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Full Length Article

A prevalence survey of *Wolbachia* in *Polytremis fukia* (Lepidoptera: Hesperiidae)

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ABSTRACT

Wolbachia are endosymbiotic bacteria that infect arthropod and nematode hosts. Only a few studies have been made on *Wolbachia* in butterflies. In this study, we identified and determined the molecular characteristics of *Wolbachia* strains in *Polytremis fukia* (Lepidoptera: Hesperiidae), from seven locations in China. We also sequenced three portions of the mitochondrial DNA from the individuals of *P. fukia* to infer the effect of *Wolbachia* on host mitochondrial variation. The results show that 47% (15/32) are *Wolbachia* positive among all specimens. The infection rates in female and male are 69% (11/16) and 25% (4/16). Phylogenetic analysis of variation in the *ftsZ* gene from the two strains of *Wolbachia* (*w*Fuk1 and *w*Fuk2) showed they belonged to supergroup A. On the phylogeny of the mitochondrial DNA from *P. fukia*, the sequences are split into three clades. Clade I and II are consistent with the distribution of geographical population. Clade III consists of eight females invariably infected with *w*Fuk1 which showed a weak association existed between mitochondrial DNA haplotypes and *w*Fuk1 infection status.

1. Introduction

Wolbachia bacteria, estimated to occur in 20–70% of all insect species as well as many other arthropods and some nematodes (Hilgenboecker et al., 2008; Werren and Windsor, 2000), where they inhabit the host cell cytoplasm and are maternally transmitted to the next generation. Although there is some evidence of mutualism, it is well known that *Wolbachia* can be involved in the parasitic manipulation of its host's reproductive system (Werren et al., 2008). *Wolbachia* may cause feminization of genetic males, may be responsible for the death of male embryos, known as male killing, and may induce the lytokous (obligate) parthenogenesis, thus favouring unisexual production of females (Werren et al., 2008).

The *Wolbachia* surface protein (*wsp*), cell division protein gene (*ftsZ*), 16S rRNA, and other genes have been characterized and used for phylogenetic studies of this endosymbiont. The *ftsZ* gene is involved in cell division regulation (Lukenhous, 1990) and was characterized and sequenced from *Wolbachia* infected strains of *Drosophila melanogaster* (Holden et al., 1993). This gene contains conserved and highly divergent regions, which makes it suitable for fine-scale phylogenetic analysis. A remarkable genetic diversity exists in *Wolbachia*, and gene phylogenies show the existence of 13 recognized supergroups (A to F and H to M) and a controversial supergroup G (Augustinos et al., 2011; Baldo and Werren, 2007; Duron et al., 2008; Bandi et al., 1998; Czarnetzki and Tebbe, 2004; Casiraghi et al., 2005; Ros et al., 2009; Haegeman et al., 2009; Gorham et al., 2003; Bordenstein and Rosengaus, 2005).

Wolbachia infections have been reported in various Lepidoptera families such as Papilionidae, Lycaenidae, Pieridae, Nymphalidae, Hesperiidae, Pyralidae, Noctuidae, and Lasiocampidae (Bipinchandra et al., 2012; Dyson et al., 2002; Hiroki et al., 2004; Jiggins et al., 2000; Russell et al., 2009; Tagami and Miura, 2004). A few butterfly species harbouring the bacteria have been thoroughly studied (Ankola et al., 2011; Charlat et al., 2007; Duplouy et al., 2010; Hornett et al., 2006; Narita et al., 2007; Nice et al., 2009). *Polytremis* Mabille, 1904 is a genus within the family Hesperiidae. All species are restricted to the southeastern Palaearctic and northern Oriental realm and are particularly concentrated in China (Chou, 1994; Evans, 1949; Huang and Xue, 2004; Zhu, 2012). We have reported the molecular phylogeny of the genus in a prior study (Jiang et al., 2013). Subsequently, we have found that at least two species of *Polytremis* (*Polytremis nascens* Leech, 1893 and *Polytremis pellucid* Murray, 1875) are infected with *Wolbachia*. The populations of *P. nascens* may have recently been subjected to a *Wolbachia* induced sweep (Jiang et al., 2014).

Polytremis fukia Evans is widely distributed in the whole southern China except Taiwan, Hainan and south Yunan Province, from Zhejiang to west Sichuan Province and considered a serious pest on agricultural and horticultural crops (Evans, 1949; Zhu, 2012; Xiong et al., 2011; Xu and Duan, 2010). The present study focused on screening *P. fukia* from different local regions for the presence of *Wolbachia*, by

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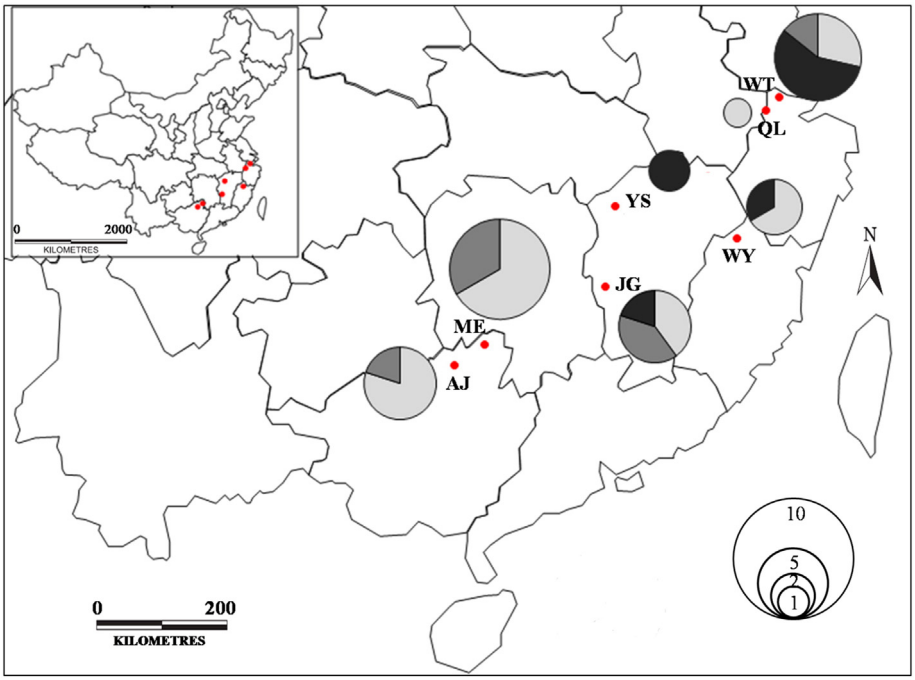

Fig. 1. Distribution of the specimens of *P. fukia* collected in China. Each population is represented by a circle (black: infected with *w*Fuk1, dark grey: infected with *w*Fuk2, light grey: uninfected). The area of the circles is proportional to the number of individuals of the population. The circles labelled 1, 2, 5 and 10 on the right hand side represent the number of individuals.

using the *ftsZ* gene, whose sequences were then used to construct a phylogenetic tree to show relationships among the *Wolbachia* infections detected. Additionally, we also look forward to understanding the possible influence of *Wolbachia* on the mitochondrial variation in *P. fukia*. The *Wolbachia* analysis will enrich our knowledge of population ecology and genetic structure of *P. fukia* and may help for future control.

2. Materials and methods

2.1. Sample collection and DNA extraction

We collected a total of 32 specimens of *P. fukia* (16♀ and 16♂) from seven local regions in China from 2008 to 2014 (Fig. 1, Table S1). All specimens were caught in the field, identified morphologically, preserved by dehydration in small envelopes and dried with silica desiccant for further processing. Two representatives of closely related species, *P. nascens* and *Polytremis discreta*, were chosen as outgroups for the phylogenetic analyses. The lower half of the abdomen of each butterfly was used for DNA extraction using a QIAamp DNA Mini kit (Qiagen, Hilden, Germany), as it contains the reproductive tissues in which *Wolbachia* is predominantly found.

2.2. Detection and identification of Wolbachia

To screen for the presence of *Wolbachia*, a region at 437 bp in length was amplified using general *Wolbachia* primers (WSpecF and WSpecR) for 16S rRNA (Werren and Windsor, 2000; Table 1). As long as positive controls were present in the same PCR, repeated unsuccessful amplification was interpreted as a lack of infection. For characterization of *Wolbachia* strains, a 750 bp segment of the *Wolbachia* cell cycle gene *ftsZ* was amplified using the *Wolbachia*-specific primers ftsZf1 and ftsZr1 (Werren et al., 1995, Table 1). PCRs were performed in a 20 μL final volume under the following conditions: 1 μL template DNA, 0.4 μM concentrations of all forward and reverse primers, 200 μM each dNTP, 1.5 mM MgCl2, 50 mM KCl, 10 mM Tris–HCl (pH 8.3) and 2.5 U Taq polymerase (Takara, Otsu, Shiga, Japan). The PCRs were run on a DNA thermal cycler (Bio-Rad, Hercules, CA, USA) with the following cycling conditions: 95 °C denaturation for 5 min followed by 40 cycles of 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min and a final extension of 72 °C for 5 min. Positive amplicons were extracted using the Wizard SV Gel and sequenced using an ABI 377 automated DNA sequencer. The sequences generated in this study have been deposited in the GenBank database (KU865650 and KU865668-KU865682). The results were analysed using Chromas 1.4 and compared with the NCBI database

Table 1
Primer sequences and amplicon lengths of PCR products of target genes.

Gene/region	Primers	Amplicon length (bp)	Reference
COI	HCO2198: 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ LCO1490: 5′-GGTCAACAAATCATAAAGATATTGG-3′	487	Rand et al., 2000
COII	PIERRE: 5′-AGAGCCTCTCCTTTAATAGAACA-3′ EVA: 5′-GAGACCATTACTTGCTTTCAGTCATCT-3′	618	Caterino and Sperling, 1999
ND5	V1: 5′-CCTGTTTCTCTGCTTTAGTTTAGTTCA-3′ A1: 5′-AATATDAGGTATAAATCATAC-3′	716	Yagi et al., 1999
16S rRNA	WSpecF: 5′-CATACCTATTCGAAGGGATAG-3′ WSpecR: 5′-AGCTTCGAGTGAAACCAATTC-3′	437	Werren and Windsor, 2000
ftsZ	ftsZf1: 5′-GTTGTCGCAAATACCGATGC-3′ ftsZr1: 5′-CTTAAGTAAGCTGGTATATC-3′	750	Werren et al., 1995