribution of reproductive parasites in ants also requires more in-depth research (Duron et al., 2008). Because *Wolbachia*, *Arsenophonus*, and *Cardinium* have all been reported in ant species (Wenseleers et al., 1998; Sirviö and Pamilo, 2010; Sebastien et al., 2012), we tested serial samples of ants from southern China to screen for these three maternally inherited endosymbionts; here, discuss the prevalence and geographical variation of reproductive parasite clades among ants in southern China.

2. Materials and methods

2.1. Sampling and DNA extraction

Workers of ants were collected by hand and in bait traps from Guangdong Province (GD), Guangxi Province (GX), Hainan Province (HN), Wuyishan Mountain National Park (WYS), Sichuan Province (SC), and Taiwan Province (TW) during 2012. Sampling sites are shown in Fig. 1. All specimens were preserved in pure ethanol and identified to the species level based on their morphological characters (Wu and Wang, 1995; Zhou, 2001; Zhao, 2006). Fifty-six ant species and 149 ant samples were collected in total for this study.

Total genomic DNA was extracted from ten individuals for each ant sample using a DNA isolation kit (Tiangen Biotech Co. Ltd., Beijing, China) following manufacturer's instructions. DNA extractions were stored at −40 °C until use.

2.2. PCR amplification and sequencing

To screen DNA extractions for the presence of *Wolbachia*, *Arsenophonus*, or *Cardinium*, polymerase chain reactions (PCRs) were conducted in 20 μl reaction mixtures including 2 μl 10× PCR buffer (Mg^{2+} Plus), 1.6 μl dNTPs mixture (2.5 mM each), 1 μl 10 mM forward and reverse primer and 0.6 units of Taq DNA polymerase (Takara, Dalian). Negative controls were used for all PCRs, and 5 μl PCR products were inspected on a 2% agarose/EtBr gel. For *Wolbachia*, we used the general *wsp* primers wsp81F (5′-TGGTCCATTAAGTGATGAAGAAAC-3′) and wsp691R (5′-AAAAATTAAACGCTACTCCA-3′), which amplify a product ranging from 590 to 632 bp (Zhou et al., 1998). *Wolbachia*-specific PCR amplification was conducted using the following thermal profile: initial denaturation at 95 °C for 2 min, followed by 35 cycles of 94 °C for 1 min, 54 °C for 1 min and 72 °C for 1 min, with a final extension at 72 °C for 5 min. For *Arsenophonus*, we used the specific 16S rDNA primers ArsF (5′-GGGTTGTAAAGTACTTTCAGTCGT-3′) and ArsR3 (5′-CCTYTATCTCTAAAGGMTTCGCTGGATG-3′), which amplify a product ranging from 581 to 804 bp (Duron et al., 2008). The *Arsenophonus*-specific PCR amplification was conducted using the following thermal profile: initial denaturation at 95 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 52 °C for 30 s and 72 °C for 1 min, with a final extension at 72 °C for 5 min. For *Cardinium*, we used the *Cardinium*-specific 16S rDNA primers carF (5′-GCGGTGTAAAATGAGCGTG-3′) and carR1 (5′-ACCTMTTCTTAACTCAAGCCT-3′) (Weeks et al., 2003). *Cardinium*-specific

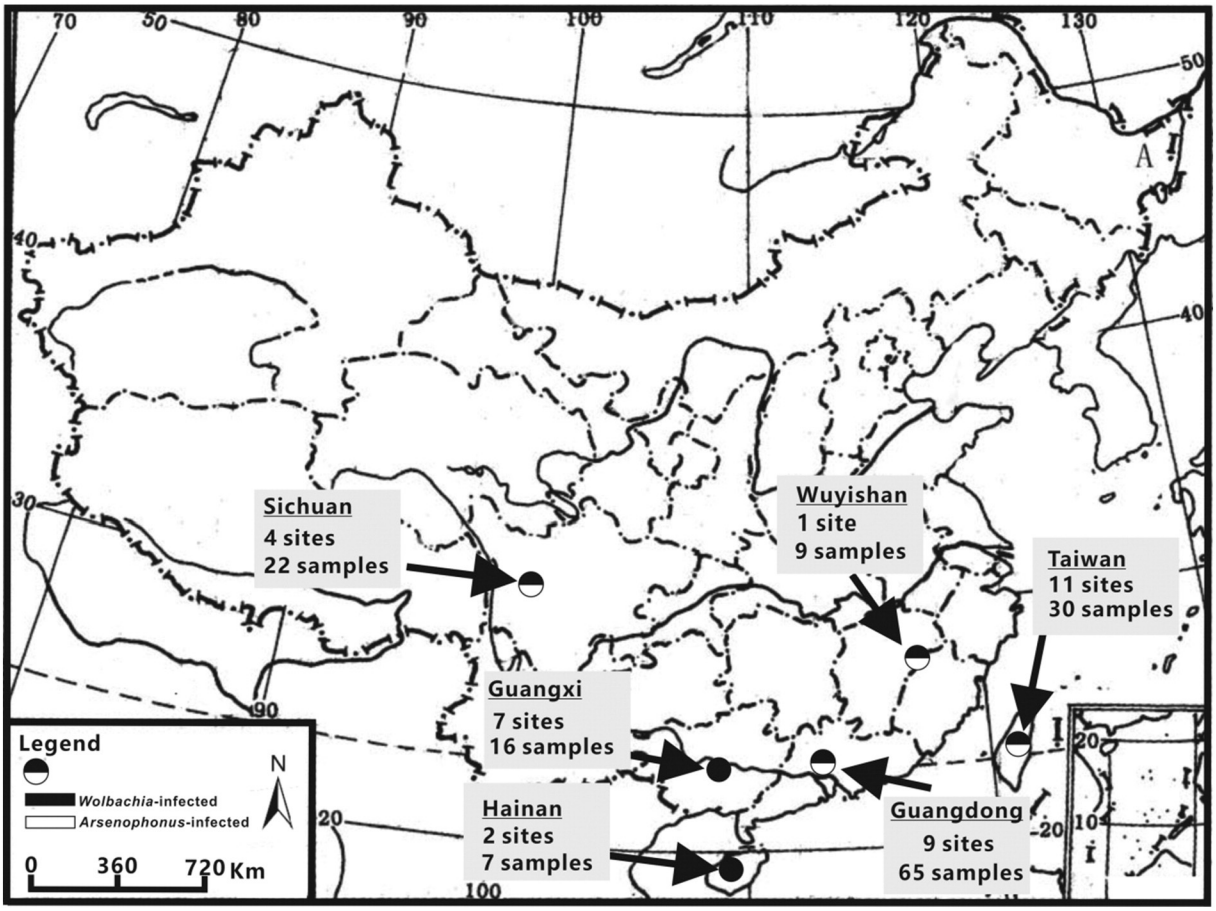

Fig. 1. Map showing the sampling sites. The number of ant samples collected from each area is indicated in gray boxes.