Temporal stability of sex ratio distorter prevalence in natural populations of the isopod *Armadillidium vulgare*

Sylvine Durand[1], Romain Pigeault[1], Isabelle Giraud[1], Anaïs Loisier[1], Nicolas Bech[1], Frédéric Grandjean[1], Thierry Rigaud[2], Jean Peccoud[1] and Richard Cordaux[1,3,*]

[1] Laboratoire Ecologie et Biologie des Interactions, Equipe Ecologie Evolution Symbiose, Université de Poitiers, UMR CNRS 7267, Bât. B31, 3 rue Jacques Fort, TSA 51106, 86073 Poitiers Cedex 9, France

[2] Laboratoire Biogéosciences, Université Bourgogne Franche-Comté, UMR CNRS 6282, 6 boulevard Gabriel, 21000 Dijon, France

[3] Université Paris-Saclay, CNRS, IRD, UMR Évolution Génomes Comportement Écologie, 91190 Gif-sur-Yvette, France

[*] Corresponding author: richard.cordaux@universite-paris-saclay.fr

Running title: Temporal stability of sex ratio distorter prevalence in *A. vulgare*

Word count: 4607

Abstract

In the terrestrial isopod *Armadillidium vulgare*, many females produce progenies with female-biased sex ratios due to two feminizing sex ratio distorters (SRD): *Wolbachia* endosymbionts and a nuclear non-mendelian locus called the *f* element. To investigate the potential impact of these SRD on the evolution of host sex determination, we analyzed their temporal distribution in six *A. vulgare* populations sampled up to six times over 12 years, for a total of 29 time points. SRD distribution was heterogeneous among populations despite their close geographic locations, so that when one SRD was frequent in a population, the other SRD was rare. In contrast with spatial heterogeneity, our results overall did not reveal substantial temporal variability in SRD prevalence within populations, suggesting equilibria in SRD evolutionary dynamics may have been reached or nearly so. Temporal stability was also generally reflected in mitochondrial and nuclear variation. Nevertheless, in a population, a *Wolbachia* strain replacement coincided with changes in mitochondrial composition but no change in nuclear composition, thus constituting a typical example of mitochondrial sweep caused by endosymbiont rise in frequency. Rare incongruence between *Wolbachia* strains and mitochondrial haplotypes suggested the occurrence of intraspecific horizontal transmission, making it a biologically relevant parameter for *Wolbachia* evolutionary dynamics in *A. vulgare*. Overall, our results provide an empirical basis for future studies on SRD evolutionary dynamics in the context of multiple sex determination factors co-existing within a single species, to ultimately evaluate the impact of SRD on the evolution of host sex determination mechanisms and sex chromosomes.

Keywords

Sex ratio distorter, endosymbiont, *Wolbachia*, *f* element, sex determination, temporal dynamics

Introduction

Sex ratio distorters (SRD) are selfish genetic elements located on sex chromosomes or transmitted by a single sex, which skew the proportion of males and females in progenies towards the sex that enhances their own vertical transmission (Beukeboom and Perrin, 2014). They are found in a wide range of animal and plant species, of which they tremendously impact the ecology and evolution (Burt and Trivers, 2006; Werren, 2011). SRD are sources of genetic conflicts because they increase their own transmission at the expense of other genetic elements of the genome (Burt and Trivers, 2006; Werren, 2011; Beukeboom and Perrin, 2014). Genetic variants are therefore selected if they mitigate the fitness costs inflicted by SRD. Hence, female-biased sex ratios impose strong selective pressure, known as sex ratio selection, favouring genotypes producing more individuals of the under-represented sex (i.e. males) and ultimately restoring Fisherian (i.e. balanced) sex ratios. Thus, SRD may promote the evolution of host sex determination mechanisms (Burt and Trivers, 2006; Werren, 2011; Cordaux *et al.*, 2011; Beukeboom and Perrin, 2014).

SRD are particularly well documented in arthropods, among which is the emblematic bacterial endosymbiont *Wolbachia* (Werren *et al.*, 2008; Kaur *et al.*, 2021). *Wolbachia* is a cytoplasmic, maternally inherited alpha-proteobacterium that often acts as a reproductive parasite by manipulating host reproduction in favor of infected females, thereby conferring itself a transmission advantage. In particular, *Wolbachia* has evolved the ability to induce female-biased sex ratios in host progenies through male killing, thelytokous (i.e. all female-producing) parthenogenesis and feminization (Werren *et al.*, 2008; Cordaux *et al.*, 2011; Hurst and Frost, 2015; Kaur *et al.*, 2021).

Feminization, causing infected (and non-transmitting) genetic males to develop into (transmitting) phenotypic females, is mostly documented in terrestrial isopods (Martin *et al.*, 1973). In the well-studied *Armadillidium vulgare*, chromosomal sex determination follows female heterogamety (ZZ males and ZW females) (Juchault and Legrand, 1972; Chebbi *et al.*, 2019; Cordaux *et al.*, 2021). In some *A. vulgare* populations, sex ratio is biased by *Wolbachia* bacteria or by a nuclear locus called the *f* element (Rigaud *et al.*, 1997; Cordaux *et al.*, 2011; Cordaux and Gilbert, 2017). Three *Wolbachia* strains are known to naturally occur in *A. vulgare*: *w*VulC, *w*VulM and *w*VulP (Rigaud *et al.*, 1991; Cordaux *et al.*, 2004; Verne *et al.*, 2007). The *f* element results from the horizontal transfer of a large portion of a feminizing *Wolbachia* genome in the *A. vulgare* genome (Leclercq *et al.*, 2016). The *f* element induces female development, as a W chromosome does, and it shows non-Mendelian inheritance, making it an SRD (Legrand and Juchault, 1984; Leclercq *et al.*, 2016). In this species, sex ratio selection has resulted in the evolution of a masculinizing allele restoring balanced sex ratios,

79 hence conferring resistance to feminization (Rigaud and Juchault, 1993). This nuclear locus
80 represents a new male sex-determining locus, thereby effectively establishing a new male
81 heterogametic system XY/XX. Thus, multiple sex determination factors co-exist in *A. vulgare* ,
82 ultimately caused by feminizing SRD (Juchault and Mocquard, 1993; Cordaux and Gilbert, 2017).
83 Given the widespread distribution of *Wolbachia* infection in terrestrial isopods (Bouchon *et al.*, 1998;
84 Cordaux *et al.*, 2012), it has been suggested that SRD may have contributed to the frequent
85 turnovers of sex chromosomes recorded in this clade (Juchault and Mocquard, 1993; Juchault and
86 Rigaud, 1995; Becking *et al.*, 2017, 2019; Russell *et al.*, 2021).

87 To elucidate the potential impact of SRD on the evolution of sex determination in terrestrial isopods,
88 it is essential to clarify the intraspecific evolutionary dynamics of SRD such as *Wolbachia* and the *f*
89 element. Previous studies have shown that: (i) *Wolbachia* and the *f* element are present at variable
90 frequencies in *A. vulgare* populations, (ii) the *f* element is overall more frequent than *Wolbachia*, (iii)
91 the two SRD usually do not co-occur at high frequency in populations, and (iv) mitochondrial
92 haplotypes (mitotypes) are tightly linked to *Wolbachia* strains (suggesting stable maternal
93 transmission), but not to the *f* element (Juchault *et al.*, 1993; Durand *et al.*, 2023). These results have
94 provided insights into the spatial distribution of *Wolbachia* and the *f* element in *A. vulgare*; in the
95 present study, we investigate their temporal dynamics.

96 Prior studies on endosymbiont temporal dynamics have shown that host population invasions can
97 occur within just a few years, e.g. *Wolbachia* in *Drosophila simulans* (Turelli and Hoffmann, 1991) and
98 *Eurema hecabe* (Miyata *et al.*, 2024), and *Rickettsia* in *Bemisia tabaci* (Himler *et al.*, 2011). Likewise,
99 nuclear suppressors of SRD can rise in frequency very quickly, e.g. *Wolbachia* suppressor in
100 *Hypolimna bolina* (Charlat *et al.*, 2007). Similar patterns of rapid spread and evolution of resistance
101 have been reported for a nuclear SRD, Paris Sex Ratio, in *D. simulans* (Helleu *et al.*, 2019).
102 Endosymbiont temporal dynamics has seldom been studied on a longer time scale. Comparison of
103 historical (i.e. museum) and contemporary sympatric populations of *H. bolina* highlighted
104 fluctuations of *Wolbachia* frequency over periods of 73-123 years (Hornett *et al.*, 2009). However, at
105 an intermediate time scale (10-20 years), *Wolbachia* was found to decrease in frequency in *Acraea*
106 *encedon* (Hassan *et al.*, 2013), while it was stably maintained in *Eurema mandarina* (Kageyama *et al.*,
107 2020) and *D. simulans* (Weeks *et al.*, 2007; Carrington *et al.*, 2011). In the case of *A. vulgare*, SRD
108 temporal dynamics has previously been tackled by a single study (Juchault *et al.*, 1992), in which
109 *Wolbachia* prevalence was found to decrease concomitantly to an increase of *f* element prevalence
110 in an *A. vulgare* population from Niort (western France) sampled at three time points over a period
111 of 23 years (1963, 1973 and 1986). However, a single population was included in the study, thus
112 limiting the breadth of its conclusions. Here we report an analysis of *Wolbachia* and *f* element

113 distribution in six *A. vulgare* populations sampled up to six times over a period of 12 years,
114 representing a total of 889 individuals from 29 time points. The studied populations were sampled in
115 a narrow geographic area in western France, within 70 km of the Niort population (Juchault *et al.*,
116 1992), to control for spatial dynamics. In contrast to most previous studies, here we analyzed SRD in
117 the context of both mitochondrial and nuclear variation. Our results highlight an overall temporal
118 stability of SRD distribution in *A. vulgare*, with few exceptions.

119

120 **Materials and Methods**

121

122 A total of 889 *A. vulgare* individuals were analyzed, from six natural populations sampled in western
123 France between 2003 and 2017 (Figure 1). DNA samples from Beauvoir-2017, Chizé-2017,
124 Coulombiers-2017, Gript-2017, La Crèche-2017 and Poitiers-2015 were available from (Durand *et al.*,
125 2023). All other individuals were collected by hand. Sex ratios in sampled individuals should not be
126 considered as reflecting those of populations, due to possible selection biases by samplers.
127 Individuals were sexed and stored in alcohol or at -20°C prior to DNA extraction. Total genomic DNA
128 of samples collected between 2003 and 2013 was extracted from gonads using phenol and
129 chloroform (Kocher *et al.*, 1989) and DNA of samples collected between 2014 and 2016 was
130 extracted from the head and legs, as described previously (Leclercq *et al.*, 2016).

131 Four molecular markers were used to assess the presence of *Wolbachia* and the *f* element in DNA
132 extracts: *Jtel* (Leclercq *et al.*, 2016), *wsp* (Braig *et al.*, 1998), *recR* (Badawi *et al.*, 2014) and *ftsZ*
133 (Werren *et al.*, 1995) (Table S1). While *Jtel* is specific to the *f* element, *wsp* and *recR* are specific to
134 *Wolbachia*, and *ftsZ* is present in both the *f* element and *Wolbachia* (Leclercq *et al.*, 2016). The
135 presence or absence of these markers was assessed by PCR assays, as described previously (Leclercq
136 *et al.*, 2016). Different amplification patterns were expected for individuals with *Wolbachia* only (*Jtel-*
137 *, wsp+*, *recR+*, *ftsZ+*), the *f* element only (*Jtel+, wsp-, recR-*, *ftsZ+*), both *Wolbachia* and the *f* element
138 present (*Jtel+, wsp+, recR+*, *ftsZ+*) or both *Wolbachia* and the *f* element lacking (*Jtel-, wsp-, recR-*,
139 *ftsZ-*). The few individuals exhibiting other amplification patterns were classified as "undetermined
140 status".

141 To characterize *Wolbachia* strain diversity, *wsp* PCR products were purified and Sanger sequenced
142 using both forward and reverse primers by GenoScreen (Lille, France). Sequences from forward and
143 reverse reads were assembled using Geneious v.7.1.9 to obtain one consensus sequence per
144 individual.

145 To evaluate mitochondrial diversity, a ~700 bp-long portion of the Cytochrome Oxidase I (*COI*) gene
146 was amplified by PCR in all individuals (Folmer *et al.*, 1994) (Table S1). PCR products were purified
147 and Sanger sequenced as described above. Mitotype networks were built using the *pegas* package
148 for R (Paradis, 2010).

149 To evaluate nuclear diversity, all individuals were genotyped at 22 microsatellite markers (Verne *et*
150 *al.*, 2006; Giraud *et al.*, 2013) distributed in five multiplexes (Multiplex 1: Av1, Av2, Av4, Av5, Av9;
151 Multiplex 2: Av6, Av3, Av8; Multiplex 3: AV0023, AV0056, AV0085, AV0086, AV0096; Multiplex 4:
152 AV0002, AV0016, AV0018, AV0032, AV0099; Multiplex 5: AV0061, AV0063, AV0089, AV0128) (Table
153 S2). PCR was performed using fluorescence-marked forward primers, as described previously
154 (Durand *et al.*, 2017). PCR fragments were separated by electrophoresis on an ABI 3730XL automated
155 sequencer by Genoscreen (Lille, France). Alleles were scored using the software GeneMapper 3.7
156 (Applied Biosystems), each genotype being independently read by two people.

157 Of the 22 amplified microsatellite markers, Av4 and Av5 could not be scored because of multiple
158 peaks, AV0096 and AV0128 did not amplify consistently, and AV0023 and AV0061 were
159 monomorphic across the dataset. The *Genepop* package for R (Rousset, 2008) detected no linkage
160 disequilibrium between the 16 remaining loci. The presence of null alleles was tested by using a
161 combination of software, as recommended previously (Dąbrowski *et al.*, 2014): Micro-Checker (Van
162 Oosterhout *et al.*, 2004), Cervus (Kalinowski *et al.*, 2007) and ML-NullFreq (Kalinowski and Taper,
163 2006). As a result, AV0099 was discarded because it consistently presented hints of null alleles in
164 many populations and sampling years. Individuals whose genotypes were available for fewer than 13
165 out of the 15 remaining markers were removed from the following analyses.

166 Hardy-Weinberg equilibrium was tested for each locus, locality and sampling year with an exact test
167 using Markov chain with the *Genepop* package for R. The Fstat software v. 2.9.3.2 (Goudet, 2001)
168 was used to calculate allelic richness and heterozygosity (based on a minimum of 3 individuals).
169 Genetic differentiation was estimated by computing Fst values for all pairs of populations (Weir and
170 Cockerham, 1984) with Fstat. Significance was calculated using global tests implemented in Fstat
171 with a level of significance adjusted for multiple tests using the standard Bonferroni correction. The
172 longitudes and latitudes of the populations were used to calculate Euclidean distances between
173 populations and to test for isolation by distance by correlating these geographical distances with the
174 genetic distances. Significance of the correlation was tested at individual scale by using a Mantel test
175 (with 9999 permutations) implemented in GENALEX v 6.2 (Peakall and Smouse, 2006). Genetic
176 clusters were also delineated without a priori with a Bayesian, individual-based approach
177 implemented in the software Structure (Pritchard *et al.*, 2000). The admixture model was selected, as

178 well as the option of correlated allele frequencies. The number of clusters (K) varied from 2 to 9. For
179 each value of K, 20 independent runs were carried out, as recommended in (Evanno *et al.*, 2005),
180 with a total number of 100,000 iterations and a burn-in of 10,000. To determine the most likely value
181 of K, the method described in (Evanno *et al.*, 2005) was applied as implemented in Structure
182 Harvester version 0.6.9 (Earl and vonHoldt, 2012). In addition, a Discriminant Analysis of Principal
183 Components (DAPC) (Jombart *et al.*, 2010) was performed on populations according to their sampling
184 locality and year with the *adegenet* package (Jombart, 2008), to search for potential discrepancies
185 across time points within populations.

186 Statistical analyses were carried out using the R software (v.4.2.1). The influence of sex, and
187 population if appropriate, on SRD prevalence was analyzed using general linear models with a
188 binomial error distribution. Maximal models, including all higher order interactions, were simplified
189 by sequentially eliminating non-significant terms and interactions to establish a minimal model
190 (Crawley, 2012). The significance of the explanatory variables was established using a likelihood ratio
191 test, which is approximately distributed as a Chi-square distribution (Bolker, 2008). The significant
192 Chi-squared values given in the text are for the minimal model, whereas non-significant values
193 correspond to those obtained before the deletion of the variable from the model. Chi-square tests
194 were used to compare the frequency of individuals infected by *Wolbachia* and the frequency of
195 individuals carrying the *f*-element in the different populations. Figures were realized with *ggplot2*
196 (Wickham *et al.*, 2020). Results from Structure were processed with the program Distruct
197 (Rosenberg, 2003) for graphical representation.

198

199 **Results**

200

201 We tested the presence of *Wolbachia* and the *f* element in 889 individuals (627 females and 262
202 males) from six populations sampled at various time points between 2003 and 2017, representing a
203 total of 29 sampling points (Tables 1 and S3). Among the 889 analyzed individuals, we failed to
204 determine the status of 29 individuals. Of the remaining 860 individuals, 29.9% carried only the *f*
205 element, 15.2% carried only *Wolbachia*, 0.6% carried both SRD and 54.3% carried none. Although
206 sometimes present in males, both SRD were significantly more frequent in females than in males
207 (*Wolbachia*: $\chi^2 = 73.59$, $p < 0.0001$; *f* element: $\chi^2 = 41.03$, $p < 0.0001$, Tables 1 and S3). *Wolbachia*-
208 infected individuals carried one of the three previously known *Wolbachia* strains of *A. vulgare*: *w*VulC
209 (n=22), *w*VulM (n=25) or *w*VulP (n=83).

210 Overall, both SRD were found at at least one time point in all six populations (Figure 2). However, the
211 populations displayed substantial variation in the distribution of the two SRD. In Beauvoir, Chizé,
212 Coulombiers and La Crèche populations, the *f* element was significantly more predominant (29-86%
213 frequency) than *Wolbachia* (2-10%) ($\chi^2 > 36.91$ and $p < 10^{-9}$ in all four populations). Conversely,
214 *Wolbachia* was significantly more frequent (63%) than the *f* element (2%) in Poitiers ($\chi^2 > 135.32$, $p <$
215 10^{-16}). Finally, both SRD were very rare in Gript (2-3%) and did not differ in frequencies ($\chi^2 = 0.10$, $p =$
216 0.748). Remarkably, *f* element prevalence did not differ significantly across time points (spanning up
217 to 12 years) in all four populations in which it was predominant ($\chi^2 = 0.002$, $p = 0.968$), although *f*
218 element prevalence was significantly different among populations ($\chi^2 = 62.55$, $p < 0.0001$). In these
219 populations, *f* element prevalence was significantly higher in females than in males ($\chi^2 = 61.87$, $p <$
220 0.0001) (Figure 2). In Poitiers, where *Wolbachia* remained globally frequent over time, females were
221 also significantly more infected than males ($\chi^2 = 106.06$, $p < 0.0001$). Interestingly, the frequency of
222 the *w*VulC strain significantly decreased over time ($\chi^2 = 12.18$, $p < 0.0001$) while the *w*VulP strain
223 exhibited the opposite trend, and significantly so ($\chi^2 = 6.03$, $p = 0.014$).

224 Sequencing of the *COI* mitochondrial gene of 884 individuals identified 12 mitotypes, nine of which
225 have previously been detected in *A. vulgare* populations (named I to VIII, and XII) (Durand *et al.*,
226 2023) and three are newly described mitotypes (XXIV to XXVI; GenBank accession numbers
227 OR074129 to OR074131, respectively) (Figure 3 and Table S3). There was an excellent congruence
228 between *Wolbachia* strains and mitotypes, as previously reported (Verne *et al.*, 2012; Durand *et al.*,
229 2023). Indeed, individuals carrying *w*VulC were associated with either mitotype V or its close relatives
230 (XII and XXVI), those carrying *w*VulP were associated with mitotype VII and those carrying *w*VulM
231 were associated with mitotype II. Exceptions included two individuals infected by *w*VulM, which
232 were associated with the distantly related mitotypes I and III. By contrast, the *f* element was found in
233 eight different mitochondrial backgrounds (I to VI, VIII and XXV) distributed across the mitochondrial
234 network.

235 All time points considered, there was between three (in Chizé) and six (in Poitiers) mitotypes per
236 population (Figures 3 and S1). Mitotype frequencies were globally stable across time points within
237 populations (e.g. Coulombiers, Figure 4A), with the notable exceptions of La Crèche and Poitiers
238 populations. In La Crèche, a shift in major mitotypes occurred between 2005 and 2012, with the
239 increasing rarity of mitotype III and V being concomitant with the rise in frequency of mitotypes I and
240 VIII, followed by stability since 2012 (Figure 4B). In Poitiers, the rise in frequency of mitotype VII
241 (associated with *Wolbachia* strain *w*VulP) coincided with a relative decrease of *w*VulC-associated
242 mitotypes V, XII and XXVI (Figure 4C).

243 To test if the patterns of SRD and mitochondrial variation were also reflected in the nuclear genome,
244 we examined variation in 667 individuals with genotype information available for at least 13 out of
245 the 15 retained microsatellite markers (see Materials and Methods), representing a total of 28
246 sampling points (Poitiers-2015 was discarded due to low genotyping success) (Table S3). None of the
247 loci significantly departed from Hardy-Weinberg equilibrium for all sampling points. Allelic richness
248 ranged from 1 to 4.78 and heterozygosity ranged from 0 to 0.91 (Table S4). Pairwise Fst values
249 ranged from 0 to 0.091 (mean Fst = 0.035) (Table S5). The majority of Fst values between populations
250 (200/321) were significant, suggesting the occurrence of genetic differentiation among populations.
251 Consistently, a Mantel test evaluating the correlation between genetic and geographic distances
252 revealed a significant isolation by distance ($r^2 = 0.034$, $p < 0.001$). Such a signal of genetic structure
253 was confirmed by Bayesian clustering analyses. Indeed, a delta-K analysis (Evanno et al., 2005)
254 inferred that the best fit to the data was obtained for K=2 genetic clusters (Figure S2). A first genetic
255 cluster mainly comprised individuals sampled in Poitiers and Coulombiers, and a second genetic
256 cluster comprised those from Beauvoir, Chizé, La Crèche and Gript (Figure 5). In agreement with
257 isolation by distance, this clustering pattern reflected the geographic distribution of populations, as
258 the former cluster encompassed eastern-most populations and the latter cluster encompassed
259 western-most populations (Figure 1). It is noteworthy that the low delta-K values obtained suggested
260 poor convergence between the 20 independent runs, which may be the result of a previously
261 highlighted effect of isolation by distance (Meirmans, 2012). By contrast, the clustering analysis did
262 not highlight any obvious change in genetic structure between time points within populations. The
263 DAPC also suggested a major population structuration (Figure 6), as the first component separated
264 Poitiers and Coulombiers (locating towards the right side of the axis) from the other populations
265 (locating towards the left side of the axis). It also supported overall homogeneity of populations
266 across time points, apart from La Crèche-2005, which was separated from the other La Crèche time
267 points in the second component of the DAPC. The exception of La Crèche-2005 was also reflected in
268 the significant pairwise Fst values between time points within population, which were generally non-
269 significant for the other populations (Table S5).

270

271 **Discussion**

272

273 In this study, we analyzed the temporal dynamics of two SRD segregating in natural populations of
274 the terrestrial isopod *A. vulgare*, to shed light on the potential impact of SRD on the evolution of host
275 sex determination mechanisms at a within-species scale. *Wolbachia* and *f* element distributions were

276 highly heterogeneous among populations in our study area, despite their modest geographic
277 distance of at most 80 km. The emerging trend is that when one SRD is frequent in a population, the
278 other SRD is rare, as noted previously (Durand *et al.*, 2023). As it causes a stronger bias toward
279 females, *Wolbachia* is expected to prevail over the *f* element in *A. vulgare* populations (Rigaud, 1997;
280 Cordaux and Gilbert, 2017). Yet, the *f* element was the dominant SRD in four out of six populations
281 we studied and was rising in frequency in Niort (Juchault *et al.*, 1992). Overall, the *f* element is more
282 widespread than *Wolbachia* in *A. vulgare* populations (Juchault *et al.*, 1993; Durand *et al.*, 2023).
283 Previously proposed explanations include a higher fitness cost entailed by *Wolbachia* relative to the *f*
284 element and occasional paternal transmission of the *f* element (which some males carry, Figure 2)
285 enabled by masculinizing epistatic alleles (Juchault *et al.*, 1992; Rigaud and Juchault, 1993; Rigaud,
286 1997; Rigaud and Moreau, 2004; Cordaux and Gilbert, 2017).

287 In contrast with spatial heterogeneity, our sampling scheme with up to six sampling time points
288 spanning 12 years per population highlights a global temporal stability in SRD prevalence within
289 populations. At first glance, this qualitative pattern differs from that previously reported for the Niort
290 population, in which *Wolbachia* prevalence was found to decrease concomitantly to an increase of *f*
291 element prevalence over a period of 23 years (Juchault *et al.*, 1992). Given *A. vulgare*'s generation
292 time of one year, our study might have spanned too few generations (12) to capture variation in SRD
293 prevalence, which the Niort study spanning 23 generations did. Nevertheless, the time scale of our
294 study enabled us to detect variation in *Wolbachia* strain prevalence, as well as mitochondrial and
295 nuclear variation within and between populations, suggesting that lack of resolution is not an issue.
296 Alternatively, most of the populations we studied may reflect some relatively stable equilibrium with
297 respect to SRD evolutionary dynamics, an equilibrium that the Niort population might not have
298 reached. Indeed, theoretical models have indicated that when feminizing factors are in competition,
299 the one that induces the strongest bias toward females is expected to spread in the population
300 (Taylor, 1990). Thus, a single SRD should remain in the population at equilibrium. Consistently, in
301 most of the populations we analyzed, a single SRD occurs at high frequency, suggesting that these
302 populations may be at or near equilibrium for an SRD. This signal of temporal stability is reminiscent
303 of that recorded in the butterfly *E. mandarina*, in which the feminizing *w*Fem *Wolbachia* strain has
304 been stably maintained at high frequency for 12 years in a Japanese population (Kageyama *et al.*,
305 2020). Similarly, the cytoplasmic incompatibility-inducing *w*Ri *Wolbachia* strain has apparently
306 reached an equilibrium frequency at ~93% in *D. simulans* populations from California (Weeks *et al.*,
307 2007; Carrington *et al.*, 2011). It has been suggested that an apparent stability could be due to
308 hidden processes such as population structure (including extinction-recolonization processes),
309 intragenomic conflicts and coevolutionary processes (Hatcher, 2000).

310 The temporal stability of SRD in most of *A. vulgare* populations is also reflected in host mitochondrial
311 and nuclear variation, with two notable exceptions. The first one is La Crèche population in 2005,
312 which differs from the other time points (2012 to 2017) on both mitochondrial and nuclear grounds.
313 Interestingly, the sampling spot in La Crèche has been altered by land remodeling between 2005 and
314 2012. This anthropogenic activity may have caused the reduction or collapse of the historic *A.*
315 *vulgare* population and the introduction of new individuals as part of the addition of materials during
316 the remodeling (e.g., soil from another location). Such an extinction-recolonization scenario may
317 explain the loss of *Wolbachia* (present at low frequency in 2005) and the increase in *f* element
318 frequency in 2012. It is noteworthy that from 2012 on, SRD, mitochondrial and nuclear variation have
319 been stable, suggesting that stabilization of the population dynamics may be reached in a few years.

320 The second case of instability is the Poitiers population, which is stable with respect to nuclear
321 variation but not to both mitochondrial and SRD variation. Poitiers is the only population in our
322 dataset in which *Wolbachia* is the dominant SRD across time points, thus highlighting a qualitative
323 pattern of temporal stability. However, our results indicate that the rise in frequency of the *w*VulP
324 strain correlates with a decrease of the *w*VulC strain, suggesting a *Wolbachia* strain replacement in
325 this population. The *w*VulP strain is characterized by a recombination event involving *w*VulC (Verne
326 *et al.*, 2007), indicating that *w*VulC is older than *w*VulP, which is consistent with the situation
327 recorded in Poitiers. Assuming the driver of this replacement is *Wolbachia* and not another
328 cytoplasmic element (like the mitochondrion), replacement of *w*VulC by *w*VulP could be due to the
329 latter strain having a transmission advantage over the former strain. Unfortunately, the *w*VulP strain
330 is not very well characterized, and while feminization induction is likely (Verne *et al.*, 2007), it has not
331 been formally demonstrated and compared to feminization induced by *w*VulC (Rigaud *et al.*, 1991;
332 Cordaux *et al.*, 2004). Neither has the respective costs of these two *Wolbachia* strains been
333 investigated. In any event, because *Wolbachia* and mitochondria are co-inherited cytoplasmic
334 entities, changes in *Wolbachia* strains associated with different mitotypes are expected to lead to
335 concomitant changes in mitochondrial variation, but no change in nuclear variation. Therefore, our
336 observations in Poitiers may constitute a typical example of mitochondrial sweep caused by
337 endosymbiont rise in frequency (Galtier *et al.*, 2009).

338 *Wolbachia* dynamics in Poitiers also illustrates that transovarial, maternal transmission is the main
339 transmission mode of *Wolbachia* in *A. vulgare*. However, non-maternal transmission may also occur,
340 as suggested by two individuals with *w*VulM from Beauvoir and La Crèche. These individuals carry
341 mitotypes I and III, respectively, unlike all other *w*VulM-infected individuals which carry mitotype II.
342 As mitotypes I, II and III are distantly related, a plausible explanation is that the two unusual
343 individuals have acquired *Wolbachia* by horizontal transfer, although the hypothesis of historical

344 infections cannot be formally discarded. Horizontal transfer of *Wolbachia* is largely documented in
345 arthropods (O'Neill *et al.*, 1992; Werren *et al.*, 1995; Heath *et al.*, 1999; Vavre *et al.*, 1999), including
346 terrestrial isopods (Bouchon *et al.*, 1998; Cordaux *et al.*, 2001, 2012). Potential mechanisms in
347 isopods include contact between wounded individuals (Rigaud and Juchault, 1995) and
348 cannibalism/predation (Le Clec'h *et al.*, 2013). In total, our results suggest that two out of 136
349 *Wolbachia*-infected individuals could conceivably have acquired their symbionts by horizontal
350 transmission. This may be an underestimate, as horizontal transfers between individuals carrying the
351 same mitotype cannot be detected with our approach. If so, horizontal transmission may occur at a
352 measurable rate in *A. vulgare*, suggesting that it is a parameter of importance in *Wolbachia*
353 evolutionary dynamics in this species.

354 To conclude, the evolutionary dynamics of SRD, mitochondrial and nuclear variation from various
355 populations over a period of up to 12 years revealed that distributions of *Wolbachia* and the *f*
356 element were much more variable spatially than temporally. This conclusion is well supported for the
357 *f* element, with four populations independently showing the same pattern. For *Wolbachia*, it has
358 remained at high frequency over time but strain genotyping identified an apparently ongoing strain
359 replacement. It is however more difficult to draw strong conclusions for this SRD as it occurred at
360 high frequency in a single population. Such geographic and temporal distribution suggests that
361 migration may not heavily influence SRD evolution in *A. vulgare*, a species exhibiting strong female
362 phylopatry (Durand *et al.*, 2019). This is in contrast with the highly dispersive *H. bolina*, in which
363 *Wolbachia* frequency has been shown to fluctuate over time (Hornett *et al.*, 2009). Overall, our
364 results provide an empirical basis for future studies on SRD evolutionary dynamics in the context of
365 multiple sex determination factors co-existing within a single species, such as modelling
366 investigations. These efforts will ultimately contribute to assess the impact of SRD on the evolution
367 of host sex determination mechanisms and sex chromosomes.

Acknowledgments

We thank Sébastien Verne and Victorien Valette for assistance with sample collection. This work was funded by Agence Nationale de la Recherche Grants ANR-15-CE32-0006 (CytoSexDet) to RC and TR, and ANR-20-CE02-0004 (SymChroSex) to JP, and intramural funds from the CNRS and the University of Poitiers.

Author contribution statement

RC, JP and TR designed the experiments. FG, NB and JP sampled populations. SD, IG and AL performed laboratory work. SD, RP and NB performed data analyses. RC and SD wrote the first draft of the manuscript. TR, JP, NB, RP and FG amended the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Data archiving

Mitotypes are available in GenBank under accession numbers OR074129 to OR074131. All other data are provided in the supplementary information.

389 **References**

390

391 Badawi M, Giraud I, Vavre F, Grève P, Cordaux R (2014). Signs of Neutralization in a Redundant Gene
392 Involved in Homologous Recombination in *Wolbachia* Endosymbionts. *Genome Biol Evol* **6**:
393 2654–2664.

394 Becking T, Chebbi MA, Giraud I, Moumen B, Laverré T, Caubet Y, *et al.* (2019). Sex chromosomes
395 control vertical transmission of feminizing Wolbachia symbionts in an isopod. *PLOS Biol* **17**:
396 e3000438.

397 Becking T, Giraud I, Raimond M, Moumen B, Chandler C, Cordaux R, *et al.* (2017). Diversity and
398 evolution of sex determination systems in terrestrial isopods. *Sci Rep* **7**: 1–14.

399 Beukeboom LW, Perrin N (2014). *The evolution of sex determination*. Oxford University Press: Oxford.

400 Bolker BM (2008). *Ecological models and data in R*. Princeton University Press: Princeton and Oxford.

401 Bouchon D, Rigaud T, Juchault P (1998). Evidence for widespread Wolbachia infection in isopod
402 crustaceans: molecular identification and host feminization. *Proc Biol Sci* **265**: 1081–90.

403 Braig HR, Zhou WG, Dobson SL, O'Neill SL (1998). Cloning and characterization of a gene encoding the
404 major surface protein of the bacterial endosymbiont Wolbachia pipientis. *J Bacteriol* **180**:
405 2373–2378.

406 Burt A, Trivers R (2006). *Genes in conflict*. The Belknap Press of Harvard University Press: Cambridge,
407 Massachusetts.

408 Carrington LB, Lipkowitz JR, Hoffmann AA, Turelli M (2011). A re-examination of Wolbachia-induced
409 cytoplasmic incompatibility in California Drosophila simulans. *PloS One* **6**: e22565.

410 Charlat S, Hornett EA, Fullard JH, Davies N, Roderick GK, Wedell N, *et al.* (2007). Extraordinary flux in
411 sex ratio. *Science* **317**: 214.

412 Chebbi MA, Becking T, Moumen B, Giraud I, Gilbert C, Peccoud J, *et al.* (2019). The Genome of
413 Armadillidium vulgare (Crustacea, Isopoda) Provides Insights into Sex Chromosome Evolution
414 in the Context of Cytoplasmic Sex Determination. *Mol Biol Evol* **36**: 727–741.

415 Cordaux R, Bouchon D, Greve P (2011). The impact of endosymbionts on the evolution of host sex-
416 determination mechanisms. *Trends Genet* **27**: 332–41.

417 Cordaux R, Chebbi MA, Giraud I, Pleydell DRJ, Peccoud J (2021). Characterization of a Sex-
418 Determining Region and Its Genomic Context via Statistical Estimates of Haplotype
419 Frequencies in Daughters and Sons Sequenced in Pools. *Genome Biol Evol* **13**: evab121.

420 Cordaux R, Gilbert C (2017). Evolutionary Significance of Wolbachia-to-Animal Horizontal Gene
421 Transfer: Female Sex Determination and the f Element in the Isopod Armadillidium vulgare.
422 *Genes* **8**: 186.

423 Cordaux R, Michel Salzat A, Bouchon D (2001). *Wolbachia* infection in crustaceans: novel hosts and
424 potential routes for horizontal transmission. *J Evol Biol* **14**: 237–243.

Cordaux R, Michel-Salzat A, Frelon-Raimond M, Rigaud T, Bouchon D (2004). Evidence for a new feminizing Wolbachia strain in the isopod Armadillidium vulgare: evolutionary implications. *Heredity* **93**: 78–84.

Cordaux R, Pichon S, Hatira HB, Doublet V, Greve P, Marcade I, *et al.* (2012). Widespread Wolbachia infection in terrestrial isopods and other crustaceans. *Zookeys* **176**: 123–31.

Crawley MJ (2012). *The R book*. John Wiley & Sons, Ltd: Chichester.

Dąbrowski MJ, Pilot M, Kruczyk M, Żmihorski M, Umer HM, Gliwicz J (2014). Reliability assessment of null allele detection: inconsistencies between and within different methods. *Mol Ecol Resour* **14**: 361–373.

Durand S, Cohas A, Braquart-Varnier C, Beltran-Bech S (2017). Paternity success depends on male genetic characteristics in the terrestrial isopod *Armadillidium vulgare*. *Behav Ecol Sociobiol* **71**: 90.

Durand S, Grandjean F, Giraud I, Cordaux R, Beltran-Bech S, Bech N (2019). Fine-scale population structure analysis in Armadillidium vulgare (Isopoda: Oniscidea) reveals strong female philopatry. *Acta Oecologica* **101**.

Durand S, Lheraud B, Giraud I, Bech N, Grandjean F, Rigaud T, *et al.* (2023). Heterogeneous distribution of sex ratio distorters in natural populations of the isopod Armadillidium vulgare. *Biol Lett* **19**: 20220457.

Earl DA, vonHoldt BM (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv Genet Resour* **4**: 359–361.

Evanno G, Regnaut S, Goudet J (2005). Detecting the number of clusters of individuals using the software structure: a simulation study. *Mol Ecol* **14**: 2611–2620.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol* **3**: 294–299.

Galtier N, Nabholz B, Glémin S, Hurst GDD (2009). Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Mol Ecol* **18**: 4541–4550.

Giraud I, Valette V, Bech N, Grandjean F, Cordaux R (2013). Isolation and characterization of microsatellite loci for the isopod crustacean *Armadillidium vulgare* and transferability in terrestrial isopods. *Plos One* **8**: e76639.

Goudet (2001). FSTAT, a program to estimate and test gene diversities and fixation indices, version 2.9.3. *http://www2.unil.ch/popgen/softwares/fstat.htm*.

Hassan SSM, Idris E, Majerus MEN (2013). Male-Killer Dynamics in the Tropical Butterfly Acraea encedon (Lepidoptera: Nymphalidae). *Afr Entomol* **21**: 209–214.

Hatcher MJ (2000). Persistence of selfish genetic elements: population structure and conflict. *Trends Ecol Evol* **15**: 271–277.

462 Heath BD, Butcher RDJ, Whitfield WGF, Hubbard SF (1999). Horizontal transfer of Wolbachia
463 between phylogenetically distant insect species by a naturally occurring mechanism. *Curr Biol*
464 **9**: 313–316.

465 Helleu Q, Courret C, Ogereau D, Burnham KL, Chaminade N, Chakir M, *et al.* (2019). Sex-Ratio Meiotic
466 Drive Shapes the Evolution of the Y Chromosome in Drosophila simulans. *Mol Biol Evol* **36**:
467 2668–2681.

468 Himler AG, Adachi-Hagimori T, Bergen JE, Kozuch A, Kelly SE, Tabashnik BE, *et al.* (2011). Rapid
469 spread of a bacterial symbiont in an invasive whitefly is driven by fitness benefits and female
470 bias. *Science* **332**: 254–256.

471 Hornett EA, Charlat S, Wedell N, Jiggins CD, Hurst GDD (2009). Rapidly shifting sex ratio across a
472 species range. *Curr Biol CB* **19**: 1628–1631.

473 Hurst GDD, Frost CL (2015). Reproductive parasitism: maternally inherited symbionts in a biparental
474 world. *Cold Spring Harb Perspect Biol* **7**: a017699.

475 Jombart T (2008). adegenet: a R package for the multivariate analysis of genetic markers.
476 *Bioinformatics* **24**: 1403–1405.

477 Jombart T, Devillard S, Balloux F (2010). Discriminant analysis of principal components: a new
478 method for the analysis of genetically structured populations. *BMC Genet* **11**: 94.

479 Juchault P, Legrand JJ (1972). Croisement de néo-mâles experimentaux chez Armadillidium vulgare
480 Latr. (Crustace, Isopode, Oniscoide). Mise en evidence d'une hétérogamétie femelle. *C R*
481 *Acad Sci Paris* **274**: 1387–1389.

482 Juchault P, Mocquard JP (1993). Transfer of a parasitic sex factor to the nuclear genome of the host:
483 A hypothesis on the evolution of sex-determining mechanisms in the terrestrial isopod
484 *Armadillidium vulgare* Latr. *J Evol Biol* **6**: 511–528.

485 Juchault P, Rigaud T (1995). Evidence for female heterogamety in two terrestrial crustaceans and the
486 problem of sex chromosome evolution in isopods. *Heredity* **75**: 466–471.

487 Juchault P, Rigaud T, Mocquard J-P (1992). Evolution of sex-determining mechanisms in a wild
488 population of *Armadillidium vulgare* Latr. (Crustacea, Isopod) : competition between two
489 feminizing parasitic sex factors. *Heredity* **69**: 382–390.

490 Juchault P, Rigaud T, Mocquard J-P (1993). Evolution of sex determination and sex ratio variability in
491 wild populations of *Armadillidium vulgare* (Latr.) (Crustacea, Isopoda): a case study in conflict
492 resolution. *Acta Oecologica* **14**: 547–562.

493 Kageyama D, Narita S, Konagaya T, Miyata MN, Abe J, Mitsuhashi W, *et al.* (2020). Persistence of a
494 Wolbachia-driven sex ratio bias in an island population of Eurema butterflies. :
495 2020.03.24.005017.

496 Kalinowski ST, Taper ML (2006). Maximum likelihood estimation of the frequency of null alleles at
497 microsatellite loci. *Conserv Genet* **7**: 991–995.

498 Kalinowski ST, Taper ML, Marshall TC (2007). Revising how the computer program cervus
499 accommodates genotyping error increases success in paternity assignment. *Mol Ecol* **16**:
500 1099–1106.

Kaur R, Shropshire JD, Cross KL, Leigh B, Mansueto AJ, Stewart V, *et al.* (2021). Living in the endosymbiotic world of Wolbachia: A centennial review. *Cell Host Microbe* **29**: 879–893.

Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, *et al.* (1989). Dynamics of mitochondrial-DNA evolution in animals: amplification and sequencing with conserved primers. *Proc Natl Acad Sci U S A* **86**: 6196–6200.

Le Clec'h W, Chevalier FD, Genty L, Bertaux J, Bouchon D, Sicard M (2013). Cannibalism and Predation as Paths for Horizontal Passage of Wolbachia between Terrestrial Isopods. *PLOS ONE* **8**: e60232.

Leclercq Sb, Thézé J, Chebbi MA, Giraud I, Moumen B, Ernenwein L, *et al.* (2016). Birth of a W sex chromosome by horizontal transfer of *Wolbachia* bacterial symbiont genome. *Proc Natl Acad Sci U S A* **113**: 15036–15041.

Legrand JJ, Juchault P (1984). Nouvelles données sur le déterminisme génétique et épigénétique de la monogénie chez le crustacés isopodes terrestres *Armadillidium vulgare* Latr. *Génét Sél Evol* **16**: 57–84.

Martin G, Juchault P, Legrand JJ (1973). Mise en évidence d'un micro-organisme intracytoplasmique symbiote de l'Oniscoide *Armadillidium vulgare* L. dont la présence accompagne l'intersexualité ou la féminisation totale des mâles génétiques de la lignée thélygène. *Comptes Rendus Académie Sci Paris* **276**: 2313–2316.

Meirmans PG (2012). The trouble with isolation by distance. *Mol Ecol* **21**: 2839–2846.

Miyata M, Nomura M, Kageyama D (2024). Rapid spread of a vertically transmitted symbiont induces drastic shifts in butterfly sex ratio. *Curr Biol CB* **34**: R490–R492.

O'Neill SL, Giordano R, Colbert AM, Karr TL, Robertson HM (1992). 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. *Proc Natl Acad Sci* **89**: 2699–2702.

Paradis E (2010). pegas: an R package for population genetics with an integrated–modular approach. *Bioinformatics* **26**: 419–420.

Peakall R, Smouse PE (2006). genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol Ecol Notes* **6**: 288–295.

Pritchard JK, Stephens M, Donnelly P (2000). Inference of Population Structure Using Multilocus Genotype Data. *Genetics* **155**: 945.

Rigaud T (1997). Inherited microorganisms and sex determination of arthropod hosts. In: O'Neill SL, Hoffmann AA, Werren JH (eds) *Influential passengers: inherited microorganisms and arthropod reproduction*, Oxford Univ. Press: Oxford, pp 81–101.

Rigaud T, Juchault P (1993). Conflict between feminizing sex ratio distorters and an autosomal masculinizing gene in the terrestrial isopod Armadillidium vulgare Latr. *Genetics* **133**: 247–252.

Rigaud T, Juchault P (1995). Success and failure of horizontal transfers of feminizing Wolbachia endosymbionts in woodlice. *J Evol Biol* **8**: 249–255.

539 Rigaud T, Juchault P, Mocquard JP (1997). The evolution of sex determination in isopods crustaceans.
540 *Bioessays* **19**: 409–416.

541 Rigaud T, Moreau M (2004). A cost of Wolbachia-induced sex reversal and female-biased sex ratios:
542 decrease in female fertility after sperm depletion in a terrestrial isopod. *Proc R Soc B-Biol Sci*
543 **271**: 1941–1946.

544 Rigaud T, Souty Grosset C, Raimond R, Mocquard JP, Juchault P (1991). Feminizing endocytobiosis in
545 the terrestrial crustacean *Armadillidium vulgare* Latr. (Isopoda): Recent acquisitions.
546 *Endocytobiosis Cell Res* **7**: 259–273.

547 Rosenberg NA (2003). DISTRUCT: a program for the graphical display of population structure. *Mol*
548 *Ecol Notes* **4**: 137–138.

549 Rousset F (2008). GENEPOP'007: a complete re-implementation of the GENEPOP software for
550 Windows and Linux. *Mol Ecol Resour* **8**: 103–106.

551 Russell A, Borrelli S, Fontana R, Laricchiuta J, Pascar J, Becking T, *et al.* (2021). Evolutionary transition
552 to XY sex chromosomes associated with Y-linked duplication of a male hormone gene in a
553 terrestrial isopod. *Heredity* **127**: 266–277.

554 Taylor DR (1990). Evolutionary consequences of cytoplasmic sex ratio distorters. *Evol Ecol* **4**: 235–
555 248.

556 Turelli M, Hoffmann AA (1991). Rapid spread of an inherited incompatibility factor in California
557 Drosophila. *Nature* **353**: 440–442.

558 Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004). Micro-checker: software for
559 identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Notes* **4**: 535–
560 538.

561 Vavre F, Fleury F, Lepetit D, Fouillet P, Boulétreau M (1999). Phylogenetic evidence for horizontal
562 transmission of Wolbachia in host-parasitoid associations. *Mol Biol Evol* **16**: 1711–1723.

563 Verne S, Johnson M, Bouchon D, Grandjean F (2007). Evidence for recombination between feminizing
564 Wolbachia in the isopod genus Armadillidium. *Gene* **397**: 58–66.

565 Verne S, Johnson M, Bouchon D, Grandjean F (2012). Effects of parasitic sex-ratio distorters on host
566 genetic structure in the Armadillidium vulgare-Wolbachia association. *J Evol Biol* **25**: 264–76.

567 Verne S, Puillandre N, Brunet G, Gouin N, Samollow PB, Anderson JD, *et al.* (2006). Characterization
568 of polymorphic microsatellite loci in the terrestrial isopod *Armadillidium vulgare*. *Mol Ecol*
569 *Notes* **6**: 328–330.

570 Weeks AR, Turelli M, Harcombe WR, Reynolds KT, Hoffmann AA (2007). From parasite to mutualist:
571 rapid evolution of Wolbachia in natural populations of Drosophila. *PLoS Biol* **5**: e114.

572 Weir BS, Cockerham CC (1984). Estimating F-Statistics for the Analysis of Population Structure.
573 *Evolution* **38**: 1358–1370.

574 Werren JH (2011). Selfish genetic elements, genetic conflict, and evolutionary innovation. *Proc Natl*
575 *Acad Sci U S A* **108 Suppl 2**: 10863–10870.

576 Werren JH, Baldo L, Clark ME (2008). Wolbachia: master manipulators of invertebrate biology. *Nat*
577 *Rev Microbiol* **6**: 741–51.

578 Werren JH, Zhang W, Guo LR (1995). Evolution and phylogeny of Wolbachia: reproductive parasites
579 of arthropods. *Proc Biol Sci* **261**: 55–63.

580 Wickham H, Chang W, Henry L, Pedersen TL, Takahashi K, Wilke C, *et al.* (2020). ggplot2: Create
581 Elegant Data Visualisations Using the Grammar of Graphics.

582

583

584 **Figure legends**

585

586 **Figure 1.** Map of France (left) showing the location of the six populations analyzed in this study and
587 zoom in the relevant geographic area (right), indicating the relative positions and distances in
588 kilometers (km) between populations.

589

590 **Figure 2.** Prevalence of sex ratio distorters in *Armadillidium vulgare* males (m) and females (f) from
591 six populations sampled at different time points.

592

593 **Figure 3.** Mitotype network of 12 mitochondrial variants from six *Armadillidium vulgare* populations
594 sampled at different time points. Each circle represents one mitotype and circle diameter is
595 proportional to the number of individuals carrying the mitotype. Branch lengths connecting circles
596 are proportional to divergence between mitotypes.

597

598 **Figure 4.** Variation in mitotype counts across years for three *Armadillidium vulgare* populations from
599 Coulombiers, La Crèche and Poitiers. Prevalence of sex ratio distorters is coded as indicated in the
600 key on the right-hand side of the figure.

601

602 **Figure 5.** Assignment of individuals from six *Armadillidium vulgare* populations sampled at different
603 time points to one of two genetic clusters (blue and pink colors) following Bayesian analysis. Each bar
604 represents an individual, and the proportion of each color represents the probability of assignment
605 to the corresponding cluster.

606

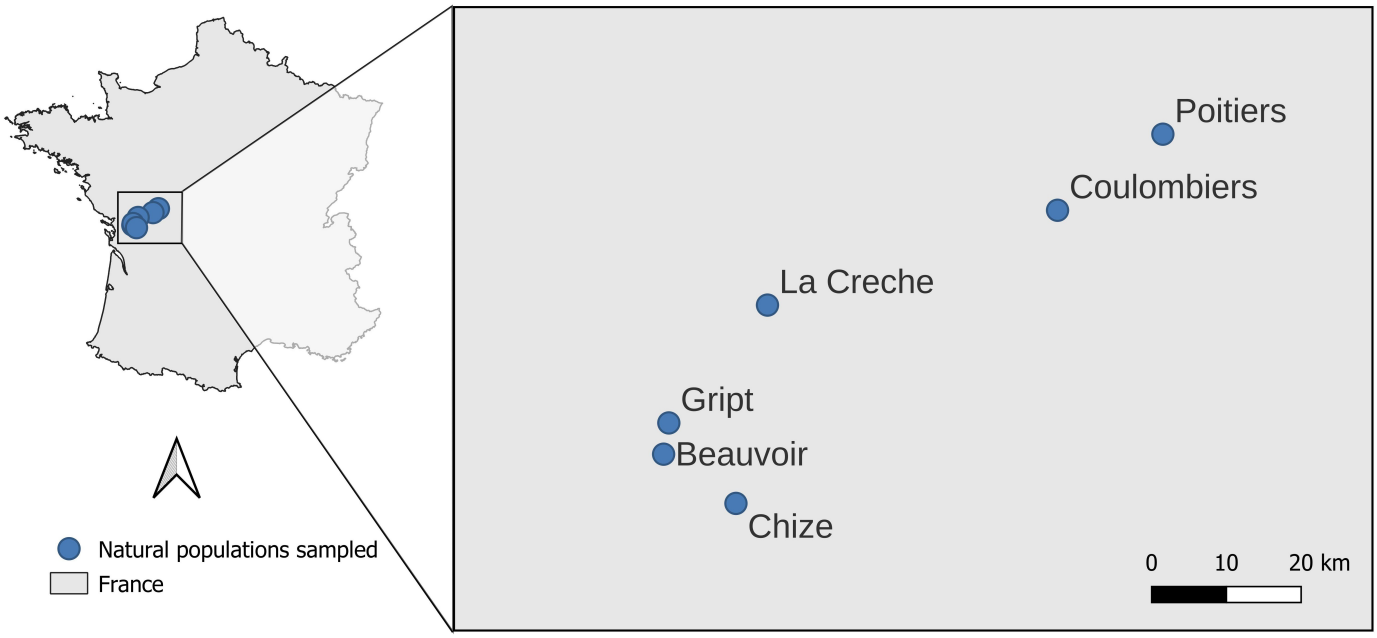
607 **Figure 6.** Discriminant Analysis of Principal Components scatterplot. The two axes represent the first
608 two principal components (PC). Dots represent individuals. Each of the 28 sampling points presents a
609 95% inertia ellipse and is labeled with two letters indicating the population and the two last digits of
610 the sampling year. The eigenvalues of the analysis (inset) show the relative amount of genetic
611 structure captured by the principal components (the first two components are highlighted in dark
612 grey).

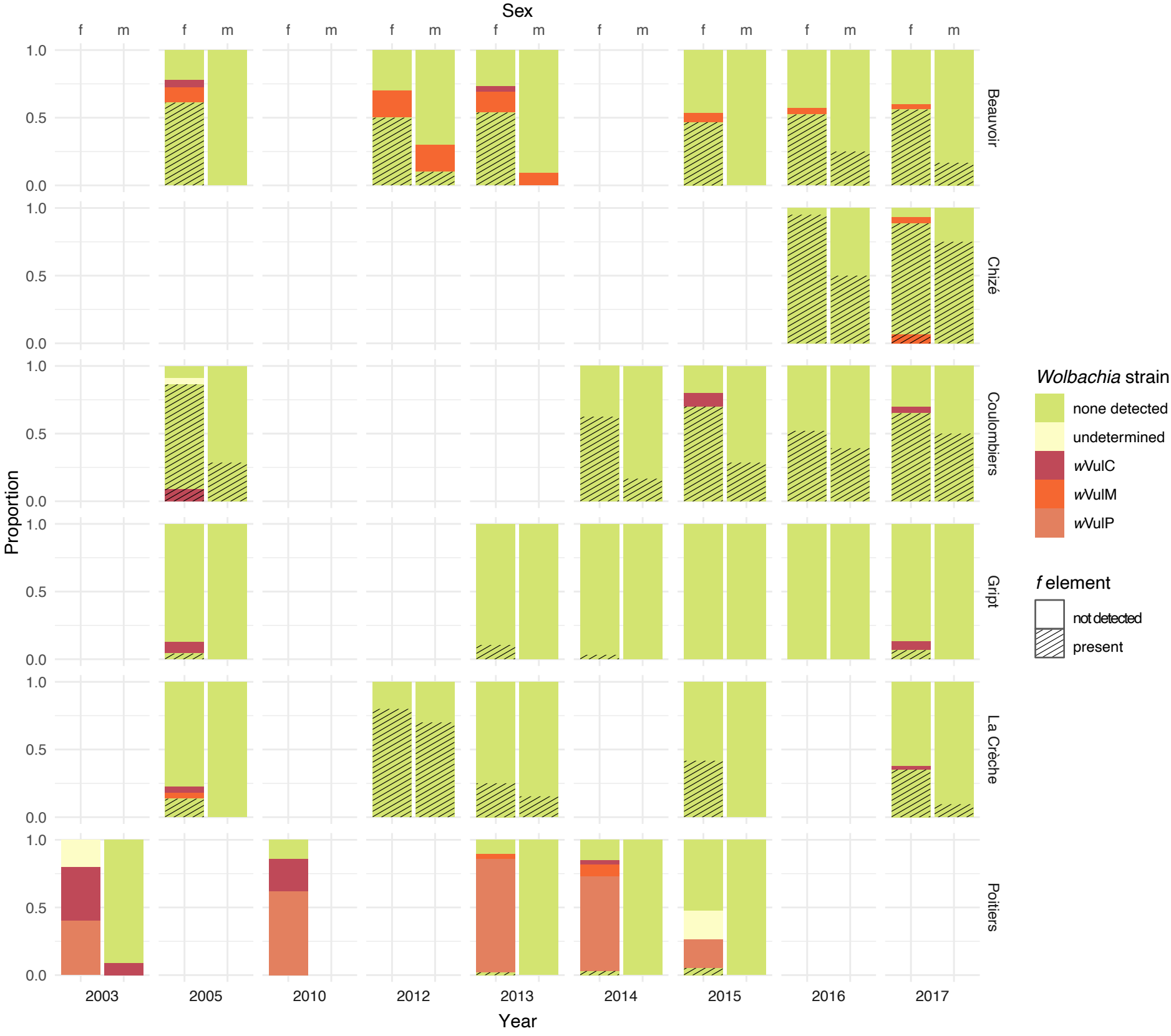
613 **Table 1.** Prevalence of *Wolbachia* and *f* element sex ratio distorters in six populations of *Armadillidium vulgare*.

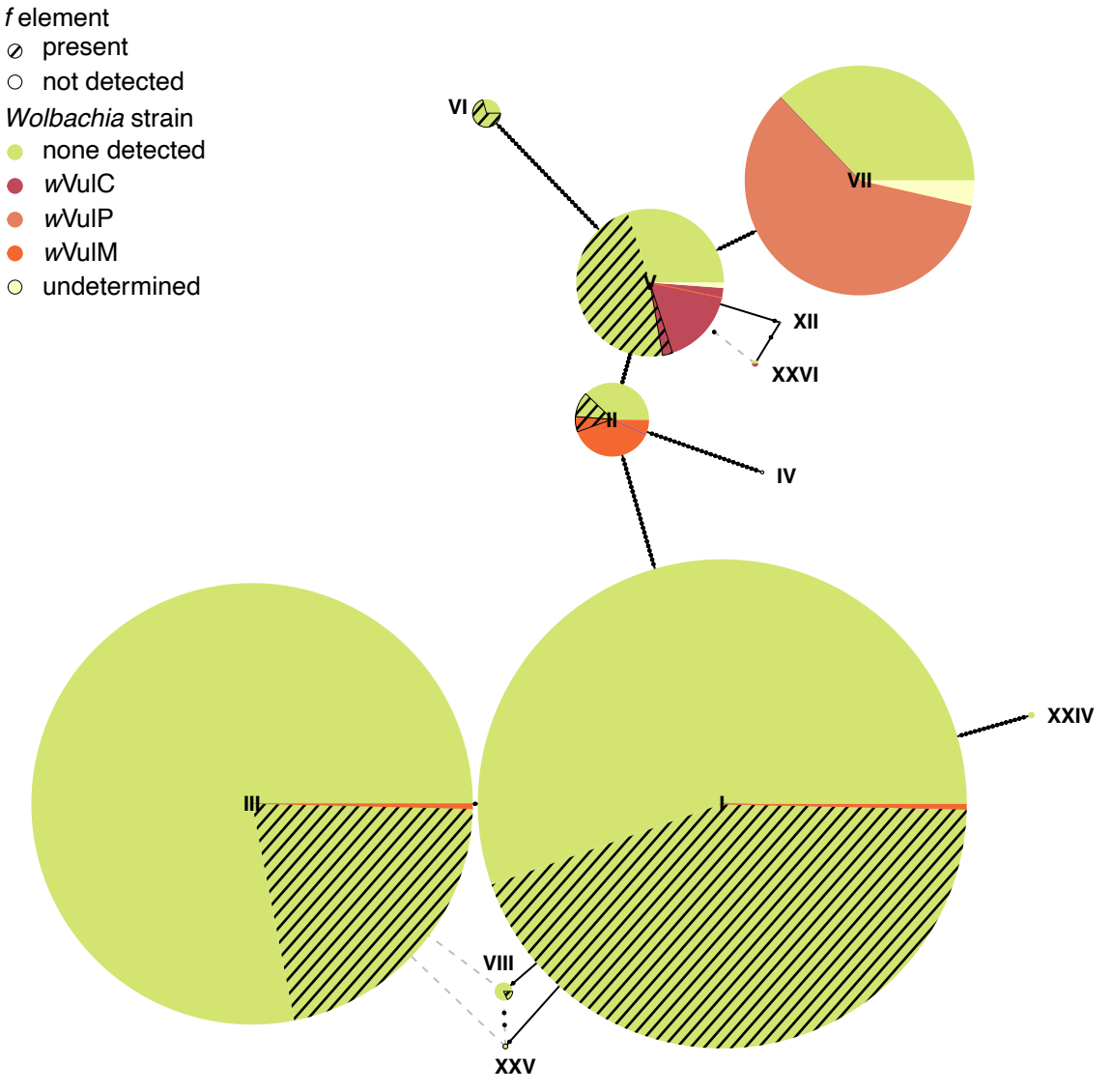
614

Population	Sampling year	Sample size	Sex	n	No *f* element, no *Wolbachia*	Only *f* element	Only *Wolbachia*				Both *w*VulM and *f* element	Both *w*VulC and *f* element	Undetermined status
							*w*VulC	*w*VulM	*w*VulP	Undetermined			
Beauvoir	2017	31	Males	6	5	1							
			Females	25	9	14		1					1
	2016	25	Males	4	3	1							
			Females	21	8	11		1					1
	2015	21	Males	6	6								
			Females	15	6	7		1					1
	2013	37	Males	11	10			1					
			Females	26	7	14	1	4					
	2012	20	Males	10	7	1		2					
			Females	10	3	5		2					
	2005	34	Males	16	16								
			Females	18	3	11	1	2					1
Chizé	2017	52	Males	8	2	6							
			Females	44	3	36		2			3		
	2016	26	Males	6	3	3							
			Females	20	1	19							
Coulombiers	2017	24	Males	4	2	2							
			Females	20	6	13	1						
	2016	48	Males	23	13	9							1
			Females	25	10	13							2
	2015	27	Males	7	5	2							
			Females	20	4	14	2						
	2014	14	Males	6	5	1							
			Females	8	3	5							
	2005	29	Males	7	5	2							
			Females	22	2	17				1		2	
Gript	2017	45	Males	15	15								
			Females	30	26	2	2						
	2016	16	Males	4	4								
			Females	12	12								

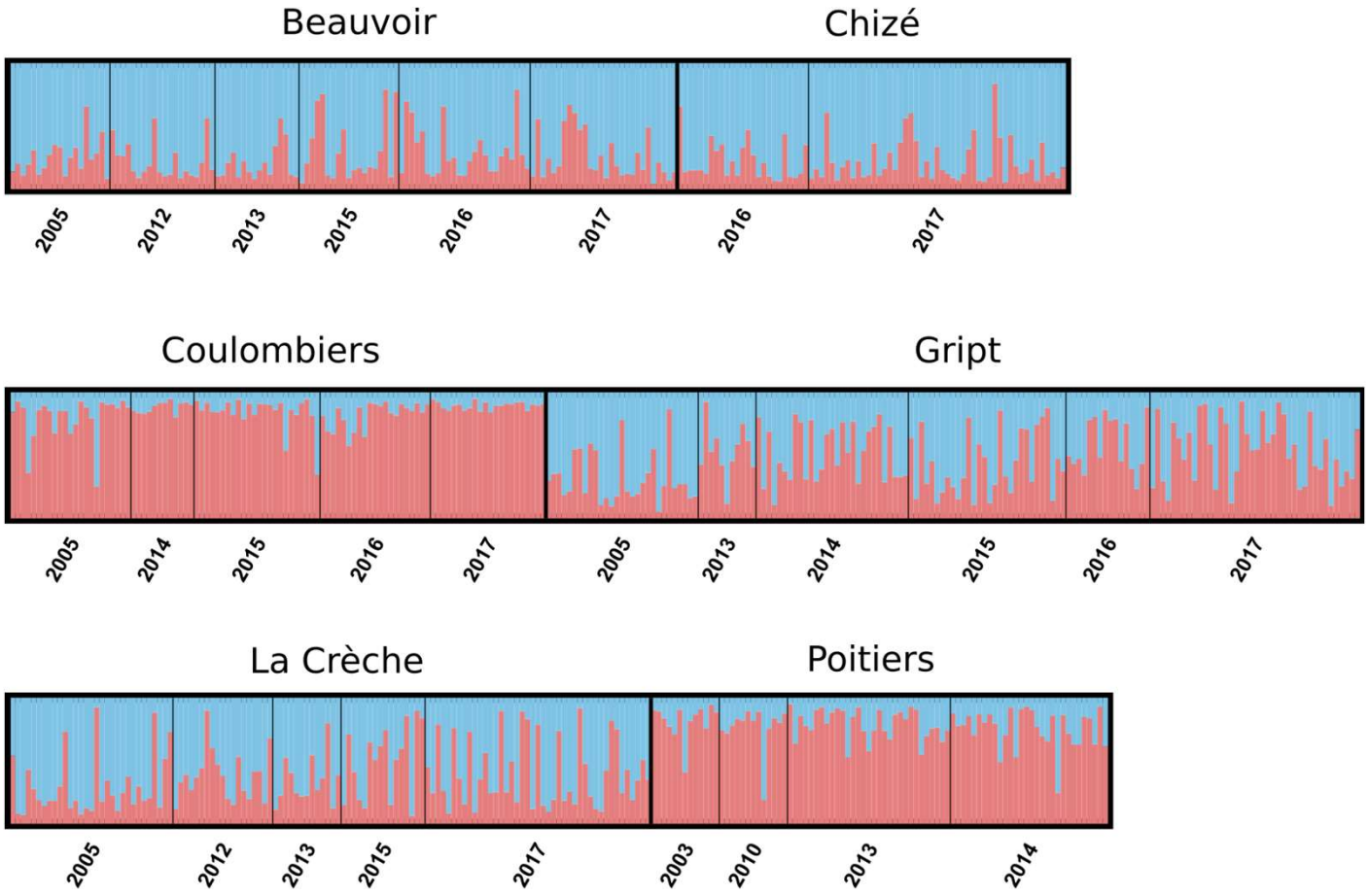
	2015	30	Males	5	5								
			Females	25	24								1
	2014	35	Males	4	4								
			Females	31	30	1							
	2013	22	Males	3	3								
			Females	19	17	2							
	2005	34	Males	10	9								1
			Females	24	18	1	2						3
La Crèche	2017	58	Males	21	19	2							
			Females	37	23	13	1						
	2015	16	Males	4	3								1
			Females	12	7	5							
	2013	17	Males	13	11	2							
			Females	4	3	1							
	2012	20	Males	10	3	7							
			Females	10	2	8							
	2005	39	Males	17	14								3
			Females	22	11	3	1	1					6
Poitiers	2015	23	Males	4	4								
			Females	19	10	1			4	4			
	2014	41	Males	8	8								
			Females	33	5	1	1	3	23				
	2013	68	Males	19	17								2
			Females	49	4	1		2	41				1
	2010	21	Males	0									
			Females	21	1		5		13				2
	2003	16	Males	11	8		1						2
			Females	5			2		2	1			
Total males				262	209	39	1	3	0	0	0	0	10
Total females				627	258	218	19	19	83	6	3	2	19
Total				889	467	257	20	22	83	6	3	2	29

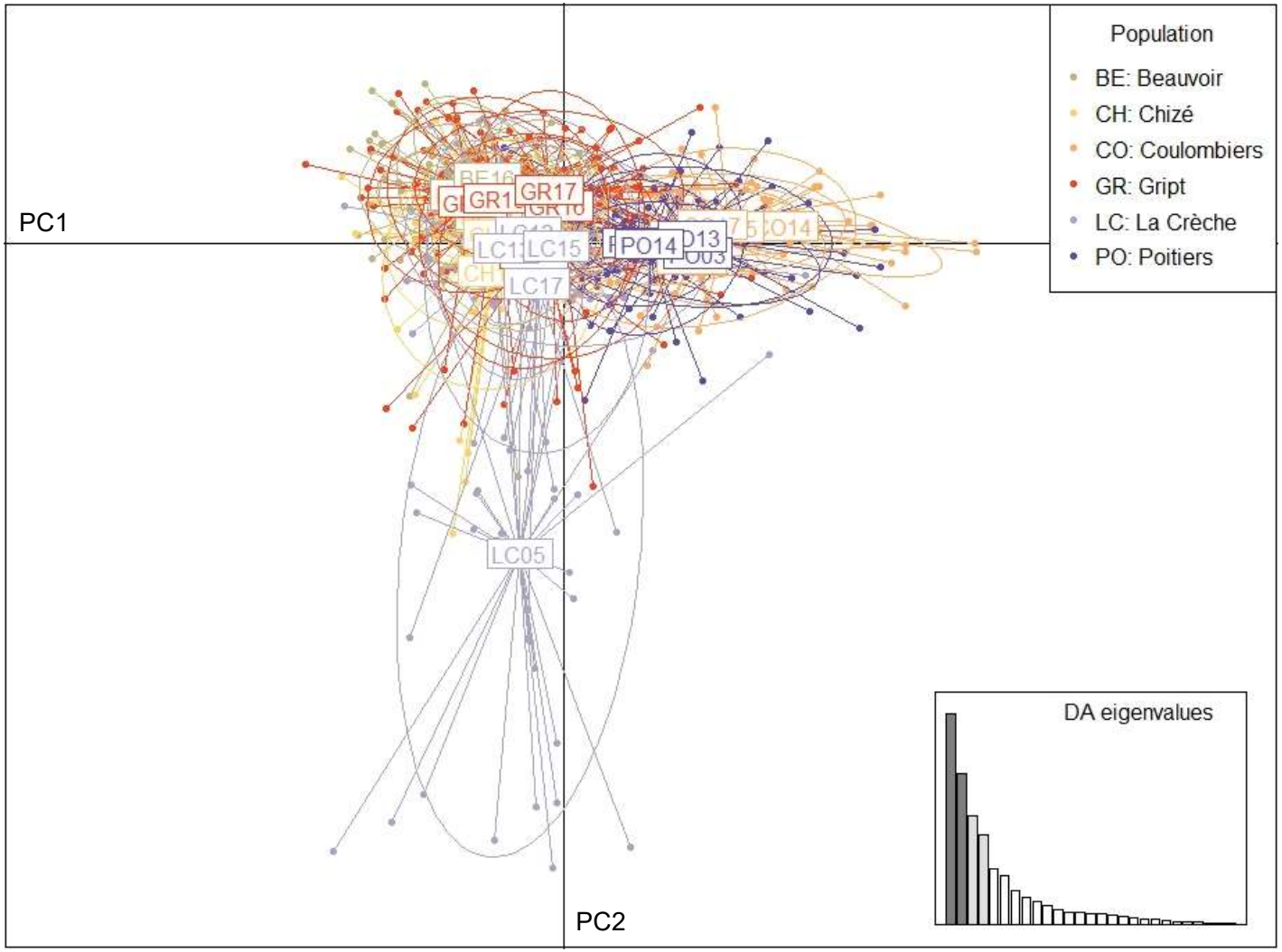

Poitiers
Coulombiers
La Creche
Gript
Beauvoir
Chize
0
10
20 km
Natural populations sampled
France


Sex
f
m
Proportion
1.0
0.5
0.0
Beauvoir
Chizé
Coulombiers
Gript
La Crèche
Poitiers
2003
2005
2010
2012
2013
2014
2015
2016
2017
Year
Wolbachia strain
none detected
undetermined
wVulC
wVulM
wVulP
f element
not detected
present


f element
present
not detected
Wolbachia strain
none detected
*w*VulC
*w*VulP
*w*VulM
undetermined
VI
VII
V
XII
XXVI
II
IV
XXIV
III
I
VIII
XXV

Coulombiers
La Crèche
Poitiers
count
year
I
III
V
VI
VII
VIII
XXIV
NA
IX
XXVI
2005
2014
2015
2016
2017
2012
2013
2003
2010
Wolbachia strain
none detected
undetermined
wVulC
wVulM
wVulP
f element
not detected
present


Beauvoir
2005
2012
2013
2015
2016
2017
Chizé
2016
2017
Coulombiers
2005
2014
2015
2016
2017
Gript
2005
2013
2014
2015
2016
2017
La Crèche
2005
2012
2013
2015
2017
Poitiers
2003
2010
2013
2014


Population
BE: Beauvoir
CH: Chizé
CO: Coulombiers
GR: Gript
LC: La Crèche
PO: Poitiers
PC1
PC2
GR17
LC15
PO14
LC17
LC05
DA eigenvalues