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Evidence for recombination between feminizing *Wolbachia* in the isopod genus *Armadillidium*

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Abstract

Wolbachia are maternally inherited endosymbiotic α-Proteobacteria infecting a wide range of arthropods. *Wolbachia* induce feminization in many terrestrial isopod species, particularly in the genus *Armadillidium* (Crustacea, Oniscidea). The diversity of *Wolbachia* strains infecting *Armadillidium* species was examined. Results reveal that natural populations of *A. vulgare* contain three different *Wolbachia* strains (*w*VulC, *w*VulM and *w*VulP). The *wsp* gene and its 3′-adjacent region show evidence that two recombination events have occurred between two of these strains. In both cases, multiple statistical analyses suggest that a small gene fragment of a strain closely related to *w*VulM (minor parent) is inserted into the genome of another strain closely related to *w*VulC (major parent). Although multiple infections in a single individual have never been demonstrated in natural population, the existence of recombination between feminizing strains suggests that bi-infections are possible, or at least that bi-infections can be maintained sufficiently long enough to allow recombination. Recombination events increase genetic diversity of *Wolbachia* found in *Armadillidium* species and may play a role in the ability of *Wolbachia* strains to invade new hosts.

Keywords: Endosymbiosis; *wsp*; Genome plasticity; Feminization

1. Introduction

Wolbachia are maternally inherited endosymbiotic α-Proteobacteria infecting a wide range of nematodes and arthropods (O'neill et al., 1992, Stouthamer et al., 1999). Depending on both the bacterial lineage and host, they may have very diverse effects on host reproduction, such as cytoplasmic incompatibility (CI), male killing, parthenogenetic reproduction or feminization of genetic males (Rigaud, 1999, Stouthamer et al., 1999). In terrestrial isopod species (Crustacea, Oniscidea), *Wolbachia* induce CI in two species, *Porcellio dilatatus petiti* and *Cylisticus convexus* (Legrand and Juchault, 1986, Moret et al., 2001) and feminization in many others, including members of the genus *Armadillidium*, such *as A. vulgare* and *A. nasatum* (Bouchon et al., 1998, Rigaud et al., 1991). Feminization occurs through conversion of genetic males into functional females, which typically produce female-biased progenies (Rigaud et al., 1997). Recently, based on *wsp* gene sequences, two distinct feminizing *Wolbachia* strains have been found in various populations of *A. vulgare* (Cordaux et al., 2004). The comparison of bacterial strains and their respective host mitochondrial phylogenies showed no concordance, indicating horizontal transmission of the *Wolbachia* strains within populations of *A. vulgare*. *A. vulgare* is to date the only known species of terrestrial isopod to host more than one *Wolbachia* strain (Bouchon et al., 1998). Despite extensive sampling efforts, no multiple infections in single individuals of isopod species have been so far recorded. This suggests that feminization establishes a high competition between *Wolbachia* and/or that multi-infection is unstable in these species. Since no multiple infections have been discovered in isopod species, recombination within and between *Wolbachia* strains is basically unexpected. However, Baldo et al. (2005a) have shown evidence

Abbreviations: bp, base pair; CI, cytoplasmic incompatibility; HVR, hypervariable region; MLST, multilocus sequence typing

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for recombination in some *Wolbachia* strains of isopods. The evolutionary context of these recombination events is difficult to understand as horizontal transfers would be rare events between some of the involved host species as they live in different habitats.

The first example of recombination was described between two *Wolbachia* strains (belonging to the supergroup B) of a parasitoid wasp and its fly host (Werren and Bartos, 2001). Since then, a growing number of publications have revealed other recombination events within *Wolbachia* chromosomal genes, a large number of which involve *wsp* (Baldo et al., 2005a,b, Jiggins, 2002, Jiggins et al., 2001, Keller et al., 2004, Malloch and Fenton, 2005, Reuter and Keller, 2003, Werren and Bartos, 2001). Recombination can also occur between highly divergent strains belonging to different supergroups (Baldo et al., 2005a,b, Jiggins, 2002, Malloch and Fenton, 2005). Therefore recombination may be common in *Wolbachia* and may confuse phylogenetic relationship reconstructions based on a single gene. Moreover, several studies in different insect species specify lateral transfer of the phage WO-B between co-infecting *Wolbachia* strains (Bordenstein and Wernegreen, 2004, Masui et al., 2000) and intragenic recombination may be common in extra-chromosomal DNA, including phage and transposable elements (Bordenstein and Wernegreen, 2004, Duron et al., 2005).

For lateral DNA transfers that are not phage-mediated, bacteria must come into close contact (Jiggins, 2002). As *Wolbachia* are vertically transmitted endosymbionts, opportunities for recombination between different types would appear to be restricted except in the case of multiple infections.

In this paper we wish to test the occurrence of genetic exchange between feminizing *Wolbachia* strains. To address this issue we analyzed *Wolbachia* polymorphism within three host species belonging to the *Armadilllidium* genus. Results strongly suggest two independent recombination events between the two *Wolbachia* strains (*w*VulC and *w*VulM) infecting sympatric *Armadillidium* species. The origin and evolutionary implications of recombination events and horizontal transfers of feminizing *Wolbachia* strains are discussed.

2. Materials and methods

2.1. Sampling and sequencing

Three populations of *A. vulgare* (originating from Poitiers, Ensoulesse and Chizé) and a population of *A. nasatum* (originating from Frelinghien) were sampled in France (Table 1). The population of Poitiers was sampled in 2003 and in 2006. The infection status of each specimen was assessed by PCR assay of the *Wolbachia wsp* gene as described below. Additionally, one infected female of *A. album* collected at Yves (France) for which the *Wolbachia* strain has previously been identified (Bouchon et al., 1998) was included in this study. This strain will be denoted *w*Album to avoid confusion with the strain infecting *Aedes albopictus* (called wAlb, according to the nomenclature of Zhou et al. (1998)).

DNA extraction, PCR amplification and sequencing of the *wsp* region from all infected individuals were performed as described in Michel-Salzat et al. (2001). To infer the size of the recombinant fragments, the 3′ adjacent region of the *wsp* gene was sequenced. PCR amplifications were performed using *wsp* 81F primer (Braig et al., 1998) and a primer RPOHR (5′-TTAAHCCYATRTTWCCTTC-3′) designed inside the heat shock σ-factor *rpoH* gene which is located downstream of the *wsp* gene in the *w*Mel genome (Wu et al., 2004). PCR cycling conditions were: 95 °C for 2 min; 38 cycles at 95 °C for 1 min, 45 °C for 1 min, 72 °C for 1 min 40 s; 72 °C for 5 min; then held at 4 °C. PCR products were purified using QiaQuick PCR purification kits (Qiagen). Sequencing was performed using a BigDye v3.1 terminator sequencing kit and an ABI 3130 Genetic Analyzer. By using primers *wsp* 81F, *wsp* 691R (Braig et al., 1998), RPOHR and an internal forward primer 411F (5′-AAATCAAACTTTACGCTGGCG-3′), we obtained complete sequences of 1136 to 1146 bp, depending on the *Wolbachia* strains. These sequences comprise 606 to 615 bp of the 3′ end of the *wsp* gene, 296 to 297 bp of a non-coding intergenic region and 234 bp of the 5′-region of the heat shock σ-factor *rpoH* gene. In order to measure the range of recombination, we also sequenced six other *Wolbachia* genes, namely *groE* operon that includes a non-coding intergenic region together with *groES* and *groEL* (with primers groEF1 5′-GAAGAAAAACAAGGTG-GAATT-3′ and groER1 5′-GTACCATCACCAACTTTGTC-3′; D. Bouchon, pers. com.) and 5 genes widely distributed across the *Wolbachia* genome and used in one of the two available multilocus sequence typing approaches (Baldo et al., 2006; Paraskevopoulos et al., 2006): *ftsZ* (with primers ftsZf1 and ftsZr1, Werren et al., 1995), *gatB*, *coxA*, *hcpA* and *fbpA*. PCR conditions and sequencing reactions were performed as those described by Baldo et al. (2006). All sequences were deposited in GenBank–EMBL databases under the accession numbers DQ778095–DQ778107 and EF451545–EF451564 (Table 2).

Table 1
Wolbachia prevalence in the sampled populations of three *Armadillidium* species

Sampled locality	Sample year	*N*, ♂/♀	Number of infected females:				
			*w*VulM	*w*VulC	*w*VulP	*w*Nas	*w*Album
Armadillidium vulgare							
Ensoulesse	2003	10/9	1		1		
Poitiers	2003	10/8	1	3	4		
Poitiers	2006	5/14	1		9		
Chizé Biological Reserve	2003	5/14	3	5			
Armadillidium nasatum							
Frelinghien	2006	5/22				6	
Mignaloux [a]	1993	0/19				4	
Amou [a]	1995	3/3				1	
Armadillidium album							
Yves	1994	1/7					6

[a] Samples from Bouchon et al. (1998).

2.2. Evolutionary analyses

All sequences were manually aligned taking into account their amino acid translation. Sequences of *wsp* and *groE* were