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1 **Heterogeneous distribution of sex ratio distorters in natural populations of**  
2 **the isopod *Armadillidium vulgare***

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16

17 **Abstract**

18

19 In the isopod *Armadillidium vulgare*, many females produce progenies with female-biased sex ratios,  
20 due to two feminizing sex ratio distorters (SRD): *Wolbachia* endosymbionts and the *f* element. We  
21 investigated the distribution and population dynamics of these SRD and mitochondrial DNA variation  
22 in 16 populations from Europe and Japan. Confirming and extending results from the 1990's, we  
23 found that the SRD are present at variable frequencies in populations, and that the *f* element is  
24 overall more frequent than *Wolbachia*. The two SRD never co-occur at high frequency in any  
25 population, suggesting an apparent mutual exclusion. We also detected *Wolbachia* or the *f* element  
26 in some males, which likely reflects insufficient titer to induce feminization or presence of  
27 masculinizing alleles. Our results are consistent with a single integration event of a *Wolbachia*  
28 genome in the *A. vulgare* genome at the origin of the *f* element, which contradicts an earlier  
29 hypothesis of frequent losses and gains. We identified strong linkage between *Wolbachia* strains and  
30 mitochondrial haplotypes, but no association between the *f* element and mitochondrial background.  
31 Our results open new perspectives on SRD evolutionary dynamics in *A. vulgare*, the evolution of  
32 genetic conflicts and their impact on the variability of sex determination systems.

33

34 **Keywords**

35

36 Sex ratio distorter, endosymbiont, *Wolbachia*, *f* element, sex determination

## 37 1. Introduction

38

39 Sex ratio distorters (SRD) are selfish genetic elements located on sex chromosomes or transmitted by  
40 a single sex, which skew the proportion of males and females in progenies towards the sex that  
41 enhances their own vertical transmission [1]. Major SRD types include sex chromosome meiotic  
42 drivers [2,3], some B chromosomes [4] and selfish cytoplasmic genetic entities [5,6,7]. Collectively,  
43 they are found in a wide range of animal and plant species and they have had a tremendous impact  
44 on the ecology and evolution of their host species [8,9]. One of the most emblematic SRD is the  
45 bacterial endosymbiont *Wolbachia* [10,11]. *Wolbachia* is a cytoplasmic, maternally inherited alpha-  
46 proteobacterium found in a wide range of arthropods and nematodes. In arthropods, *Wolbachia*  
47 often manipulates host reproduction in favor of infected females, thereby conferring itself a  
48 transmission advantage. This is achieved through various strategies, three of which causing sex ratio  
49 distortions towards females: male killing, thelytokous parthenogenesis and feminization of genetic  
50 males [6,7,10,11].

51 In the terrestrial isopod *Armadillidium vulgare*, chromosomal sex determination follows female  
52 heterogamety (ZZ males and ZW females) [12–14]. However, many females produce progenies with  
53 female-biased sex ratios, due to the presence of two feminizing SRD: *Wolbachia* endosymbionts and  
54 a locus called the *f* element [6,15,16]. *Wolbachia* symbionts cause ZZ genetic males to develop as  
55 phenotypic females [17]. Three *Wolbachia* strains have been described in *A. vulgare*, for which  
56 feminization induction has been demonstrated (*wVulC* and *wVulM* strains [18,19]) or is strongly  
57 suspected (*wVulP* strain [20]). The *f* element is a nuclear insert of a large portion of a feminizing  
58 *Wolbachia* genome in the *A. vulgare* genome [21]. The *f* element induces female development, as a  
59 W chromosome does, and it shows non-Mendelian inheritance, making it an SRD [21,22]. These SRD  
60 may cause turnovers in sex determination mechanisms [6,15,23] and they could explain why sex  
61 chromosome systems are so variable in terrestrial isopods [24–27].

62 Testing this hypothesis requires characterizing the evolutionary dynamics of SRD such as *Wolbachia*  
63 and the *f* element in natural populations. In *A. vulgare*, this characterization is quite limited because  
64 prior studies were mostly restricted to a narrow geographic area (western France), sometimes  
65 focusing solely on *Wolbachia* [20,28–31]. The only exception is a 1993 study [32], which collated and  
66 extended results from the early 1980's [33,34]. The main observations were that *Wolbachia* and the *f*  
67 element are present at variable frequencies in field populations, and the *f* element is more frequent  
68 than *Wolbachia*. However, earlier studies were limited by the lack of molecular tests for *Wolbachia*  
69 and/or the *f* element, preventing any direct assessment of SRD presence. Instead, the authors used a

70 complex, indirect procedure combining a physiological test and crossings [32]. In addition to being  
71 tedious and time-consuming (generation time is one year in this species), this procedure did not  
72 allow direct and undisputable assessment of SRD presence. Moreover, it could only be run on  
73 females and therefore provided no information on SRD presence in males. Finally, it could not reveal  
74 individuals potentially carrying both SRD.

75 Here, we took advantage of the availability of molecular markers to directly assess SRD presence in  
76 males and females from *A. vulgare* field populations from Europe and Japan. This approach allowed  
77 us to circumvent the limitations of previous studies, and to revisit the population dynamics of  
78 *Wolbachia* and the *f* element in this species and their association mitochondrial lineages.

79

## 80 **2. Materials and Methods**

81

82 647 *A. vulgare* individuals from 16 natural populations across Europe and Japan were collected by  
83 hand. Individuals were sexed and stored in alcohol or at -20°C prior to DNA extraction. Total genomic  
84 DNA was extracted from the head and legs of each individual, as described previously [21]. We used  
85 four molecular markers to assess the presence of *Wolbachia* and the *f* element in DNA extracts: *Jtel*  
86 [21], *wsp* [35], *recR* [36] and *ftsZ* [37] (Table S1). While *Jtel* is specific to the *f* element, *wsp* and *recR*  
87 are specific to *Wolbachia*, and *ftsZ* is present in both the *f* element and *Wolbachia* [21]. We assessed  
88 the presence or absence of these markers by PCR, as described previously [21]. Different  
89 amplification patterns were expected for individuals with *Wolbachia* only (*Jtel*-, *wsp*+, *recR*+, *ftsZ*+),  
90 the *f* element only (*Jtel*+, *wsp*-, *recR*-, *ftsZ*+), both *Wolbachia* and the *f* element present (*Jtel*+, *wsp*+,  
91 *recR*+, *ftsZ*+) or both *Wolbachia* and the *f* element lacking (*Jtel*-, *wsp*-, *recR*-, *ftsZ*-). The few  
92 individuals exhibiting other amplification patterns were classified as “undetermined status”. A  
93 quantitative-PCR assay was used to measure *Wolbachia* titer in some individuals (see supplementary  
94 Methods). To characterize *Wolbachia* strain diversity, *wsp* PCR products were purified and Sanger  
95 sequenced using both forward and reverse primers by GenoScreen (Lille, France). Forward and  
96 reverse reads were assembled using Geneious® v.7.1.9 to obtain one consensus sequence per  
97 individual. To evaluate mitochondrial diversity, we amplified by PCR a ~700 bp-long portion of the  
98 Cytochrome Oxidase I (*COI*) gene in all individuals [38]. PCR products were purified and Sanger  
99 sequenced as described above. Haplotype network analysis was performed using the *pegas* package  
100 [39]. All statistical analyses were performed with R v.3.6.0 [40]. Figures were realized with *ggplot2*  
101 [41].

102

### 103 3. Results

104

105 We tested the presence of *Wolbachia* and the *f* element in 423 females and 224 males from 16  
106 populations across Europe and Japan (Tables 1, S2). While most males lacked both SRD, 48% of  
107 females carried at least one of them. The remaining females presumably carry W chromosomes,  
108 although the existence of other feminizing elements cannot be formally excluded. As expected for  
109 feminizing elements, the SRD were mostly found in females, the *f* element being more frequent than  
110 *Wolbachia* overall. Both SRD were found in the same individuals in only 3 females from a single  
111 population (Chizé). *Wolbachia*-infected individuals carried one of the three previously known  
112 *Wolbachia* strains of *A. vulgare*: wVulC (n=62), wVulM (n=23) or wVulP (n=4).

113 *Wolbachia* and *f* element distribution in females was highly heterogenous among populations (Figure  
114 1a). These SRD were found in 10 and 11 out of 16 populations, but they reached frequencies >10% in  
115 only 6 and 7 populations, respectively. The two SRD coexisted in 8 populations. A generalized linear  
116 model predicting the frequency of the *f* element as a binomial response by the proportion of  
117 individuals carrying *Wolbachia* (each statistical unit being a population) showed that the prevalence  
118 of the two SRD was significantly negatively correlated (9.8% of deviance explained, Chi-squared test,  
119  $p < 7.9 \times 10^{-8}$ , 14 df) (Figure 1b). Hence, in Floirac, Poitiers, Saint Julien l'Arç and Pisa populations,  
120 *Wolbachia* was frequent (23-94% frequency in females) and the *f* element was rare (0-8%). By  
121 contrast, the *f* element was frequent (35-96%) and *Wolbachia* was rare (0-11%) in Prague, Beauvoir,  
122 Chizé, Coulombiers and La Crèche populations. In the other populations, both SRD were found at low  
123 to moderate frequency (0-19%), including 3 populations devoid of both SRD (Lastovo, Hyogo and  
124 Bucharest).

125 Males carrying *Wolbachia* or the *f* element were found in 2 and 4 out of 16 populations, respectively.  
126 In all cases, these males occurred in populations in which the corresponding SRD were the most  
127 prevalent ones in females: Beauvoir, Chizé, Coulombiers and La Crèche for the *f* element, and Floirac  
128 and Saint Julien l'Arç for *Wolbachia*. Overall, these males had much lower *Wolbachia* titer than  
129 females from their respective populations (Figure S1, Table S3).

130 The 642 individuals sequenced at the *COI* gene presented a total of 92 segregating sites defining 23  
131 haplotypes (named I to XXIII; GenBank accession numbers in Table S4), with 1 to 7 haplotypes per  
132 population (Table S2, Figure 2). The most frequent and widespread haplotype (I) was found in 188  
133 individuals from 10 populations. The second most frequent and widespread haplotype (V) was found

134 in 106 individuals from 7 populations. We found 21 out of the 23 haplotypes among individuals  
135 lacking both *Wolbachia* and the *f* element (Table 2, Figure 2). Among individuals carrying the *f*  
136 element, 6 haplotypes were found, all but one (I, II, III, V and VI) being shared with individuals lacking  
137 both *Wolbachia* and the *f* element, and one (IV) being carried by a single individual in the entire  
138 dataset. Among *Wolbachia*-infected individuals, all those carrying *wVulC* were associated with either  
139 haplotype V or its close relatives (XI and XII). All individuals carrying *wVulM* were associated with  
140 haplotype II and those carrying *wVulP* with haplotype VII. Of the 5 haplotypes found in *Wolbachia*-  
141 infected individuals, 4 were shared with individuals lacking both *Wolbachia* and the *f* element (II, V,  
142 VII and XII), 2 of which were also shared with individuals carrying the *f* element (II and V), and one  
143 (XI) was present in a single individual in the entire dataset.

144

#### 145 **4. Discussion**

146

147 Our results provide direct evidence that the *f* element is overall more frequent than *Wolbachia* in the  
148 sampled *A. vulgare* populations. We detected the *f* element in 11 *A. vulgare* natural populations from  
149 4 European countries (Czech Republic, France, Germany and The Netherlands) and Japan. Together  
150 with its previous detection in Denmark [21], our results indicate that the *f* element has spread to a  
151 wide geographical range. The relative frequencies of the *f* element and *Wolbachia* are highly variable  
152 among populations and, in general, when one SRD is frequent, the other SRD is rare. Overall, these  
153 results are consistent with earlier results from the 1990's [32], although no molecular assay allowing  
154 direct testing was available at that time and SRD presence or absence was inferred indirectly.

155 As the *Jtel* marker is located across the site of integration of the *f* element in the *A. vulgare*  
156 chromosome [21], *f* element presence in various populations can be explained by a single event of  
157 integration of a *Wolbachia* genome in the *A. vulgare* genome. A less parsimonious scenario would  
158 require independent insertions at the same chromosomal site, which is highly unlikely. The former  
159 scenario contradicts an earlier hypothesis on the evolutionary dynamics of the *f* element, which  
160 suggested that the *f* element was unstably integrated in the *A. vulgare* genome, experiencing  
161 frequent loss from oocytes and recurrent gain from *Wolbachia* endosymbionts [22,23,42–44]. Under  
162 this scenario, multiple independent *f*-like elements would be expected to segregate at low  
163 frequencies in populations, they should be integrated in different genomic locations and they should  
164 all be able to induce feminization [16]. While our results do not formally invalidate the possibility of  
165 additional *f*-like integrations in *A. vulgare* populations, which the *Jtel* marker would not detect, it

166 does not appear to be the most parsimonious hypothesis. Examination of sex ratios from progenies  
167 of wild-caught females lacking both SRD may offer further insight into this issue.

168 Using molecular assays allowed us to circumvent two limitations of the previously used physiological  
169 test: the impossibility to detect *Wolbachia* and the *f* element in males, and the impossibility to detect  
170 individuals carrying both SRD. Regarding *Wolbachia* presence in males, the historic protocol was only  
171 applicable to females per design [29,30,32] and subsequent PCR screens for *Wolbachia* infection  
172 have mostly focused on testing females [20,30,31]. In fact, males have seldom been tested and found  
173 to carry *Wolbachia* [45]. Here, we detected *Wolbachia* in 7 males from 2 populations (Florac and  
174 Saint Julien l'Ars), carrying either wVulC or wVulM strains. The failure of feminization by *Wolbachia*  
175 most certainly reflects insufficient bacterial titer to induce feminization (Figure S1). These field  
176 observations hence support the view that titer is an important factor for successful feminization, as  
177 low titer is linked to incomplete feminization and intersexual phenotypes [42,46].

178 We also detected the presence of the *f* element in 11 males from 4 populations. Historically, the  
179 presence of the *f* element in males has been indirectly inferred from crossings and the resulting sex-  
180 ratios biases of progenies [22,43,47]. Our results constitute the first direct evidence for the presence  
181 of the *f* element in *A. vulgare* males. In all 4 populations in which *f*-carrying males were found, the *f*  
182 element was also frequent in females. Altogether, these observations suggest that the 11 males  
183 carrying the *f* element also carry the masculinizing dominant *M* allele [16,43,47]. Indeed, the *M* allele  
184 is able to restore a male phenotype in individuals carrying the *f* element [16,43,47]. Because of sex  
185 ratio selection, the *M* allele is thought to have been selected to restore males in response to female-  
186 biased sex ratios caused by the *f* element [47]. Thus, the *M* allele is expected to rise in frequency  
187 when the *f* element is frequent in a population [47], which is consistent with our observations.  
188 Unfortunately, no molecular marker of the *M* allele is currently available, which prevents any direct  
189 assessment of its actual presence in these populations. Thus, we cannot exclude that males carrying  
190 the *f* element simply carry non-feminizing variants of this SRD.

191 Our results show that *Wolbachia* and the *f* element never co-occur at high frequency in any  
192 population. This apparent mutual exclusion can be explained considering that co-occurrence of  
193 multiple feminizing factors in a population should favor the most transmitted one [16,48]. Hence,  
194 *Wolbachia* is expected to lead to the loss of nuclear feminizing elements in *A. vulgare* populations.  
195 This situation does not result from an interference between chromosomes and *Wolbachia* within  
196 individuals, but from counter selection of nuclear feminizing alleles in a population that becomes  
197 increasingly biased towards females. Hence, the rise of *Wolbachia* would associate with the decline



198 of the *f* element in a population. Why, under these circumstances, *Wolbachia* has not invaded all *A.*  
199 *vulgare* populations is still unclear and may reflect fitness effects or possible resistance genes.

200 As a result, only very few individuals were found to carry both *Wolbachia* and the *f* element. They  
201 represent only 3 females, all from the Chizé population (Figure 1a). These were likely born from  
202 mothers carrying *Wolbachia* and fathers carrying the *f* element, which are frequent at Chizé. The  
203 apparent absence of carriers of both SRD in other populations where these SRD are present could  
204 simply be explained by the paucity of males carrying the *f* element.

205 Mapping SRD distribution onto mitochondrial genealogy showed excellent congruence between  
206 *Wolbachia* strains and mitochondrial haplotypes (*wVulC-V*, *wVulM-II* and *wVulP-VII*). Such strong  
207 association has previously been noted in *A. vulgare-Wolbachia* interactions at a smaller geographic  
208 scale [30,31] and, more generally, in many arthropod-*Wolbachia* interactions [49]. This result  
209 corroborates the rarity of non-maternal transmission of *Wolbachia* in *A. vulgare*. By contrast, the *f*  
210 element was found in 6 different mitochondrial backgrounds (I-VI) scattered across the  
211 mitochondrial phylogeny, indicating no particular association between the *f* element and  
212 mitochondria. This result confirms and extends earlier data focused on western France and in which *f*  
213 element presence in females was indirectly inferred based on sex ratios of their progenies [30]. This  
214 observation can be explained by the occasional paternal transmission of the *f* element, which breaks  
215 its association with mitochondrial background [16,22,30].

216

217 **Data accessibility**

218

219 All data are provided in the electronic supplementary material.

220

221

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223

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226

227

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229

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232 the University of Poitiers.

233

234 **Figure legends**

235

236 **Figure 1. (A)** Prevalence of *Wolbachia* and the *f* element in males (m) and females (f) from 16  
237 *Armadillidium vulgare* populations. **(B)** Relative proportions of *Wolbachia* and the *f* element in 16 *A.*  
238 *vulgare* populations (represented by open circles).

239

240 **Figure 2.** Haplotype network of 23 mitochondrial variants (I-XXIII) from 16 *Armadillidium vulgare*  
241 populations. Each circle represents one haplotype and circle diameter is proportional to the number  
242 of individuals carrying the haplotype. Branch lengths connecting circles are proportional to  
243 divergence between haplotypes. Sex ratio distorter frequencies are color-coded for each haplotype.

244

245 **Table 1. Prevalence of *Wolbachia* and *f* element sex ratio distorters in 16 populations of *Armadillidium vulgare*.**

246

Population (Country)	Sampling year	Sample size	Sex	Number of individuals	No <i>f</i> element, no <i>Wolbachia</i>	Only <i>f</i> element	<i>Only Wolbachia</i>				Both wVulM and <i>f</i> element	Undetermined status
							wVulC	wVulM	wVulP	Undetermined		
Lastovo (Croatia)	2017	54	Males	30	30							
			Females	24	24							
Prague (Czech Republic)	2018	36	Males	9	9							
			Females	27	1	26						
Beauvoir (France)	2017	31	Males	6	5	1						
			Females	25	9	14		1				1
Chizé (France)	2017	52	Males	8	2	6						
			Females	44	3	36		2			3	
Coulombiers (France)	2017	24	Males	4	2	2						
			Females	20	6	13	1					
Floirac (France)	2016	114	Males	38	34		2					2
			Females	76	21	6	40	9				
Gript (France)	2017	45	Males	15	15							
			Females	30	26	2	2					
La Crèche (France)	2017	58	Males	21	19	2						
			Females	37	23	13	1					
Poitiers (France)	2015	23	Males	4	4							
			Females	19	10	1			4	4		
Saint Julien l'Ars (France)	2016	31	Males	14	9		1	3		1		
			Females	17	1		12	3				1
Göttingen (Germany)	2017	24	Males	7	3							4
			Females	17	11	3		2				1
Pisa (Italy)	2017	28	Males	15	15							
			Females	13	10		3					
Hyogo	2018	50	Males	21	18							3

(Japan)			Females	29	26						3	
Tottori	2018	49	Males	21	21							
(Japan)			Females	28	26	2						
Bucharest	2017	17	Males	9	9							
(Romania)			Females	8	8							
Wageningen	2018	11	Males	2	2							
(The Netherlands)			Females	9	7	1					1	
Total males				224	197	11	3	3	1		9	
Total females				423	212	117	59	17	4	4	3	7
Total				647	409	128	62	20	4	5	3	16

247

248

249 **Table 2. Distribution of mitochondrial haplotypes in 642 *Armadillidium vulgare* individuals from 16 populations.**

250

Sex ratio distorter status	Number of individuals	Haplotype number	Haplotype list
No <i>f</i> element, no <i>Wolbachia</i>	404	21	I, II, III, V, VI, VII, VIII, IX, X, XII, XIII, XIV, XV, XVI, XVII, XVIII, XIX, XX, XXI, XXII, XXIII
<i>f</i> element only	128	6	I, II, III, IV, V, VI
<i>Wolbachia</i> (wVulC strain) only	62	3	V, XI, XII
<i>Wolbachia</i> (wVulM strain) only	20	1	II
<i>Wolbachia</i> (wVulP strain) only	4	1	VII
<i>Wolbachia</i> (undetermined strain) only	5	2	II, VII
Both wVulM and <i>f</i> element	3	1	II
Undetermined status	16	4	I, V, VI, XIX

251

252

253 **References**

254

- 255 1. Beukeboom LW, Perrin N. 2014 *The evolution of sex determination*. Oxford: Oxford University  
256 Press.
- 257 2. Jaenike J. 2001 Sex chromosome meiotic drive. *Annu. Rev. Ecol. Syst.* **32**, 25–49.  
258 (doi:10.1146/annurev.ecolsys.32.081501.113958)
- 259 3. Helleu Q, Gérard PR, Montchamp-Moreau C. 2014 Sex chromosome drive. *Cold Spring Harb.*  
260 *Perspect. Biol.* **7**, a017616. (doi:10.1101/cshperspect.a017616)
- 261 4. Camacho JPM, Schmid M, Cabrero J. 2011 B chromosomes and sex in animals. *Sex. Dev. Genet.*  
262 *Mol. Biol. Evol. Endocrinol. Embryol. Pathol. Sex Determ. Differ.* **5**, 155–166.  
263 (doi:10.1159/000324930)
- 264 5. Chase CD. 2007 Cytoplasmic male sterility: a window to the world of plant mitochondrial-nuclear  
265 interactions. *Trends Genet.* **23**, 81–90. (doi:10.1016/j.tig.2006.12.004)
- 266 6. Cordaux R, Bouchon D, Greve P. 2011 The impact of endosymbionts on the evolution of host sex-  
267 determination mechanisms. *Trends Genet* **27**, 332–41.
- 268 7. Hurst GDD, Frost CL. 2015 Reproductive parasitism: maternally inherited symbionts in a biparental  
269 world. *Cold Spring Harb. Perspect. Biol.* **7**, a017699. (doi:10.1101/cshperspect.a017699)
- 270 8. Burt A, Trivers R. 2006 *Genes in conflict*. Cambridge, Massachusetts: The Belknap Press of Harvard  
271 University Press.
- 272 9. Werren JH. 2011 Selfish genetic elements, genetic conflict, and evolutionary innovation. *Proc.*  
273 *Natl. Acad. Sci. U. S. A.* **108 Suppl 2**, 10863–10870. (doi:10.1073/pnas.1102343108)
- 274 10. Werren JH, Baldo L, Clark ME. 2008 Wolbachia: master manipulators of invertebrate biology.  
275 *Nat Rev Microbiol* **6**, 741–51. (doi:10.1038/nrmicro1969)
- 276 11. Kaur R, Shropshire JD, Cross KL, Leigh B, Mansueto AJ, Stewart V, Bordenstein SR,  
277 Bordenstein SR. 2021 Living in the endosymbiotic world of Wolbachia: A centennial review. *Cell*  
278 *Host Microbe* **29**, 879–893. (doi:10.1016/j.chom.2021.03.006)
- 279 12. Juchault P, Legrand JJ. 1972 Croisement de néo-mâles expérimentaux chez *Armadillidium*  
280 *vulgare* Latr. (Crustace, Isopode, Oniscoïde). Mise en évidence d'une hétérogamétie femelle. *C R*  
281 *Acad Sci Paris* **274**, 1387–1389.
- 282 13. Chebbi MA, Becking T, Moumen B, Giraud I, Gilbert C, Peccoud J, Cordaux R. 2019 The  
283 Genome of *Armadillidium vulgare* (Crustacea, Isopoda) Provides Insights into Sex Chromosome  
284 Evolution in the Context of Cytoplasmic Sex Determination. *Mol. Biol. Evol.* **36**, 727–741.  
285 (doi:10.1093/molbev/msz010)
- 286 14. Cordaux R, Chebbi MA, Giraud I, Pleydell DRJ, Peccoud J. 2021 Characterization of a Sex-  
287 Determining Region and Its Genomic Context via Statistical Estimates of Haplotype Frequencies in  
288 Daughters and Sons Sequenced in Pools. *Genome Biol. Evol.* **13**, evab121.  
289 (doi:10.1093/gbe/evab121)

- 290 15. Rigaud T, Juchault P, Mocquard JP. 1997 The evolution of sex determination in isopods  
291 crustaceans. *Bioessays* **19**, 409–416.
- 292 16. Cordaux R, Gilbert C. 2017 Evolutionary Significance of Wolbachia-to-Animal Horizontal Gene  
293 Transfer: Female Sex Determination and the f Element in the Isopod *Armadillidium vulgare*. *Genes*  
294 **8**, 186. (doi:10.3390/genes8070186)
- 295 17. Martin G, Juchault P, Legrand JJ. 1973 Mise en évidence d'un micro-organisme  
296 intracytoplasmique symbiote de l'Oniscoïde *Armadillidium vulgare* L. dont la présence  
297 accompagne l'intersexualité ou la féminisation totale des mâles génétiques de la lignée thélygène.  
298 *Comptes Rendus Académie Sci. Paris* **276**, 2313–2316.
- 299 18. Rigaud T, Souty Grosset C, Raimond R, Mocquard JP, Juchault P. 1991 Feminizing  
300 endocytobiosis in the terrestrial crustacean *Armadillidium vulgare* Latr. (Isopoda): Recent  
301 acquisitions. *Endocytobiosis Cell Res* **7**, 259–273.
- 302 19. Cordaux R, Michel-Salzat A, Frelon-Raimond M, Rigaud T, Bouchon D. 2004 Evidence for a  
303 new feminizing Wolbachia strain in the isopod *Armadillidium vulgare*: evolutionary implications.  
304 *Heredity* **93**, 78–84. (doi:10.1038/sj.hdy.6800482)
- 305 20. Verne S, Johnson M, Bouchon D, Grandjean F. 2007 Evidence for recombination between  
306 feminizing Wolbachia in the isopod genus *Armadillidium*. *Gene* **397**, 58–66.  
307 (doi:10.1016/j.gene.2007.04.006)
- 308 21. Leclercq Sb, Thézé J, Chebbi MA, Giraud I, Moumen B, Ernenwein L, Greve P, Gilbert C,  
309 Cordaux R. 2016 Birth of a W sex chromosome by horizontal transfer of *Wolbachia* bacterial  
310 symbiont genome. *Proc. Natl. Acad. Sci. U. S. A.* **113**, 15036–15041.  
311 (doi:10.1073/pnas.1608979113)
- 312 22. Legrand JJ, Juchault P. 1984 Nouvelles données sur le déterminisme génétique et  
313 épigénétique de la monogénie chez le crustacés isopodes terrestres *Armadillidium vulgare* Latr.  
314 *Génét Sél Evol* **16**, 57–84.
- 315 23. Juchault P, Mocquard JP. 1993 Transfer of a parasitic sex factor to the nuclear genome of the  
316 host: A hypothesis on the evolution of sex-determining mechanisms in the terrestrial isopod  
317 *Armadillidium vulgare* Latr. *J Evol Biol* **6**, 511–528.
- 318 24. Juchault P, Rigaud T. 1995 Evidence for female heterogamety in two terrestrial crustaceans  
319 and the problem of sex chromosome evolution in isopods. *Heredity* **75**, 466–471.
- 320 25. Becking T, Giraud I, Raimond M, Moumen B, Chandler C, Cordaux R, Gilbert C. 2017 Diversity  
321 and evolution of sex determination systems in terrestrial isopods. *Sci. Rep.* **7**, 1–14.  
322 (doi:10.1038/s41598-017-01195-4)
- 323 26. Becking T, Chebbi MA, Giraud I, Moumen B, Laverré T, Caubet Y, Peccoud J, Gilbert C,  
324 Cordaux R. 2019 Sex chromosomes control vertical transmission of feminizing Wolbachia  
325 symbionts in an isopod. *PLOS Biol.* **17**, e3000438. (doi:10.1371/journal.pbio.3000438)
- 326 27. Russell A, Borrelli S, Fontana R, Laricchiuta J, Pascar J, Becking T, Giraud I, Cordaux R,  
327 Chandler CH. 2021 Evolutionary transition to XY sex chromosomes associated with Y-linked  
328 duplication of a male hormone gene in a terrestrial isopod. *Heredity* **127**, 266–277.  
329 (doi:10.1038/s41437-021-00457-2)

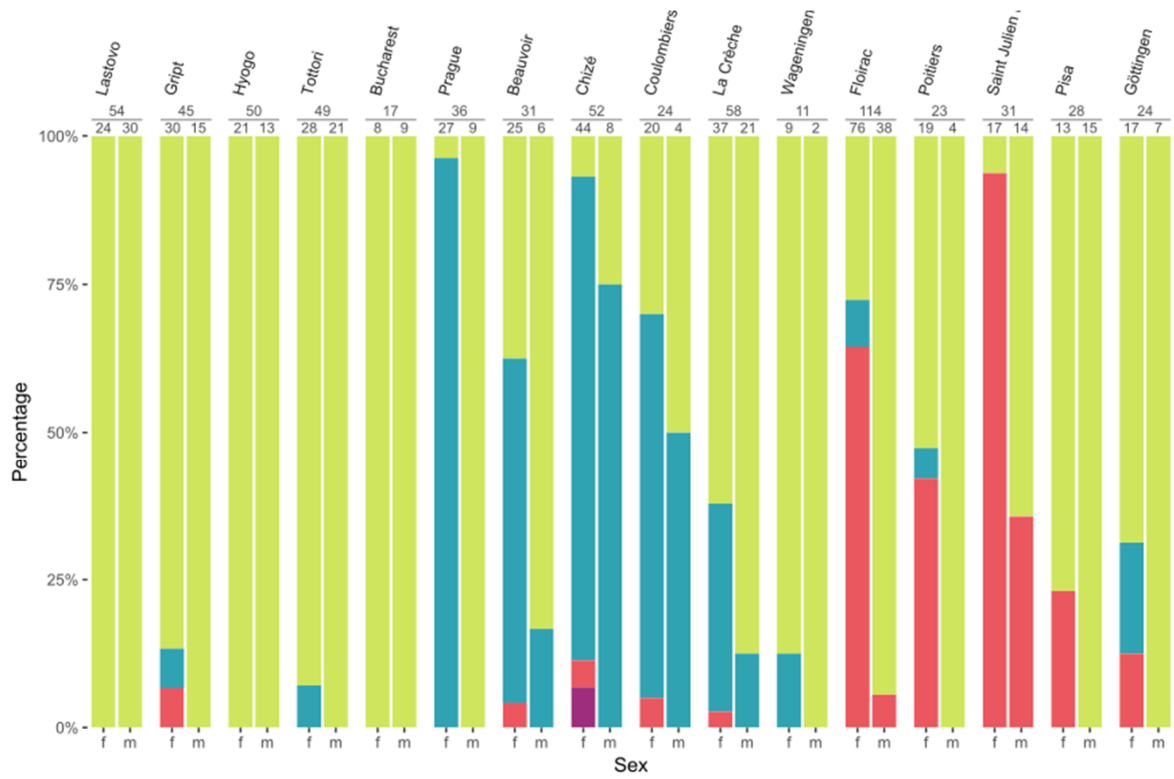


- 330 28. Juchault P, Legrand JJ, Mocquard JP. 1980 Contribution à l'étude qualitative et quantitative  
331 des facteurs contrôlant le sexe dans les populations du crustacé isopode terrestre *Armadillidium*  
332 *vulgare* Latreille. I. La population de Niort (Deux Sèvres). *Arch Zool Exp Gen* **121**, 3–27.
- 333 29. Grandjean F, Rigaud T, Raimond R, Juchault P, Souty-Grosset C. 1993 Mitochondrial DNA  
334 polymorphism and feminizing sex factor dynamics in a natural population of *Armadillidium*  
335 *vulgare* (Crustacea, Isopoda). *Genetica* **92**, 55–60.
- 336 30. Rigaud T, Bouchon D, Souty-Grosset C, Raimond R. 1999 Mitochondrial DNA polymorphism,  
337 sex ratio distorters and population genetics in the isopod *Armadillidium vulgare*. *Genetics* **152**,  
338 1669–1677.
- 339 31. Verne S, Johnson M, Bouchon D, Grandjean F. 2012 Effects of parasitic sex-ratio distorters on  
340 host genetic structure in the *Armadillidium vulgare*-*Wolbachia* association. *J. Evol. Biol.* **25**, 264–  
341 76. (doi:10.1111/j.1420-9101.2011.02413.x)
- 342 32. Juchault P, Rigaud T, Mocquard JP. 1993 EVOLUTION OF SEX DETERMINATION AND SEX-  
343 RATIO VARIABILITY IN WILD POPULATIONS OF ARMADILLIDIUM-VULGARE (LATR) (CRUSTACEA,  
344 ISOPODA) - A CASE-STUDY IN CONFLICT-RESOLUTION. *Acta Oecologica-Int. J. Ecol.* **14**, 547–562.
- 345 33. Juchault P, Legrand JJ. 1981 Contribution à l'étude qualitative et quantitative des facteurs  
346 contrôlant le sexe dans les populations du Crustacé Isopode terrestre *Armadillidium vulgare* Latr.  
347 II - Populations hébergeant le facteur féminisant F (bactérie intracytoplasmique). *Arch Zool Exp*  
348 *Gén* **122**, 65–74.
- 349 34. Juchault P, Legrand JJ. 1981 Contribution à l'étude qualitative et quantitative des facteurs  
350 contrôlant le sexe dans les populations du Crustacé isopode terrestre *Armadillidium vulgare*  
351 Latreille. III. Populations n'hébergeant pas le facteur féminisant F (bactéroïde intracytoplasmique).  
352 *Arch Zool Exp Gén* **122**, 117–131.
- 353 35. Braig HR, Zhou WG, Dobson SL, O'Neill SL. 1998 Cloning and characterization of a gene  
354 encoding the major surface protein of the bacterial endosymbiont *Wolbachia pipientis*. *J.*  
355 *Bacteriol.* **180**, 2373–2378.
- 356 36. Badawi M, Giraud I, Vavre F, Grève P, Cordaux R. 2014 Signs of Neutralization in a Redundant  
357 Gene Involved in Homologous Recombination in *Wolbachia* Endosymbionts. *Genome Biol. Evol.* **6**,  
358 2654–2664. (doi:10.1093/gbe/evu207)
- 359 37. Werren JH, Zhang W, Guo LR. 1995 Evolution and phylogeny of *Wolbachia*: reproductive  
360 parasites of arthropods. *Proc. Biol. Sci.* **261**, 55–63. (doi:10.1098/rspb.1995.0117)
- 361 38. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994 DNA primers for amplification of  
362 mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar.*  
363 *Biol. Biotechnol.* **3**, 294–299.
- 364 39. Paradis E. 2010 pegas: an R package for population genetics with an integrated–modular  
365 approach. *Bioinformatics* **26**, 419–420.
- 366 40. R Development Core Team. 2013 *R: A language and environment for statistical computing*.  
367 See <http://www.R-project.org/>.
- 368 41. Wickham H *et al.* 2020 ggplot2: Create Elegant Data Visualisations Using the Grammar of  
369 Graphics.

- 370 42. Juchault P, Legrand JJ. 1989 Sex determination and monogeny in terrestrial isopods  
371 *Armadillidium vulgare* (Latreille, 1804) and *Armadillidium nasatum* bundde-lund, 1885. *Monit.*  
372 *Zool Ital NS Monogr* **4**, 359–375.
- 373 43. Juchault P, Rigaud T, Mocquard J-P. 1992 Evolution of sex-determining mechanisms in a wild  
374 population of *Armadillidium vulgare* Latr. (Crustacea, Isopod) : competition between two  
375 feminizing parasitic sex factors. *Heredity* **69**, 382–390.
- 376 44. Rigaud T, Mocquard J-P, Juchault P. 1992 The spread of parasitic sex factors in populations of  
377 *Armadillidium vulgare* Latr. (Crustacea, Oniscidae): effects on sex ratio. *Génét Sél Evol* **24**, 3–18.
- 378 45. Dittmer J, Lesobre J, Moumen B, Bouchon D. 2016 Host origin and tissue microhabitat  
379 shaping the microbiota of the terrestrial isopod *Armadillidium vulgare*. *FEMS Microbiol. Ecol.* **92**,  
380 fiw063. (doi:10.1093/femsec/fiw063)
- 381 46. Legrand JJ, Juchault P. 1986 Rôle des bactéries symbiotiques dans l'intersexualité, la  
382 monogénie et la spéciation chez les crustacés oniscoïdes. *Boll Zool* **53**, 161–172.
- 383 47. Rigaud T, Juchault P. 1993 Conflict between feminizing sex ratio distorters and an autosomal  
384 masculinizing gene in the terrestrial isopod *Armadillidium vulgare* Latr. *Genetics* **133**, 247–252.
- 385 48. Taylor DR. 1990 Evolutionary consequences of cytoplasmic sex ratio distorters. *Evol. Ecol.* **4**,  
386 235–248.
- 387 49. Galtier N, Nabholz B, Glémin S, Hurst GDD. 2009 Mitochondrial DNA as a marker of molecular  
388 diversity: a reappraisal. *Mol. Ecol.* **18**, 4541–4550. (doi:10.1111/j.1365-294X.2009.04380.x)
- 389

Figure 1

A)



SRD  
 no f, no Wolbachia  
 f  
 Wolbachia  
 both f and Wolbachia

B)

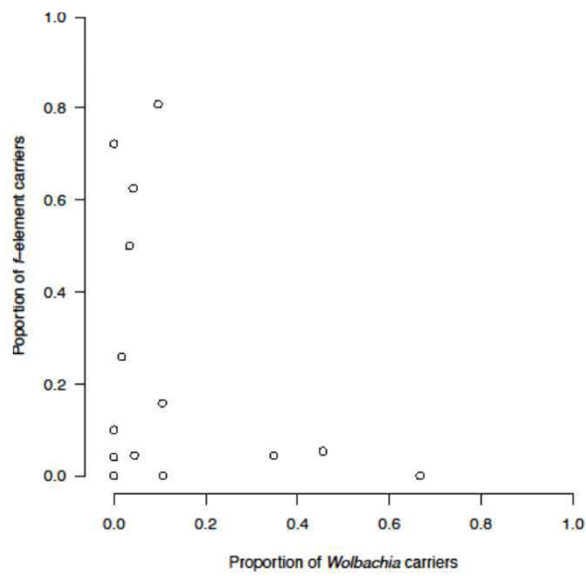


Figure 2

