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Research paper

Discovery of a new *Wolbachia* supergroup in cave spider species and the lateral transfer of phage WO among distant hosts

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ABSTRACT

Wolbachia are widespread intracellular bacteria infecting the major classes of arthropods and some filarial nematodes. In arthropods, *Wolbachia* have evolved various intriguing reproductive manipulations, including cytoplasmic incompatibility, parthenogenesis, feminization, and male killing. Sixteen supergroups of *Wolbachia* have been identified, named A–Q (except G). Though *Wolbachia* present great diversity in arthropods, spiders, especially cave spiders, are still a poorly surveyed group of *Wolbachia* hosts. Here, we report a novel *Wolbachia* supergroup from nine *Telema* cave spiders (Araneae: Telemidae) based on five molecular markers (*16S rRNA*, *ftsZ*, *gltA*, *groEL*, and *coxA*). In addition, phage WO, which was previously reported only in *Wolbachia* supergroups A, B, and F, infects this new *Wolbachia* supergroup. We detected a 100% infection rate for phage WO and *Wolbachia* in *Telema* species. The phylogenetic trees of phage WO and *Wolbachia* are not congruent, which suggests that horizontal transfer of phage WO has occurred in these secluded species. Additionally, these data indicate *Telema–Wolbachia*-phage WO may be a good model for exploring the horizontal transfer history of WO among different host species.

1. Introduction

Wolbachia are maternally inherited rickettsial endosymbiotic bacteria in the class Alphaproteobacteria and are one of the most widespread obligate intracellular bacteria in some classes of arthropods and nematodes (Ferri et al., 2011; Hilgenboecker et al., 2008; Jeyaprakash and Hoy, 2000; Werren, 1997). A meta-analysis has suggested that 40% of insect species are infected with *Wolbachia* (Zug and Hammerstein, 2012). Wolbachia use an arsenal of reproductive manipulations in hosts, including feminization (Legrand et al., 1987), parthenogenesis (Stouthamer et al., 1990), male killing (Werren et al., 1994), and cytoplasmic incompatibility (Breeuwer and Werren, 1990). These phenotypes contribute to increasing the frequency of infected females in a host population, thus propagating *Wolbachia* worldwide.

Wolbachia are highly divergent and have been divided into 16 supergroups (A–Q, except for G, which is a combination of A and B) (Augustinos et al., 2011; Baldo et al., 2007; Bing et al., 2014; Bordenstein and Rosengaus, 2005; Glowska et al., 2015; Haegeman et al., 2009; Lo et al., 2002; Ros et al., 2009). The *Wolbachia* supergroups are classified mainly based on the genetic distance of the molecular markers *16S rRNA*, *gltA* (encoding citrate synthase), *groEL* (encoding heat-shock protein 60), *coxA* (encoding cytochrome c oxidase), *ftsZ* (encoding cell division protein), and *wsp* (encoding *Wolbachia* surface protein) (Casiraghi et al., 2005; O'Neill et al., 1992; Werren and Windsor, 2000). *Wolbachia* genotyping is inferred from multi locus sequence typing (MLST) of genes (*gatB*, *coxA*, *hcpA*, *fbpA*, and *ftsZ*) and the four hypervariable regions of WSP protein (Baldo et al., 2006, 2005).

Genome reduction is the predominant evolutionary trend in obligate intracellular bacteria and most are bacteriophage absent, like *Buchnera* (Moran and Bennett, 2014; Shigenobu et al., 2000). In *Wolbachia*, phage WO is widespread, with about 89% *Wolbachia* strains harboring WO (Bordenstein and Wernegreen, 2004). However, almost all of the phage WO infections are within *Wolbachia* supergroups A, B, and F. Based on genomic analyses, *Wolbachia* supergroups C and D have lost phage WO (Darby et al., 2012; Foster et al., 2005). Whether phage WO plays some role in *Wolbachia* reproductive manipulation or can be developed to be a genetic vector for *Wolbachia* research are two hot topics (Kent and Bordenstein, 2010). Indeed, some sex specific (Sinkins et al., 2005) and stage-specific expression of WO genes (Sanogo and Dobson, 2006; Wang et al., 2014) has been shown, which indicates that these genes may play an active role in *Wolbachia* biology. Phage WO is a temperate phage that can transfer within and between discrete *Wolbachia* supergroups (A and B) (Gavotte et al., 2004; Kent et al., 2011), which suggests that phage WO might mediate gene transfer. However, we still have little knowledge of whether other *Wolbachia* supergroups are infected with phage WO, and if yes, whether phage WO

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can transfer between more discrete *Wolbachia* supergroups except between previously reported supergroups A and B.

Although *Wolbachia* is well characterized in insects, there are few reports of *Wolbachia* diversity in spiders. Studies have shown that some spiders are infected with *Wolbachia* belonging to supergroups A and B (Duron et al., 2008a; Goodacre et al., 2006; Lo et al., 2007; Rowley et al., 2004; Vanthournout et al., 2011). In the present study, we address *Wolbachia* diversity in cave spiders. Cave environments are regarded as an extreme habitat characterized by cool and moist air, permanent darkness, scarce energy sources, and constant environment compared with surface conditions (Gabriel and Northup, 2013; Zhang and Li, 2014). Usually, endosymbionts are critical for host adaptation and survival in cave environments, and there are novel endosymbionts compared to the external biota (Morse et al., 2012; Paoletti et al., 2013). Thus, *Wolbachia* diversity in cave spiders may have been affected by long-term isolation from external *Wolbachia* strains. In this study, we examine the distribution of *Wolbachia* in *Telema cordata* and eight species of the *Telema cucurbitina* "species complex" collected from Guangxi Province, China. All of these spider species are typical cave spiders. We not only detected 100% prevalence of *Wolbachia* and phage WO in these *Telema* species, but also identified that the *Wolbachia* infecting the *Telema* species belong to a novel supergroup (supergroup R). In addition, we reveal the horizontal transfer of phage WO among the distant *Wolbachia* supergroups A and R.

2. Materials and methods

2.1. Spider collection and identification

All of the spiders used in the study (see Table 1) were collected in Guangxi Province, China from April to July, 2013. The specimens were carefully morphologically identified, and the given names were cited from Zhang et al.'s study (Zhang and Li, 2014). All identified spiders were initially immersed in 95% ethanol and subsequently maintained at −20 °C until DNA extraction.

2.2. Isolation, amplification, and sequencing of genomic DNA

Before DNA extraction, each specimen was washed several times with 70% ethanol followed by sterile water to remove surface contaminants. DNA was isolated from each spider using an EasyPure Genomic DNA extraction kit (TransGen, Beijing, China) following the manufacturer's recommendations. The quality of the DNA templates was confirmed by the amplification of a partial fragment of cytochrome *c* oxidase subunit I with the primers LCO1490 and HCO2198 (see Table S1) (Vrijenhoek, 1994). DNA templates of poor quality were discarded.

All species (Table 1) were screened for the presence of *Wolbachia* strains by amplification of 16S *rRNA* and/or *wsp* using the primers shown in Table S1. When *Wolbachia* were identified, additional PCR was carried out based on *Wolbachia* protein-coding genes (*ftsZ*, *gltA*, *groEL*, *coxA*, *gatB*, *fbpA*, and *hcpA*) and phage WO minor capsid gene *orf7* (Table 1). All the primers are shown in Table S1.

The PCR program was 5 min at 94 °C; 30 cycles of 30 s at 94 °C, 40 s at 47–60 °C, and 25 s at 72 °C; and 10 min at 72 °C for the final extension step. Negative controls with sterile water as template were used for all PCR experiments. The PCR components were added as recommended by the manufacturer of TransTaq DNA Polymerase HiFi Fidelity (TransGen, Beijing, China). The PCR products were electrophoresed using 1% agarose gels in Tris-CH_3COOH buffer. Following electrophoresis, the gels were dyed with GelStain (TransGen, Beijing, China) and imaged on a VILBER FUSION FX5 (Vilber Lourmat, France). If there was a single amplified band, the PCR products were purified with the EasyPure PCR purification kit (TransGen, Beijing, China) and directly sequenced with an ABI 3730 sequencer (Biosune, Beijing, China). If there were more than one amplification band, the expected band was excised from the gels and purified with the EasyPure Quick Gel PCR purification kit (TransGen, Beijing, China) and cloned with the Peasy-T5 vector (TransGen, Beijing, China); a minimum of three positive clones were sequenced. For each gene, at least two specimens per species were sequenced. All de novo nucleotide sequences in this study were deposited in GenBank under accession numbers KT319068–KT319104 and KU057803–KU057809.

2.3. Sequence analyses

We used representative *Wolbachia* supergroups described in the literature to classify *Wolbachia* strains from *Telema* species. The representative strains for each *Wolbachia* supergroup were chosen for which at least two of the five *Wolbachia* loci (*16S rRNA*, *ftsZ*, *gltA*, *groEL*, and *coxA*) were available from the GenBank (http://www.ncbi.nlm.nih.gov/genbank/). We translated the protein coding sequences into amino acid sequences by the TranslatorX version 1.1 (Abascal et al., 2010). Then we aligned the protein sequences using MUSCLE

Table 1
Screening of *Wolbachia* and phage WO in *Telema* species.

Telema species	Location[a]	*16S rRNA*	*fbpA*	*gatB*	*hcpA*	*gltA*	*groEL*	*coxA*	*ftsZ*	*wsp*	*orf7*	No. of specimens	*Wolbachia* infection rate (%)	WO infection rate (%)
Telema cucurbitina complex sp. Cave_num1	Lingui country (25°12.819′N, 110°12.050′E)	+	+	−	−	−	−	+	−	+	+	12	100	100
Telema cucurbitina complex sp. Cave_num2	Liuzhou city (24°13.782′N, 109°24.663′E)	+	+	−	−	−	−	+	−	+	+	11	100	100
Telema cucurbitina complex sp. Cave_num6	Yangshuo country (24°56.686′N, 110°36.369′E)	+	+	−	+	−	−	+	+	−	+	10	100	100
Telema cucurbitina complex sp. Cave_num7	Laibin city (22°43.648′N, 109°05.447′E)	+	+	−	−	−	−	+	−	−	+	15	100	100
Telema cucurbitina complex sp. Cave_num10	Xiangzhou country (23°57.278′N, 109°39.696′E)	−	−	−	−	−	−	−	−	+	+	3	100	100
Telema cucurbitina complex sp. Cave_num11	Lingchuan country (25° 18.575′N, 110° 13.875′E)	+	−	−	−	−	−	+	−	+	+	13	100	100
Telema cucurbitina complex sp. Cave_num14	Guizhou city (25° 16.33′N, 110° 18.25′E)	+	−	−	−	−	−	+	−	+	+	4	100	100
Telema cucurbitina complex sp. Cave_num15	Liuzhou city (24° 13.782′N, 109° 24.663′E)	+	−	−	−	−	−	+	−	+	+	3	100	100
Telema cordata	Xiangzhou country (23° 57.278′N, 10° 39.696′E)	+	+	−	+	−	+	+	+	+	+	16	100	100

+: positive amplification,
−: failure to detect amplification product.
[a] All of the places are in Guangxi Province, China.