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

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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 sex determination, we analyzed their temporal distribution in 6 *A. vulgare* populations sampled up to 6 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. SRD temporal stability was also reflected in mitochondrial and nuclear variation, with two exceptions. One population was affected by anthropogenic alteration at the sampling spot between the earliest and second time points, likely explaining a rapid shift in genetic composition. However, subsequent global stability suggested that population dynamics equilibrium may be reached in a few generations. In another 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 further highlighted the occurrence of intraspecific horizontal transmission, making it a biologically relevant parameter for *Wolbachia* evolutionary dynamics in *A. vulgare*.

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 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 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). It has been suggested that SRD may have contributed to the frequent turnovers of sex chromosomes recorded in terrestrial isopods (Juchault and Mocquard, 1993; Juchault and Rigaud, 1995; Becking *et al.*, 2017, 2019; Russell *et al.*, 2021).

To elucidate the potential impact of SRD on the evolution of sex determination in terrestrial isopods, it is essential to clarify the intraspecific evolutionary dynamics of SRD such as *Wolbachia* and the *f* element. Previous studies have shown that: (i) *Wolbachia* and the *f* element are present at variable frequencies in *A. vulgare* populations, (ii) the *f* element is overall more frequent than *Wolbachia*, (iii) the two SRD usually do not co-occur at high frequency in populations, and (iv) mitochondrial haplotypes are tightly linked to *Wolbachia* strains (suggesting stable maternal transmission), but not

to the *f* element (Juchault *et al.*, 1993; Durand *et al.*, 2023). While these results have provided insights into the spatial distribution of *Wolbachia* and the *f* element in *A. vulgare*, in the present study we investigate their temporal dynamics. This issue has previously been tackled by a single study from 1992 (Juchault *et al.*, 1992), in which *Wolbachia* prevalence was found to decrease concomitantly to an increase of *f* element prevalence in an *A. vulgare* population from Niort (western France) sampled at three time points over a period of 23 years (1963, 1973 and 1986). However, a single population was included in the study, thus limiting the breadth of its conclusions. Here we report an analysis of *Wolbachia* and *f* element distribution in 6 *A. vulgare* populations sampled up to 6 times over a period of 12 years, representing a total of 889 individuals from 29 time points. The studied populations were sampled in a narrow geographic area in western France, within 70 km of the Niort population (Juchault *et al.*, 1992), to control for spatial dynamics. Analyzed in the context of mitochondrial and nuclear variation, our results highlight an overall temporal stability of SRD distribution in *A. vulgare*, with few exceptions.

Materials and Methods

We analyzed 889 *A. vulgare* individuals from 6 natural populations sampled in western France between 2003 and 2017. DNA samples from Beauvoir-2017, Chizé-2017, Coulombiers-2017, Gript-2017, La Crèche-2017 and Poitiers-2015 were available from (Durand *et al.*, 2023). All other individuals were collected by hand. Individuals were sexed and stored in alcohol or at -20°C prior to DNA extraction. Total genomic DNA of samples collected between 2003 and 2013 was extracted from gonads using phenol and chloroform (Kocher *et al.*, 1989) and DNA of samples collected between 2014 and 2016 was extracted from the head and legs, as described previously (Leclercq *et al.*, 2016).

We used four molecular markers to assess the presence of *Wolbachia* and the *f* element in DNA extracts: *Jtel* (Leclercq *et al.*, 2016), *wsp* (Braig *et al.*, 1998), *recR* (Badawi *et al.*, 2014) and *ftsZ* (Werren *et al.*, 1995) (Table S1). While *Jtel* is specific to the *f* element, *wsp* and *recR* are specific to *Wolbachia*, and *ftsZ* is present in both the *f* element and *Wolbachia* (Leclercq *et al.*, 2016). We assessed the presence or absence of these markers by PCR, as described previously (Leclercq *et al.*, 2016). Different amplification patterns were expected for individuals with *Wolbachia* only (*Jtel-, wsp+*, *recR+*, *ftsZ+*), the *f* element only (*Jtel+, wsp-, recR-*, *ftsZ+*), both *Wolbachia* and the *f* element present (*Jtel+, wsp+, recR+*, *ftsZ+*) or both *Wolbachia* and the *f* element lacking (*Jtel-, wsp-, recR-*, *ftsZ-*). The few individuals exhibiting other amplification patterns were classified as “undetermined status”.

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

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

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

129 Of the 22 amplified microsatellite markers, Av4 and Av5 could not be scored because of multiple
130 peaks, AV0096 and AV0128 did not amplify consistently, and AV0023 and AV0061 were
131 monomorphic across the dataset. The *Genepop* package for R (Rousset, 2008) detected no linkage
132 disequilibrium between the 16 remaining loci. We tested for the presence of null alleles using a
133 combination of software, as recommended previously (Dąbrowski *et al.*, 2014). We used Micro-
134 Checker (Van Oosterhout *et al.*, 2004), Cervus (Kalinowski *et al.*, 2007) and ML-NullFreq (Kalinowski
135 and Taper, 2006). As a result, AV0099 was discarded because it consistently presented hints of null
136 alleles in many populations and sampling years. For the following analyses, we removed individuals
137 whose genotypes were available for fewer than 13 out of the 15 remaining markers.

138 Hardy-Weinberg equilibrium was tested for each locus, locality and sampling year with an exact test
139 using Markov chain with the *Genepop* package for R. We used the Fstat software to calculate allelic
140 richness (based on a minimum of 3 individuals). To test whether the same genetic populations have
141 been sampled each year, we searched for genetic clusters without a priori with a Bayesian method
142 using the software Structure (Pritchard *et al.*, 2000). We selected the admixture model as well as the
143 option of correlated allele frequencies. We varied the number of clusters (K) from 2 to 9. For each
144 value of K, we carried out 20 independent runs, as recommended in (Evanno *et al.*, 2005), with a
145 total number of 100,000 iterations and a burn-in of 100,000. To determine the most likely value of K,

146 we applied the method described in (Evanno *et al.*, 2005) and implemented in Structure Harvester
147 version 0.6.9 (Earl and vonHoldt, 2012). In addition, we performed a Discriminant Analysis of
148 Principal Components (DAPC) (Jombart *et al.*, 2010) on populations according to their sampling
149 locality and year with the *adegenet* package (Jombart, 2008) to search for potential discrepancies
150 across time points within populations.

151 All analyses were performed with R v.3.6.0 (R Development Core Team, 2013). When multiple
152 statistical tests of the same hypothesis were performed, we corrected p-values with the Benjamini-
153 Yekutieli procedure (Benjamini and Yekutieli, 2001). Figures were realized with *ggplot2* (Wickham *et*
154 *al.*, 2020). Results from Structure were processed with the program Distruct (Rosenberg, 2003) for
155 graphical representation.

156

157 **Results**

158

159 We tested the presence of *Wolbachia* and the *f* element in 889 individuals (627 females and 262
160 males) from 6 populations sampled at various time points between 2003 and 2017, representing a
161 total of 29 sampling points (Tables 1 and S3). We found that 29.9% of individuals carried only the *f*
162 element, 15.2% carried only *Wolbachia*, 0.6% carried both SRD and 54.3% carried none. While both
163 SRD were mostly found in females, they were also sometimes present in males, as previously
164 reported (Durand *et al.*, 2023). *Wolbachia*-infected individuals carried one of the three previously
165 known *Wolbachia* strains of *A. vulgare*: *w*VulC (n=22), *w*VulM (n=25) or *w*VulP (n=83).

166 Overall, both SRD were found in at least one time point in all 6 populations (Figure 1). However, the
167 populations displayed substantial variation in the distribution of the two SRD. In Beauvoir, Chizé,
168 Coulombiers and La Crèche populations, the *f* element was predominant (29-86% frequency) and
169 *Wolbachia* rare (2-10%). Conversely, *Wolbachia* was frequent (63%) and the *f* element very rare (2%)
170 in Poitiers. Finally, both SRD were very rare in Gript (2-3%). Remarkably, SRD prevalence was
171 qualitatively stable across time points (spanning up to 12 years) within all populations (Figure 1). Yet,
172 in Poitiers where *Wolbachia* remained globally frequent overtime, the *w*VulC strain progressively
173 decreased in frequency while the *w*VulP strain exhibited the opposite trend.

174 Sequencing of the *COI* mitochondrial gene of 884 individuals identified 12 haplotypes, 9 of which
175 have previously been detected in *A. vulgare* populations (named I to VIII, and XII) (Durand *et al.*,
176 2023) and three are newly described haplotypes (XXIV to XXVI; GenBank accession numbers
177 OR074129 to OR074131, respectively) (Figure 2 and Table S3). There was an excellent congruence

178 between *Wolbachia* strains and mitochondrial haplotypes, as previously reported (Verne *et al.*, 2012;
179 Durand *et al.*, 2023). Indeed, individuals carrying *w*VulC were associated with either haplotype V or
180 its close relatives (XII and XXVI), those carrying *w*VulP were associated with haplotype VII and those
181 carrying *w*VulM were associated with haplotype II. Exceptions included two individuals infected by
182 *w*VulM, which were associated with the distantly related haplotypes I and III. By contrast, the *f*
183 element was found in 8 different mitochondrial backgrounds (I to VI, VIII and XXV) distributed across
184 the mitochondrial network.

185 All time points considered, there was between 3 (in Chizé) and 6 (in Poitiers) haplotypes per
186 population (Figures 3 and S1). Haplotype frequencies were globally stable across time points within
187 populations (e.g. Coulombiers, Figure 3A), with the notable exceptions of La Crèche and Poitiers
188 populations. In La Crèche, a shift in major haplotypes occurred between 2005 and 2012, with the
189 rarefaction of haplotype III and V being concomitant with the rise in frequency of haplotypes I and
190 VIII, followed by stability since 2012 (Figure 3B). In Poitiers, the rise in frequency of haplotype VII
191 (associated with *Wolbachia* strain *w*VulP) coincided with a relative decrease of *w*VulC-associated
192 haplotypes V, XII and XXVI (Figure 3C).

193 To test if the patterns of SRD and mitochondrial variation were also reflected in the nuclear genome,
194 we examined variation in 667 individuals with genotype information available for at least 13 out of
195 the 15 retained microsatellite markers (see Materials and Methods), representing a total of 28
196 sampling points (Poitiers-2015 was discarded due to low genotyping success) (Table S3). Allelic
197 richness ranged from 1 to 4.78 (Table S4) and all loci were at Hardy-Weinberg equilibrium for all
198 sampling points.

199 To evaluate whether the same genetic populations had been sampled at each time point, we
200 delineated genetic clusters. A Delta-K analysis (Evanno *et al.*, 2005) inferred that the best fit to the
201 data was obtained for K=2 genetic clusters (Figure S2). Indeed, the major structuration of the data
202 grouped Poitiers and Coulombiers on the one hand, and Beauvoir, Chizé, La Crèche and Gript on the
203 other hand in a Structure analysis (Figure 4). No obvious change in genetic structure was apparent
204 between time points within populations in the Structure analysis. The DAPC confirmed the major
205 population structuration, as the first component separated Poitiers and Coulombiers from the other
206 populations (Figure 5). It also supported overall homogeneity of populations across time points,
207 apart from La Crèche-2005, which was separated from the other La Crèche time points in the second
208 component of the DAPC.

209

210 **Discussion**

Wolbachia and *f* element distributions are highly heterogeneous among populations, despite their modest geographic distance of at most 80 km. The emerging trend is that when one SRD is frequent in a population, the other SRD is rare, as noted previously (Durand *et al.*, 2023). As it causes a stronger bias toward females, *Wolbachia* is expected to prevail over the *f* element in *A. vulgare* populations (Rigaud, 1997; Cordaux and Gilbert, 2017). Yet, the *f* element was the dominant SRD in 4 out of 6 populations we studied and was rising in frequency in Niort (Juchault *et al.*, 1992). Overall, the *f* element is more widespread than *Wolbachia* in *A. vulgare* populations (Juchault *et al.*, 1993; Durand *et al.*, 2023). Previously proposed explanations include a higher fitness cost entailed by *Wolbachia* relative to the *f* element and occasional paternal transmission of the *f* element (which some males carry, Figure 1) enabled by masculinizing epistatic alleles (Juchault *et al.*, 1992; Rigaud and Juchault, 1993; Rigaud, 1997; Rigaud and Moreau, 2004; Cordaux and Gilbert, 2017).

In contrast with spatial heterogeneity, our sampling scheme with up to 6 sampling time points spanning 12 years per population highlights a global temporal stability in SRD prevalence within populations. At first glance, this qualitative pattern differs from that previously reported for the Niort population, in which *Wolbachia* prevalence was found to decrease concomitantly to an increase of *f* element prevalence over a period of 23 years (Juchault *et al.*, 1992). Given *A. vulgare*'s generation time of one year, our study might have spanned too few generations (12) to capture variation in SRD prevalence, which the Niort study spanning 23 generations did. Yet, the time scale of our study enabled us to detect variation in *Wolbachia* strain prevalence, as well as mitochondrial and nuclear variation within and between populations, suggesting that lack of resolution is not an issue. Alternatively, most of the populations we studied may reflect some relatively stable equilibrium with respect to SRD evolutionary dynamics, an equilibrium that the Niort population might not have reached. Indeed, theoretical models have indicated that when feminizing factors are in competition, the one that induces the strongest bias toward females is expected to spread in the population (Taylor, 1990). Thus, a single SRD should remain in the population at equilibrium. Consistently, in most of the populations we analyzed, a single SRD occurs at high frequency, suggesting that these populations may be at or near equilibrium for an SRD. It has also been suggested that an apparent stability could be due to hidden processes such as population structure (including extinction-recolonization processes), intragenomic conflicts and coevolutionary processes (Hatcher, 2000).

The temporal stability of SRD in most of *A. vulgare* populations is also reflected in host mitochondrial and nuclear variation, with two notable singularities. The first is La Crèche population in 2005, which differs from the other time points (2012 to 2017) on both mitochondrial and nuclear grounds.

244 Interestingly, the sampling spot in La Crèche has been altered by land remodeling between 2005 and
245 2012. This anthropogenic activity may have caused the reduction or collapse of the historic *A.*
246 *vulgare* population and the introduction of new individuals as part of the addition of materials during
247 the remodeling (e.g., soil from another location). Such an extinction-recolonization scenario may
248 explain the loss of *Wolbachia* (present at low frequency in 2005) and the increase in *f* element
249 frequency in 2012. It is noteworthy that from 2012 on, SRD, mitochondrial and nuclear variation have
250 been stable, suggesting that stabilization of the population dynamics may be reached in a few years,
251 i.e., in a few generations.

252 The second case of instability is the Poitiers population, which is stable with respect to nuclear
253 variation but not to both mitochondrial and SRD variation. Poitiers is the only population in our
254 dataset in which *Wolbachia* is the dominant SRD across time points, thus highlighting a qualitative
255 pattern of temporal stability. However, our results indicate that the rise in frequency of the *w*VulP
256 strain correlates with a decrease of the *w*VulC strain, suggesting a *Wolbachia* strain replacement in
257 this population. The *w*VulP strain is characterized by a recombination event involving *w*VulC (Verne
258 *et al.*, 2007), indicating that *w*VulC is older than *w*VulP, which is consistent with the situation
259 recorded in Poitiers. Assuming the driver of this replacement is *Wolbachia* and not another
260 cytoplasmic element (like the mitochondrion), replacement of *w*VulC by *w*VulP could be due to the
261 latter strain having a transmission advantage over the former strain. Unfortunately, the *w*VulP strain
262 is not very well characterized, and while feminization induction is likely (Verne *et al.*, 2007), it has not
263 been formally demonstrated and compared to feminization induced by wVulC (Rigaud *et al.*, 1991;
264 Cordaux *et al.*, 2004). The respective costs of these two *Wolbachia* strains has not been investigated
265 neither. In any event, because *Wolbachia* and mitochondria are co-inherited cytoplasmic entities,
266 changes in *Wolbachia* strains associated with different mitochondrial haplotypes are expected to
267 lead to concomitant changes in mitochondrial variation, but no change in nuclear variation.
268 Therefore, our observations in Poitiers may constitute a typical example of mitochondrial sweep
269 caused by endosymbiont rise in frequency (Galtier *et al.*, 2009).

270 *Wolbachia* dynamics in Poitiers also illustrates that transovarial, maternal transmission is the main
271 transmission mode of *Wolbachia* in *A. vulgare*. However, non-maternal transmission also appears to
272 occur, as testified by two individuals with *w*VulM from Beauvoir and La Crèche. These individuals
273 carry mitochondrial haplotypes I and III, respectively, unlike all other *w*VulM-infected individuals
274 which carry haplotype II. As haplotypes I, II and III are distantly related, the most likely explanation is
275 that the two unusual individuals have acquired *Wolbachia* by horizontal transfer. Horizontal transfer
276 of *Wolbachia* is largely documented in arthropods (O'Neill *et al.*, 1992; Werren *et al.*, 1995; Heath *et*
277 *al.*, 1999; Vavre *et al.*, 1999), including terrestrial isopods (Bouchon *et al.*, 1998; Cordaux *et al.*, 2001,

278 2012). Potential mechanisms in isopods include contact between wounded individuals (Rigaud and
279 Juchault, 1995) and cannibalism/predation (Le Clec'h *et al.*, 2013). In total, we infer that 2 out of 136
280 *Wolbachia*-infected individuals may have acquired their symbionts by horizontal transmission. This
281 may be an underestimate, as horizontal transfers between individuals carrying the same
282 mitochondrial haplotype cannot be detected with our approach. Although it is apparently rare,
283 horizontal transmission occurs at a measurable rate in *A. vulgare*, suggesting that it is a parameter of
284 importance in *Wolbachia* evolutionary dynamics in this species.

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Author contribution statement

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

Conflict of interest

The authors declare no conflict of interest.

Data archiving

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

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467

468

469 **Figure legends**

470

471 **Figure 1.** Prevalence of sex ratio distorters in *Armadillidium vulgare* males (m) and females (f) from
472 six populations sampled at different time points.

473

474 **Figure 2.** Haplotype network of 12 mitochondrial variants from six *Armadillidium vulgare* populations
475 sampled at different time points. Each circle represents one haplotype and circle diameter is
476 proportional to the number of individuals carrying the haplotype. Branch lengths connecting circles
477 are proportional to divergence between haplotypes.

478

479 **Figure 3.** Variation in haplotype counts across years for three *Armadillidium vulgare* populations
480 from (A) Coulombiers, (B) La Crèche and (C) Poitiers. Prevalence of sex ratio distorters is color-coded.

481

482 **Figure 4.** Assignment of individuals from six *Armadillidium vulgare* populations sampled at different
483 time points to one of two genetic clusters (blue and pink colors) following Bayesian analysis. Each bar
484 represents an individual, and the proportion of each color represents the probability of assignment
485 to the corresponding cluster.

486

487 **Figure 5.** Discriminant Analysis of Principal Components scatterplot. Dots represent individuals. Each
488 of the 29 sampling points presents a 95% inertia ellipse and is labeled with two letters indicating the
489 population and the two last digits of the sampling year.

490

491 **Table 1.** Prevalence of *Wolbachia* and *f* element sex ratio distorters in 6 populations of *Armadillidium vulgare*.

492

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

Figure 1

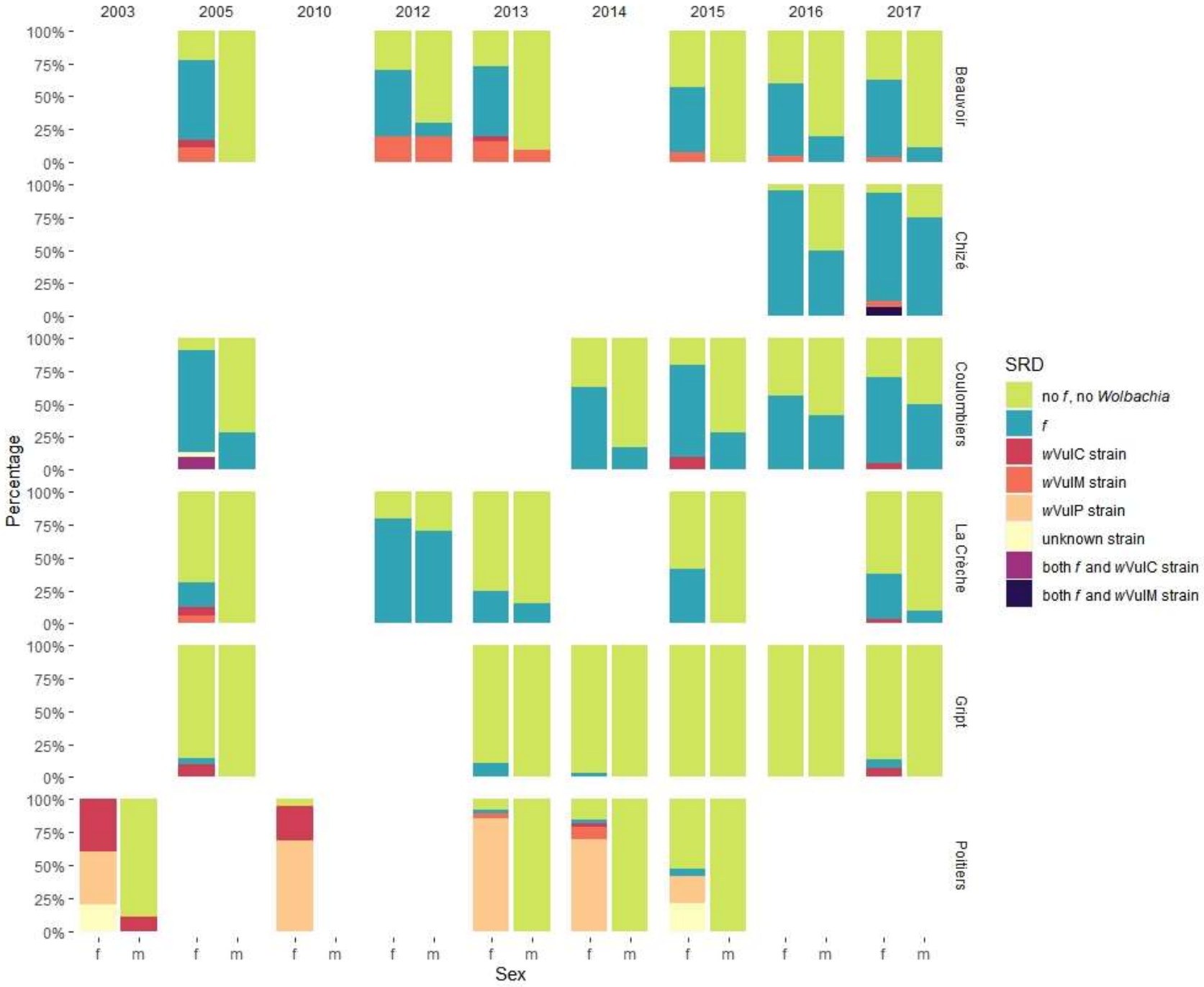

2003
2005
2010
2012
2013
2014
2015
2016
2017
Beauvoir
Chizé
Coulombiers
La Crèche
Gript
Poitiers
Percentage
100%
75%
50%
25%
0%
f
m
Sex
SRD
no f, no Wolbachia
f
wVulC strain
wVulM strain
wVulP strain
unknown strain
both f and wVulC strain
both f and wVulM strain

Figure 2

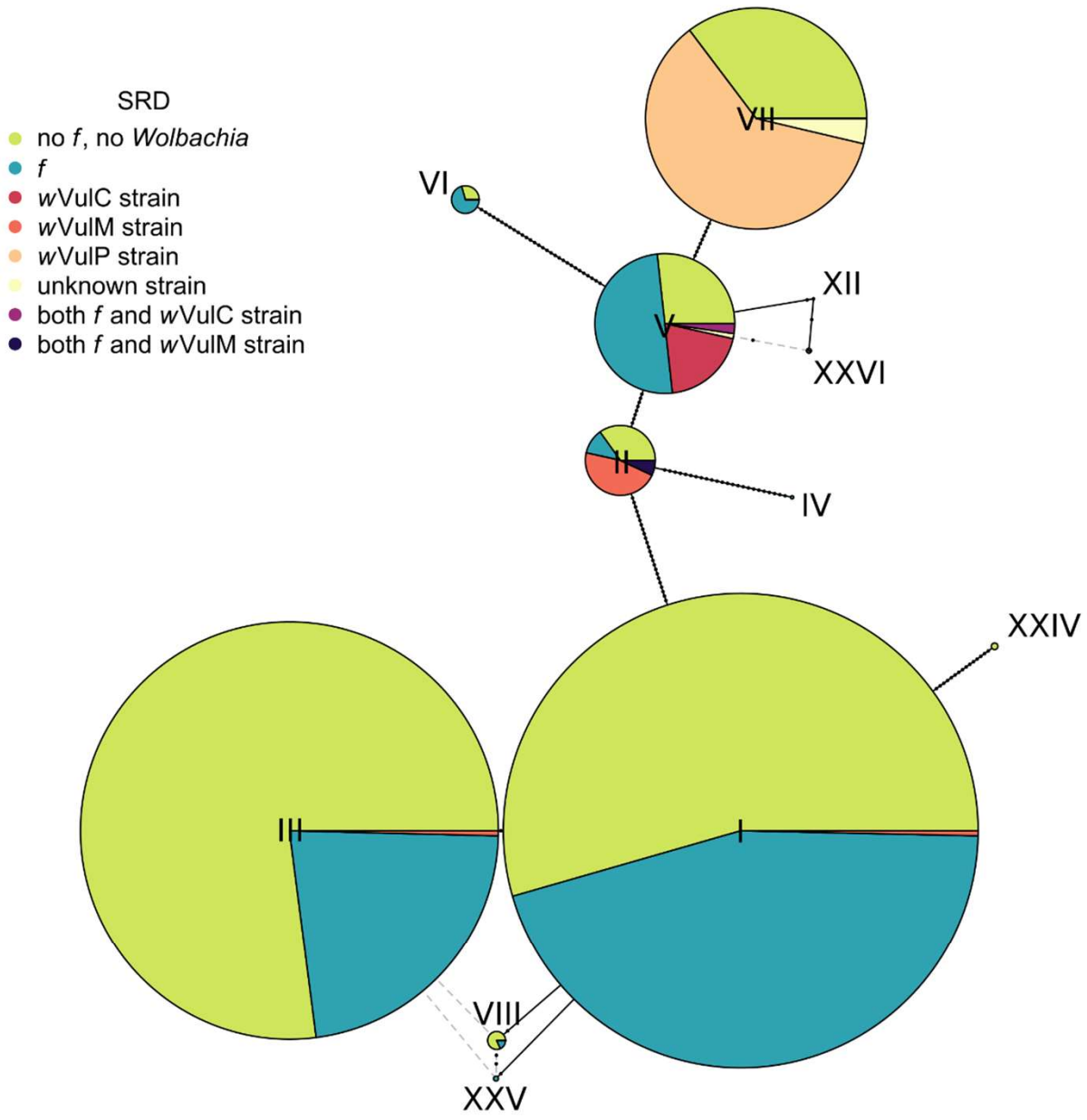

SRD
no *f*, no *Wolbachia*
f
*w*VulC strain
*w*VulM strain
*w*VulP strain
unknown strain
both *f* and *w*VulC strain
both *f* and *w*VulM strain
VII
VI
XII
V
XXVI
II
IV
XXIV
III
I
VIII
XXV

Figure 3

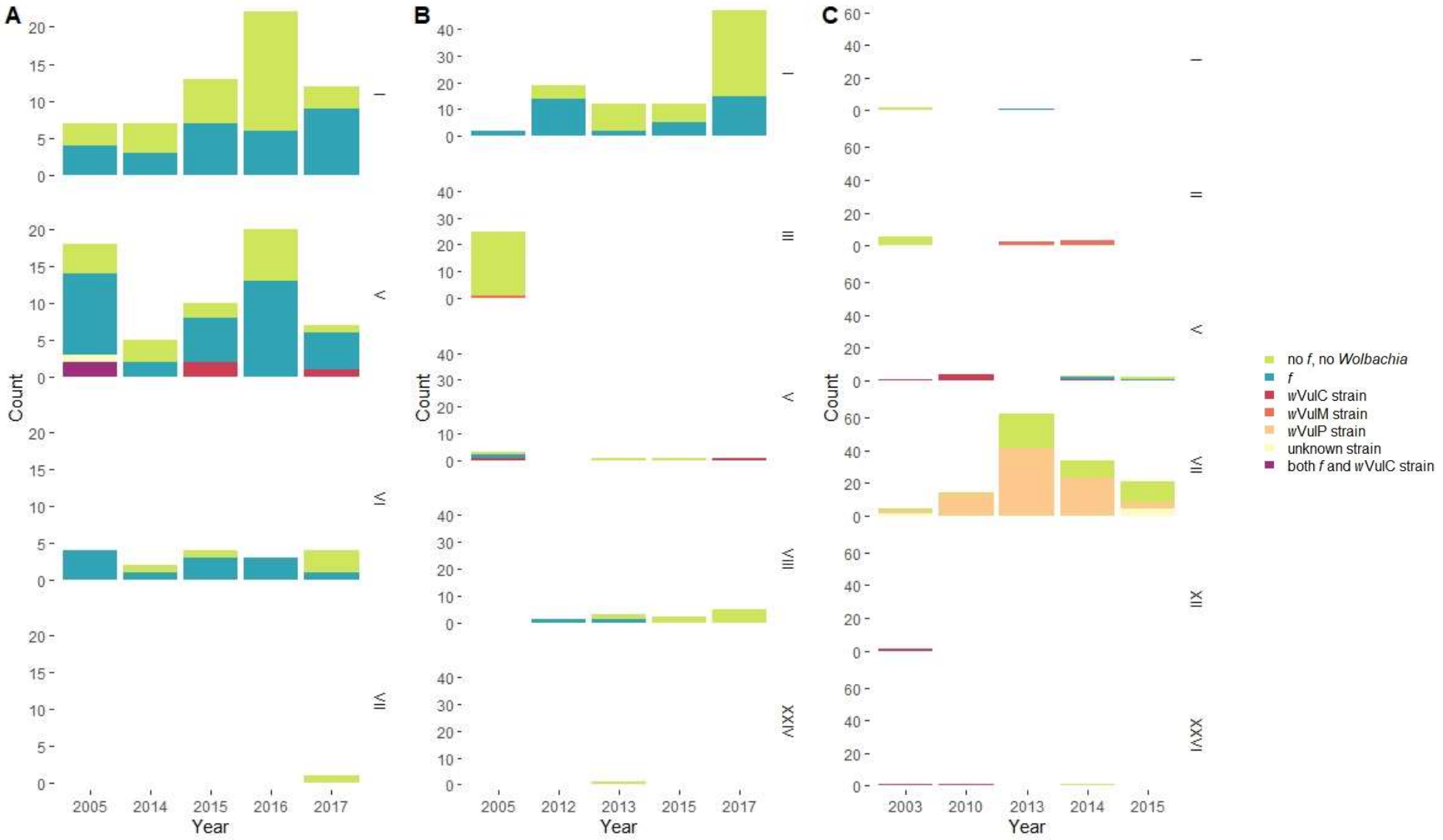
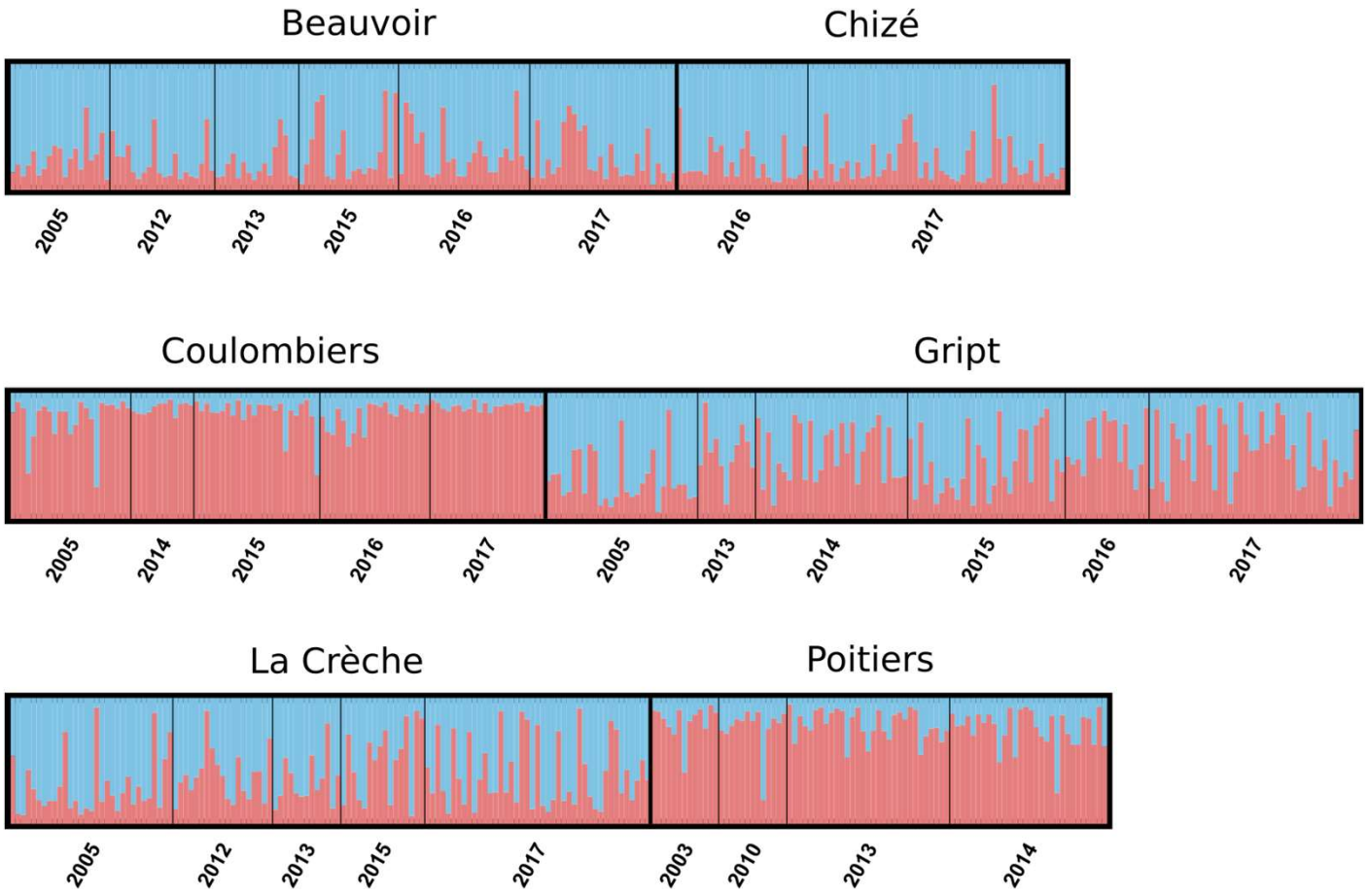

A
B
C
Count
Year
I
V
VI
VII
III
VIII
XXIV
II
XII
XXVI
2005
2014
2015
2016
2017
2012
2013
2003
2010
no f, no Wolbachia
f
wVulC strain
wVulM strain
wVulP strain
unknown strain
both f and wVulC strain

Figure 4


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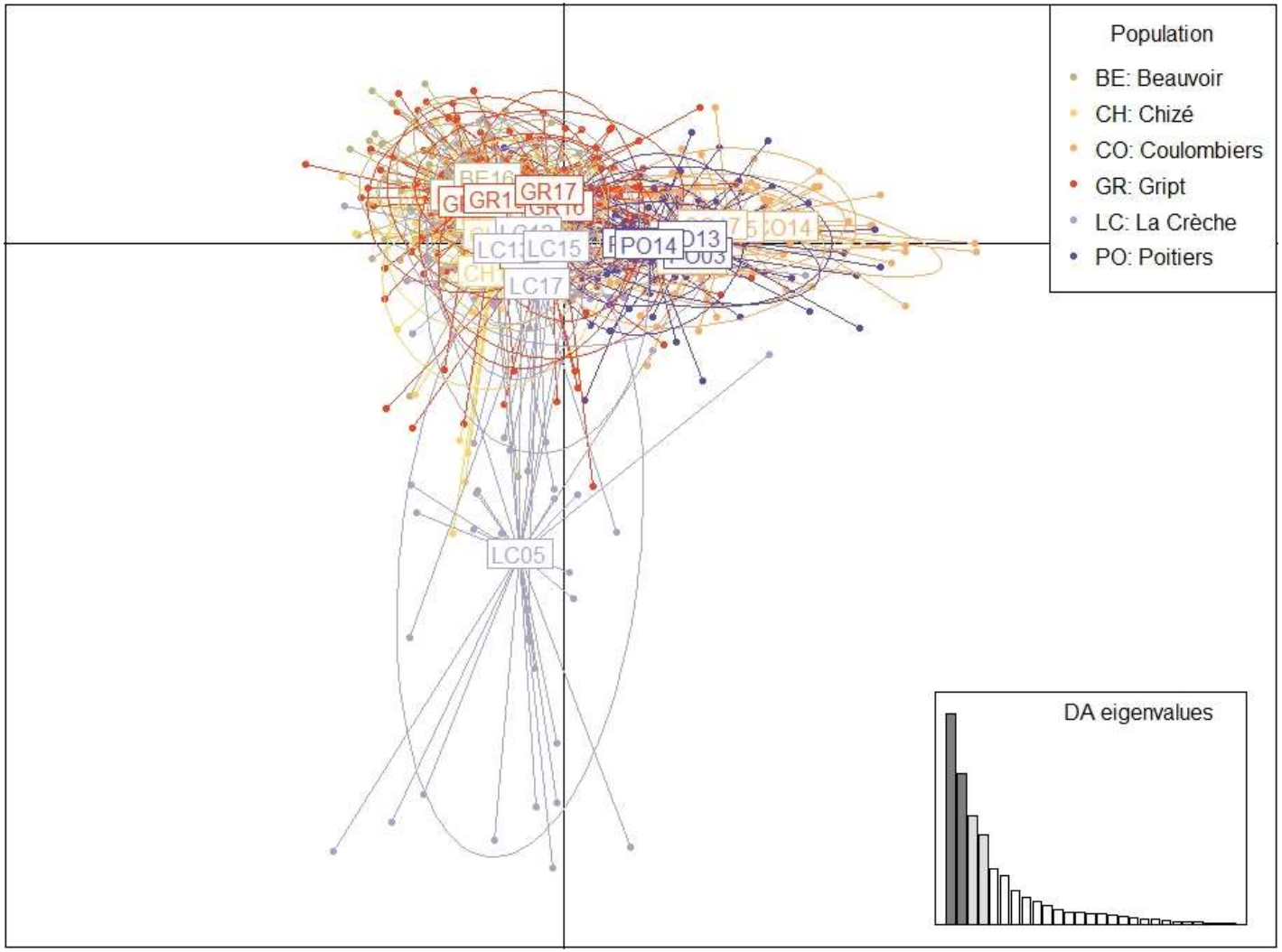
2017
Poitiers
2003
2010
2013
2014

Figure 5


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