

# Mitochondrial DNA variability and *Wolbachia* infection in two sibling woodlice species

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Several morphological races and subspecies have been described and later included within the terrestrial isopod species *Porcellionides pruinosus*. During our study of this species, we have worked on specimens from France, Greece, Tunisia and Réunion island. Laboratory crosses have revealed two separate groups of populations: French populations (four localities) in one group, and those from Tunisia, Réunion island and Greece in the other. French individuals were reproductively isolated from those of the other populations. We have undertaken a survey of mitochondrial DNA (mtDNA) polymorphism in these seven populations. We observed two groups of mitotypes corresponding to the two groups of populations. Interfertility experiments between populations and the mitochondrial genetic distances between mitotypes both suggest the presence of two different species, one in France and one in Greece, Tunisia and Réunion island. The two species harbour, respectively, two different *Wolbachia* lines. Another feature of the molecular genetic analysis was the apparent mitochondrial monomorphism in the French populations and the low variability in the other three populations. The result can be related to the possibility of *Wolbachia*-induced genetic hitchhiking in these populations.

**Keywords:** laboratory crosses, mitochondrial DNA polymorphism, *Porcellionides pruinosus*, terrestrial crustacean, *Wolbachia*.

## Introduction

*Porcellionides pruinosus* is assumed to be the most widely distributed terrestrial isopod across the world (Vandel, 1960). However, this species has had a complex and confused taxonomic history. Because of their morphological variation, specimens of *P. pruinosus* from Europe and from Africa have been given subspecific rank (Verhoeff, 1918; Strouhal, 1938). Additionally, Garthwaite & Sassaman (1985) in their studies of North American terrestrial isopods described a new species, *Porcellionides floria*, for specimens previously included within *P. pruinosus*. They suggest that *P. pruinosus* (as formerly defined) may consist of a number of distinct and localized species rather than one unique cosmopolitan species. We have undertaken a survey of *P. pruinosus* individuals from seven different localities. Different approaches give congruent results, showing the existence of two sibling species.

In parallel with the survey of mitochondrial polymorphism, we have investigated the intensity of *Wolbachia*

infection in *P. pruinosus*. The intracytoplasmic *Wolbachia* bacterium has been found in several species of insects, in crustacean isopods, in mites and in nematodes (Rousset *et al.*, 1992; Bandi *et al.*, 1998). These maternally inherited microorganisms cause cytoplasmic incompatibility in many insects, parthenogenesis in *Trichogramma* wasps and feminization of genetic males in terrestrial isopods (review in Werren & O'Neill, 1997). The isopod suborder Oniscidea (woodlice) is strongly affected by the presence of these endocyttoplasmic bacteria (Bouchon *et al.*, 1998). Their effect on the control of sex determination has been well studied in *Armadillidium vulgare*, where *Wolbachia* is believed to inhibit the activity of 'male genes' by suppressing androgenic gland development (Martin *et al.*, 1973; Juchault *et al.*, 1993). Infected eggs develop into functional females, that can transmit bacteria, regardless of their sex chromosome composition (review in Rigaud, 1997). The main consequence of this cytoplasmic sex determination is distortion of the sex ratio, with infected females producing highly female-biased broods. The same phenomenon also occurs in the woodlouse *P. pruinosus* (Juchault *et al.*, 1994; Rigaud *et al.*, 1997). In the present study, we found no mtDNA

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polymorphism in the populations entirely infected by the feminizing *Wolbachia*, but a small amount of variation in mtDNA in populations where *Wolbachia* infection was incomplete. This is consistent with a reduction in mitochondrial diversity resulting from hitchhiking following the spread of the intracytoplasmic symbiont.

## Materials and methods

### Sampling

The four French populations investigated were from the following locations: Mignaloux (Vienne), Nevers (Nièvre), Poitiers (Vienne) and St Martin-du-Fouilloux (Deux-Sèvres). All gravid females collected from the wild were reared in the laboratory (20°C; LD: 18:6) and sex-ratios of their progenies noted. Specimens from Réunion island, Indian ocean (at St Paul), Greece (Athens) and Tunisia (Tunis) were reared in the same conditions. A scanning microscope survey of cuticular ornaments revealed that individuals from these populations conformed morphologically to the description of the species *P. pruinus* (Vandel, 1960; Hadley & Hendricks, 1985) (results not shown).

### Interfertility experiments

Crosses between the different French locations were fully interfertile (data not shown). To test interfertility between individuals from distant locations, males and females from France (St Martin-du-Fouilloux), Greece, Tunisia and Réunion island were combined to obtain 12 interpopulation crosses and four intrapopulation crosses (Table 1). In each crossing type, eight couples of one male and one virgin female were mated under laboratory conditions (20°C, LD 18 : 6).

**Table 1** Number of fertilized *Porcellionides pruinus* females out of the eight tested, in crosses involving males and females from different locations

Origin of females	Origin of males			
	France	Tunisia	Réunion	Greece
France	8†	1‡	0	0
Tunisia	0	8†	8†	8†
Réunion	0	8†	8†	8†
Greece	0	8†	8†	8†

† indicates fertilized females that produced offspring.

‡ indicates fertilized female that produced no offspring (eggs abort).

### Mitochondrial DNA analysis

The total mitochondrial DNA polymorphism was analysed by restriction fragment length polymorphism. As mitochondrial DNA is maternally inherited, offspring from all broods produced by each mother were pooled in isofemale lines. Total mitochondrial DNA was extracted from gonads, nervous tissue and fat body, according to Souty-Grosset *et al.* (1992). The mtDNA was then digested with the following restriction enzymes: *KpnI*, *AccII*, *ClaI*, *BglII*, *PvuII*, *HincII*, *ScaI*, *EcoRI*, *BstEII*, *SstI*. Most of them recognize six-base sequences, except *AccII*, which recognizes a four-base sequence. The digested DNA patterns were run on agarose gels under the conditions described in Grandjean *et al.* (1993) and fragment migration patterns were visualized by staining with SYBR Green I FMC (BioProducts). This sensitive fluorescent stain detected as little as 60 pg nucleic acid per band (using 300 nm transillumination). The number of nucleotide substitutions per site, *d*, was estimated by the method of Nei & Tajima (1983).

In addition, a PCR-RFLP survey was made on the mitochondrial 16S rDNA (mt 16S rDNA). The total DNA was extracted from each individual's gonads, fat tissue and nervous system, according to Wilson *et al.* (1985). Amplification of the mt 16S rDNA was carried out according to Kocher *et al.* (1989) on a thermocycler Trio thermoblock (Biometra). The mixture contained the two primers, 16 SAR and 16 SBR, that targeted the mitochondrial 16S ribosomal RNA genes (Simon *et al.*, 1991), and *Taq* polymerase (Promega Biotech). PCR conditions were described in Bouchon *et al.* (1994). Amplified portions of mt 16S rDNA were purified by the chloroform method and digested with the following 11 restriction endonucleases: *AccII*, *HhaI*, *MspI*, *RsaI*, *HaeIII*, *DraII*, *SspI*, *NdeII*, *TaqI*, *HinfI* and *AluI*. Digested amplification products were run on 1.5% agarose gel in TBE buffer for 45 min at 100 V. After staining with ethidium bromide, fragments were visualized with UV light.

### Detection of *Wolbachia* microorganisms

The prevalence of *Wolbachia* has previously been investigated in the Mignaloux and Nevers populations (Rigaud *et al.*, 1997). The prevalence of *Wolbachia* in the populations from Poitiers, St-Martin-du-Fouilloux, Réunion island, Greece and Tunisia, was determined in wild-caught mothers, when possible, or in two offspring. The bacteria were detected by PCR amplification of part of the bacterial 16S ribosomal DNA gene, using specific primers (99f and 994r) amplifying a 900-bp fragment (O'Neill *et al.*, 1992). The total DNA extraction from

**Table 2** Mitochondrial DNA digestion profiles produced by restriction enzymes in all the populations of *Porcellionides pruinosus* (band sizes in kb)

<i>Kpn</i> I		<i>Acc</i> II		<i>Cla</i> I		<i>Bgl</i> II		<i>Pvu</i> II		<i>Hinc</i> II			<i>Sca</i> I		<i>Eco</i> RI		<i>Bst</i> EII		<i>Sst</i> I	
A	B	A	B	A	B	A	B	A	B	A	B	C	A	B	A	B	A	–	A	B
17.0	22.6	6.7†	18.4	10.6	10.2	5.4†	6.0	19.6	18.6	9.2	4.2	4.2	12.2	8.6	19.0	19.0	20.0	28.5	6.6	22.4
11.1	11.3	3.5†	9.2	6.9†	6.7†	5.0	3.2†	9.8	9.3	4.6	3.9†	3.1†	6.6†	5.8†	9.5	9.5	10.0	14.4	5.2	11.2
8.5	1.2	2.3†	4.2†	5.3	5.1	3.7†	3.0	7.0	4.8	3.0†	2.8†	2.8†	6.1	4.3	9.2	8.6	4.2		4.1†	5.6
5.5	0.6	1.4	1.4	3.6	1.9†	3.4	1.5†	3.5	2.4	2.4†	2.7†	2.7†	2.6	2.6	4.6	4.3	2.1		4.0†	2.8
	0.3†	0.7	0.7	1.8	0.8	2.5	1.2†	0.7†	2.3†	2.2	2.1	2.1	1.3	2.6†				1.9†		3.3
					0.4	1.7	1.2†			1.1	1.3†	1.3†		1.3						2.6
						0.8†	1.0†			0.8†	0.8	0.9†								
							0.8			0.7†	0.7†	0.8								
							0.7†			0.5†	0.6†	0.7†								
							0.4			0.4†	0.5†	0.6†								
											0.4	0.5†								
												0.4								
<b>42.0</b>	35.7	14.5	33.8	28.3	25.1	22.4	18.8	40.6	37.4	24.8	19.8	19.9	28.8	25.3	<b>42.3</b>	<b>41.4</b>	38.3	<b>42.9</b>	25.9	<b>42.0</b>
	<b>38.1</b>	<b>39.4</b>	<b>42.2</b>	<b>42.1</b>	<b>42.1</b>	<b>42.0</b>	<b>36.2</b>	<b>42.0</b>	<b>42.0</b>	<b>40.2</b>	<b>41.3</b>	<b>41.4</b>	<b>42.0</b>	<b>42.0</b>			<b>42.0</b>		<b>42.1</b>	

†Bands must be multiplied by three to obtain the real size of the mtDNA molecule. The total size of the mtDNA molecule is given in the last lines of the table: numbers in italics indicate the simple addition of the bands; the numbers in bold indicate the real size according to the trimeric model of Raimond *et al.* (1999). “–” indicates an enzyme that did not cleave.

Three mitotypes are defined from these profiles: **M1** AAAAAAAAAA; **M2** BBBB BBBB–B; **M3** BBBB BCBB–B

the gonads and the PCR conditions were as described in Bouchon *et al.* (1998). Amplification products were run on a 1.5% agarose gel in TBE, stained with ethidium bromide and visualized under UV light.

From two *Wolbachia* strains (Réunion island and Poitiers), we amplified a fragment of 16S rDNA gene homologous to positions 8–973 of the *E. coli* sequence, by using 27f and 973r primers (Rousset *et al.*, 1992). From amplification products, direct double strand sequencing was performed as described by Bouchon *et al.* (1998). The primers used for sequencing were 27f, 685r, 530f and 973r (Rousset *et al.*, 1992). Using these primers, 812 nucleotide sequences of the rRNA gene were determined on the both strands. The GenBank, EMBL and DDBJ accession numbers for the nucleotide sequences of the two isolate of *Wolbachia* are AJ133196 (Réunion island) and AJ223242 (Poitiers).

**Results**

*Interfertility analysis*

As shown in Table 1, there was no interbreeding between French specimens and the others. In these series of crossing no mating behaviour was observed. The reproductive isolation was therefore prezygotic, which was confirmed by the fact that no sperm was found in the females’ genital tracts after the experiment. In comparison, individuals from Réunion island, Greece and Tunisia were totally interfertile. Throughout, no

distinguishable morphological characters were detected, especially on the male copulatory appendix.

*Mitochondrial DNA analysis*

The *P. pruinosus* mtDNA was atypical in size, around 42 kb. The size, and the restriction patterns, were similar to those of the mtDNA from another terrestrial isopod *A. vulgare* (Raimond *et al.*, 1999). The mtDNA seems to be composed of three identical 14 kb monomers. One of these is linear, whereas the other two form a circle, with one monomer reversed with respect to the other. A single restriction site per monomer generates a four-band pattern in a restriction profile and additional restriction sites generate three identical fragments leading to a single band (Raimond *et al.*, 1999). These fragments have to be counted triply to obtain a correct estimation of the size of the mtDNA (Table 2).

The RFLP analysis revealed three mitotypes: M1, M2 and M3. There was no mtDNA polymorphism in the four French populations, all the isofemale lines (20 for St Martin, nine for Poitiers, 10 for Nevers and 11 for Mignaloux) having the same mitotype, M1. Mitotype M1 was strictly confined to the French populations and was not found in the Réunion island, Greek and Tunisian populations, whereas M2 and M3 were shared by these last three populations. All of the enzymes exhibited a distinct profile between the mitotype M1 and the two mitotypes M2 and M3. The M2 and M3

**Table 3** Calculated fragment sizes, in number of base pairs, resulting from restriction enzyme digestion of mitochondrial 16S rDNA amplification product for specimens of *Porcellionides pruinosus*

Restriction enzyme	Population	
	France	Tunisia-Réunion-Greece
<i>AluI</i>	384 + 111	dnc
<i>RsaI</i>	345 + 160	345 + 160
<i>HhaI</i>	dnc	dnc
<i>MspI</i>	362 + 133	dnc
<i>HaeIII</i>	416 + 79	373 + 122
<i>TaqI</i>	416 + 79	416 + 79
<i>NdeII</i>	247	dnc
<i>AccII</i>	439 + 56	439 + 56
<i>HinfI</i>	dnc	416 + 79
<i>DraII</i>	439 + 56	dnc
<i>SspI</i>	dnc	dnc

dnc, did not cleave.

mitotypes were closely related ( $d=0.0092$ ). M1 was much more distantly related to each of them ( $d=0.4448$  between M1 and M2; and  $d=0.4490$  between M1 and M3).

The PCR-RFLP analysis of the mitochondrial ribosomal gene revealed two distinct mitotypes, one for the four French populations and one for the three others. As shown in Table 3, six restriction enzymes out of the 11 distinguish the French mitotypes from the others: *AluI*, *MspI*, *HaeIII*, *NdeII*, *HinfI* and *DraII*. We obtained identical restriction patterns for Tunisian, Greek and Réunion island individuals. The nucleotide distance estimated according to Nei & Tajima (1983) was 0.133 between the mitotype harboured by the French population and the mitotype harboured by the Réunion island, Tunisian and Greek populations.

### Sex ratios, *Wolbachia* infection and relationship with mtDNA variability

Analysis of progeny sex ratios showed that females infected with *Wolbachia* produced female-biased offspring, whereas those that were not infected produced a more balanced sex ratio (Table 4). The differences in the sex ratios of offspring from infected and uninfected females were highly significant in Tunisia ( $\chi^2_4=26.22$ ;  $P < 0.01$ ) and Réunion ( $\chi^2_7=60.41$ ;  $P < 0.01$ ), as had been seen in Nevers (Rigaud *et al.*, 1997).

*Wolbachia* studies in French populations (Rigaud *et al.*, 1997) showed that almost all females in the wild harboured *Wolbachia*, with one population, Mignaloux, being 100% infected. Only one uninfected female was found in Nevers (Table 5). Surveys of the Poitiers and St-Martin-du-Fouilloux populations showed that all the females tested were infected with *Wolbachia* (Table 5), so that 97.4% of the French females tested harboured *Wolbachia*. They also all harboured the same mitotype, M1. The other populations of *P. pruinosus* tested had a lower incidence of *Wolbachia*; the Réunion island and Tunisia populations contained about 60% infected females (Table 5). The animals from Greece, Tunisia and Réunion island that were infected with *Wolbachia* had the M2 mitotype, whereas the uninfected ones had the M3 mitotype (Table 4).

The *Wolbachia* transmission level was also detected by PCR in males and females from the progenies of infected mothers (Table 5). *Wolbachia* transmission rates are significantly different between populations ( $\chi^2_6=33$ ;  $P < 0.001$ ). A logistic regression, comparing France against Greece, Réunion island and Tunisia, showed significant effects of origin (Wald-test=9.85;  $P=0.0017$ ) and of sex (Wald-test=14.65;  $P=0.0001$ ). Males of the Greek, Réunion island and Tunisian populations are always uninfected by *Wolbachia*, whereas in the French population males harbour *Wolbachia*

**Table 4** *Wolbachia* infection, progeny sex ratios and mitochondrial types in wild-caught females of *Porcellionides pruinosus*

Locations	<i>Wolbachia</i> -infected lines			Uninfected lines		
	$N^\dagger$	%m $^\ddagger$	Mitotypes $^\S$	$N$	%m	Mitotypes
Mignaloux	11 $^\P$	17.8 ± 4.7 $^\P$	M1	0 $^\P$		
Nevers	9 $^\P$	35.6 ± 2.9 $^\P$	M1	1 $^\P$	46.4 $^\P$	M1
St. Martin	22	28.4 ± 5.1 (815)	M1	0		
Poitiers	4	22.0 ± 6 (100)	M1	0		
Réunion	5	5.6 ± 12.1 (213)	M2	3	34.9 ± 3.6 (289)	M3
Tunisia	3	19.9 ± 10.8 (251)	M2	2	48.3 ± 2.4 (87)	M3
Greece	3	8.8 ± 9.6 (80)	M2	3	46.5 ± 4.3 (226)	M3

$^\dagger$ Number of isofemale lines tested (one mother was at the origin of a given line);  $^\ddagger$ proportion of males in progenies produced by the mothers (the total number of offspring is given in parentheses);  $^\S$ mitochondrial haplotype linked with each mother;  $^\P$ results from Rigaud *et al.* (1997).

**Table 5** *Wolbachia* infestation detected by PCR in wild-caught females of *Porcellionides pruinosus* and *Wolbachia* transmission detected by PCR in offspring from infected mothers in various populations (the number of individuals tested is given parentheses)

Locations	Infestation rate	Transmission rate			
		Males		Females	
Mignaloux	100% (11)†	87.5% (8)	91.6% (12)		
Nevers	90% (10)†	80% (15)	100% (13)		
St. Martin	100% (29)†	100% (12)	90.9% (11)		
Poitiers	100% (4)	100% (9)	100% (10)		
Réunion island	60% (8)	0% (4)	87.5% (16)		
Tunisia	62.5% (5)	0% (4)	93.3 (15)		
Greece	60% (10)	0% (2)	92.3 (13)		

†Results from Rigaud *et al.* (1997)

endosymbionts at an equal rate to that of females. On average, 90.9% of the males from French populations harboured the symbionts, and all the males were infected in Poitiers and St-Martin-du-Fouilloux (Table 5).

Finally, the sequence of the bacterial 16S rRNA genes revealed important differences between *Wolbachia* strains from geographically distant locations. The *Wolbachia* strain from Poitiers showed an identical sequence to that of *P. pruinosus* previously published, isolated from Celles sur Belle (France), a locality close to St Martin-du-Fouilloux (40 km) and Poitiers (50 km) (Bouchon *et al.*, 1998). The sequence of *Wolbachia* from Réunion island exhibited differences from French symbionts (Table 6). The two sequences showed a pairwise distance of 2%, which was the highest divergence recorded in oniscidean symbionts (Bouchon *et al.*, 1998). Even if sequence patterns were typical of the B group of *Wolbachiae* (Werren & O'Neill, 1997), the high divergence recorded here indicates that the two strains are distantly related.

## Discussion

Over the last few years, many cases of 'species complexes' have been described in isopods: for example, the *Orithoniscus flavus* complex (Dalens *et al.*, 1996) and the *Oniscus asellus* complex (Bilton, 1997). Even though the specimens collected in Greece, Réunion island, Tunisia and France were morphologically indistinct and all corresponded to the *P. pruinosus* description, it was clear that they could be separated into two groups. There were no crosses between individuals from France and those from Réunion island, Greece and Tunisia; this reproductive isolation was precopulatory in that no mating occurred. Greek, Réunion island and Tunisian populations were interfertile and genetically closely related. This suggested that these three populations belong to the same species. What has previously been considered to be a single species consists, in fact, of two sibling species. These two species are geographically distinct: one is commonly found in France ('French group' in the following discussion) and the other one was collected in Tunisia, Réunion island and Greece ('southern group' in the following discussion). These two sibling species may be included in a '*Porcellionides pruinosus* species complex', but it appears that the taxonomic status of *P. pruinosus* needs re-evaluation on a worldwide basis.

The data from the mitochondrial DNA analysis also strengthen the distinction between two species. Using RFLP analysis of the total mtDNA, we obtained three mitotypes of which one was strictly restricted to the French group. The two remaining mitotypes were shared in the three other populations. The PCR-RFLP analysis of the mt 16S rDNA showed two different patterns: one is only found in the French group and the other in the southern group. The mitochondrial diversity in *P. pruinosus*, with a considerable genetic distance ( $d \approx 0.45$ ) between the 'French' mitotype and the 'southern' mitotypes, suggests great divergence between the two sets of populations. Another line of evidence

**Table 6** Sequence comparison of the *P. pruinosus* symbiont from Réunion island (AJ133196) with known *Wolbachia* representatives, in 812 bp partial sequence of 16S rDNA

<i>Wolbachia</i> strain (origin)	Nucleotide change	<i>Wolbachiae</i> group	Phenotype	Accession number
<b>Isopods</b>				
<i>Porcellionides pruinosus</i> (Poitiers)	17	B	F	AJ223242
<i>Armadillidium vulgare</i> (Angoulême)	0	B	F	AJ223238
<i>Porcellio dilatatus petiti</i> (St Honorat)	9	B	CI	X65673
<b>Insects</b>				
<i>Culex pipiens</i>	19	B	CI	X61768
<i>Drosophila simulans</i> (Mont d'Ambre)	18	B	CI	X64266
<i>Drosophila simulans</i> (Riverside)	19	A	CI	X64264

confirming separated species is the nucleotide distance of 0.13 between the mitotypes, obtained with the mt 16S RNA gene portion. Tam *et al.* (1996) observed similar nucleotide distance based on mt 16S rDNA between different species of the Decapoda genus *Emerita*: 0.15 between *E. analoga* and *E. talpoida*; 0.13 between *E. analoga* and *E. portoricensis*; and 0.15 between *E. analoga* and *E. benedicti*.

The idea that *P. pruinosus* has been dispersed throughout the world by the actions of man, because similar forms are observed throughout an extensive range, is no longer acceptable. Such a spread may have occurred for the southern group which could have been transported by human activities from Greece or Africa (Tunisia) to Réunion island, even if historical evidence for such an introduction is lacking. The dispersal of the 'P. pruinosus complex' may be ancient and may predate the divergence of the sibling species. We propose that the French group has been isolated from its native locality, the Mediterranean region (Vandel, 1960), and has genetically diverged from the southern group. An isolating mechanism has evolved between these two species, so that hybridization is no longer possible between them.

Additionally, the *Wolbachia* harboured by the French and southern groups of the *P. pruinosus* complex are genetically different. Since both the hosts and *Wolbachia* strains are different, the differences in feminizing pattern (presence vs. absence in males) and in prevalence between the two associations could result from differences in coevolutionary patterns. Cross-infections are needed to discriminate which partner of the association plays a crucial role in these differences. Nevertheless, the pattern of *Wolbachia* infection in the southern group of the *P. pruinosus* complex is more closely related to that of *Wolbachia* infection in *A. vulgare*, where the males are never infected (Juchault *et al.*, 1993). This is confirmed by the closeness of ribosomal gene sequences of *A. vulgare* and French *P. pruinosus* symbionts.

Variations in mitochondrial DNA are often used to trace the evolutionary history of populations (Avise *et al.*, 1983). However, several selective forces may act on this genetic marker, including parasite-induced selective sweeps, to confound the interpretation of data (Ballard & Kreitman, 1995). The host reproductive changes caused by *Wolbachia* enhance the spread of the symbiont in infected populations (Werren & O'Neill, 1997). As a consequence, the infected cytoplasm increases in frequency by 'hitchhiking', at the expense of uninfected cytoplasm (Turelli *et al.*, 1992). This is very similar to the genetic hitchhiking associated with the fixation of an advantageous mutation by selection (Maynard Smith & Haigh, 1974) and will

usually cause a reduction in genetic polymorphism. Its maternal inheritance and the absence of recombination makes mitochondrial DNA sensitive to such hitchhiking and a maternally inherited factor, such as *Wolbachia*, could influence haplotype diversity (Ballard & Kreitman, 1995). Genetic hitchhiking could have caused the pattern of mtDNA variation in *P. pruinosus*. The feminizing effect of *Wolbachia* imparts a selective advantage to the infected females, which could have led to a selective sweep of the associated cytoplasm, carried passively as the micro-organism spreads through the population. Populations almost entirely infected with *Wolbachia* (French species) showed no mitochondrial polymorphism, suggesting that the polymorphism had disappeared when invasion was complete. This hypothesis is strengthened by the presence of a unique lineage of symbionts in French populations according to the sequence data. The only uninfected French wild-caught female shared the same mitotype with females infected by the symbiont. This could be caused by a secondary loss of *Wolbachia* in this female. On the other hand, populations with polymorphism for *Wolbachia* infection (southern group) had slight mitochondrial polymorphism, and there was a link between *Wolbachia* infection status and mtDNA types. Here, a single mitotype was associated with the symbionts, which might be explained by a single *Wolbachia* infection event. In these populations, uninfected individuals showed no mtDNA polymorphism. This might be because of the small size of our samples, and/or bottlenecks experienced by populations.

The general pattern of mtDNA polymorphism and *Wolbachia* infection in *P. pruinosus* contrasts with that found in another woodlouse: *A. vulgare*. The mtDNA in *A. vulgare* shows considerable polymorphism, despite the presence of sex ratio distorters including a *Wolbachia* symbiont (Grandjean *et al.*, 1993; Rigaud *et al.* unpublished observation). *Wolbachia* prevalence is always low in *A. vulgare* populations (Juchault *et al.*, 1993), and another sex ratio distorter is partially paternally inherited. These two traits contribute to the mtDNA polymorphism (Grandjean *et al.*, 1993). Such phenomena were not found in *P. pruinosus*, and the pattern of mitochondrial variation must be related to the strong prevalence of *Wolbachia* in this complex of species.

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