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

Sylvine Durand[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

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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”.

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

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

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

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

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

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

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

## Results

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

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

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

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

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

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

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

## Discussion

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

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

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

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

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

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

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

493

494

Figure 1

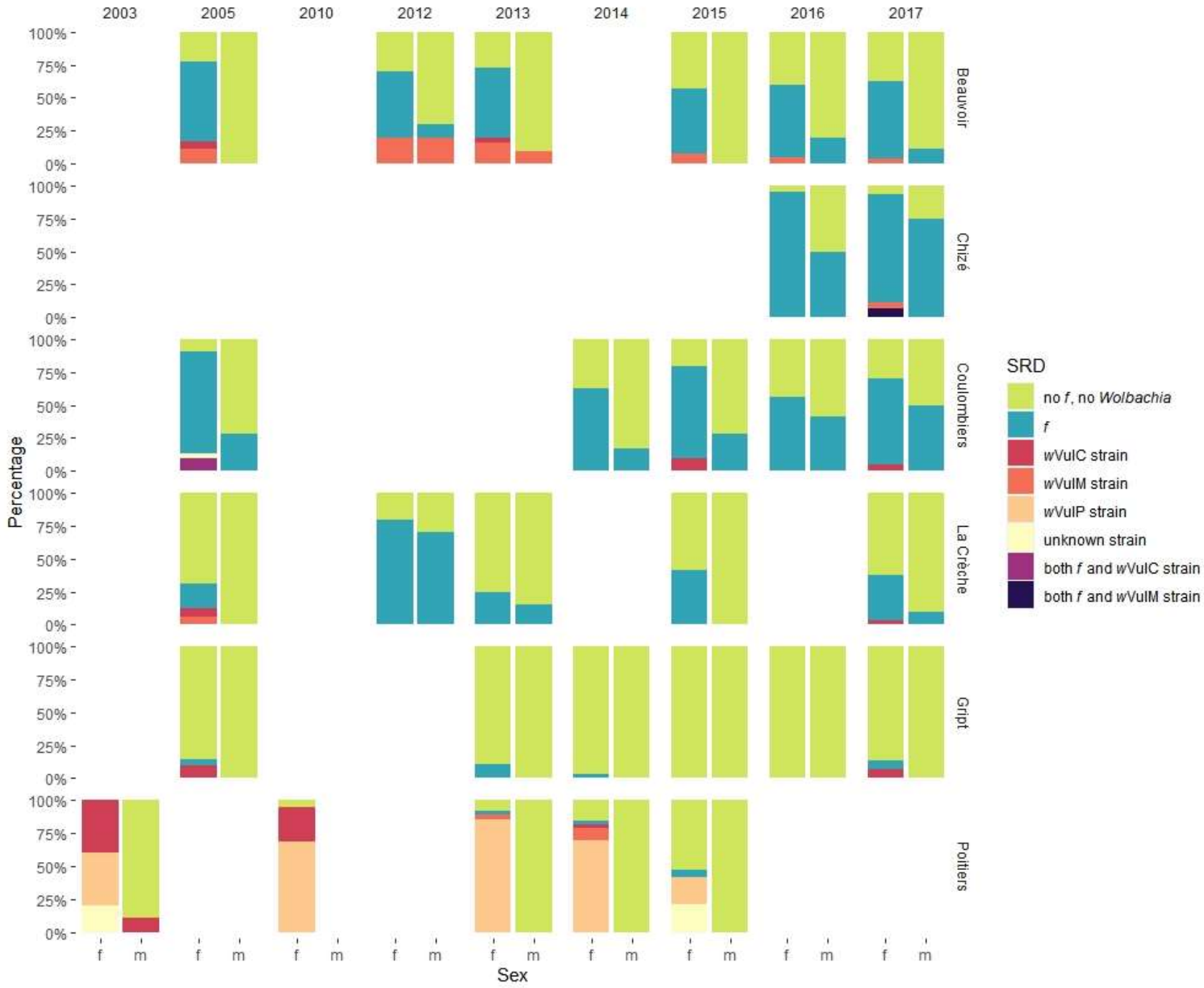
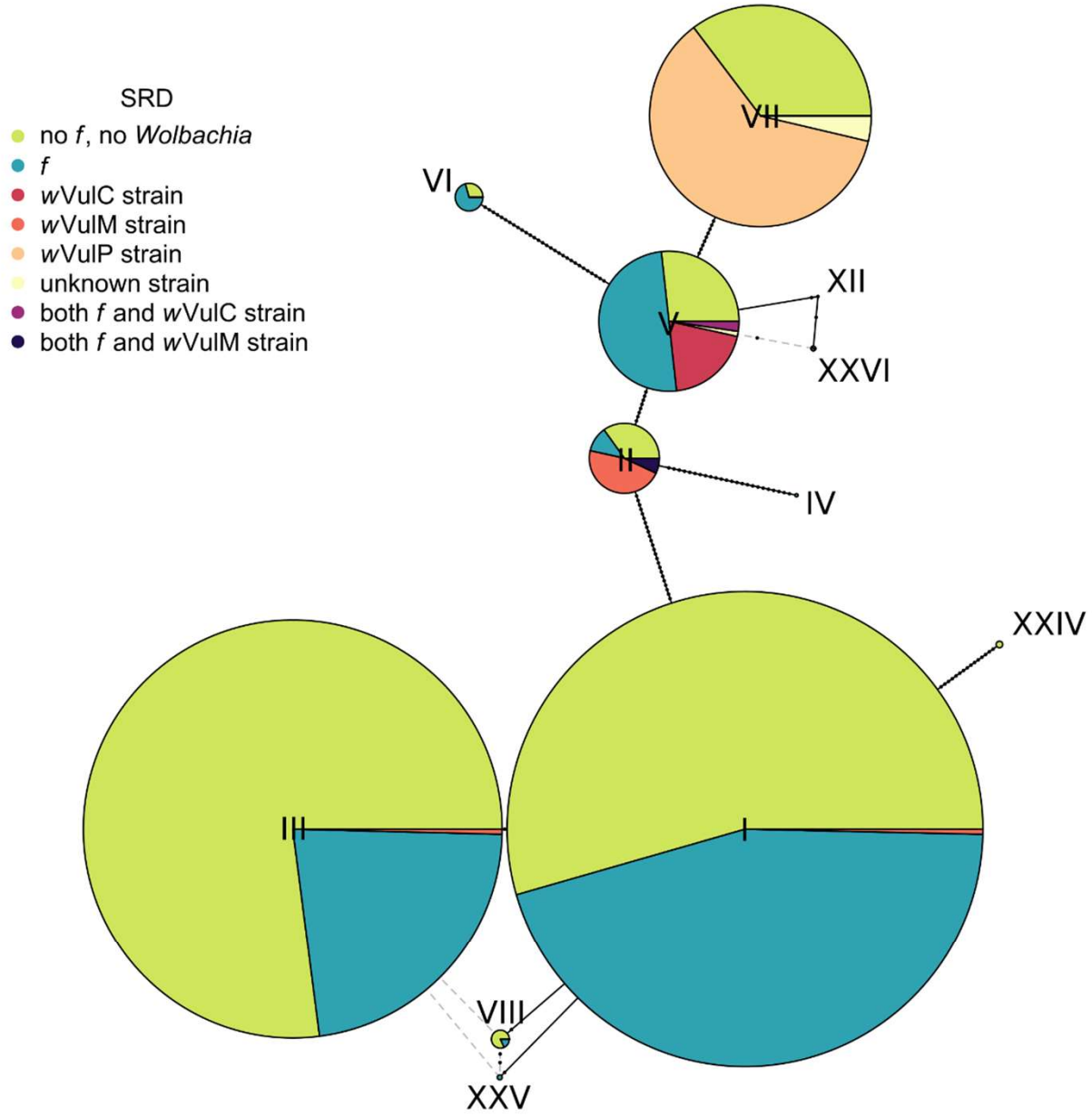

2003
2005
2010
2012
2013
2014
2015
2016
2017
100%
75%
50%
25%
0%
Percentage
Beauvoir
Chizé
Coulombiers
La Crèche
Gript
Poitiers
f
m
Sex
SRD
no f, no Wolbachia
f
wVulC strain
wVulM strain
wVulP strain
unknown strain
both f and wVulC strain
both f and wVulM strain

Figure 2


SRD
no f, no Wolbachia
f
wVulC strain
wVulM strain
wVulP strain
unknown strain
both f and wVulC strain
both f and wVulM strain
VII
VI
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XXVI
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VIII
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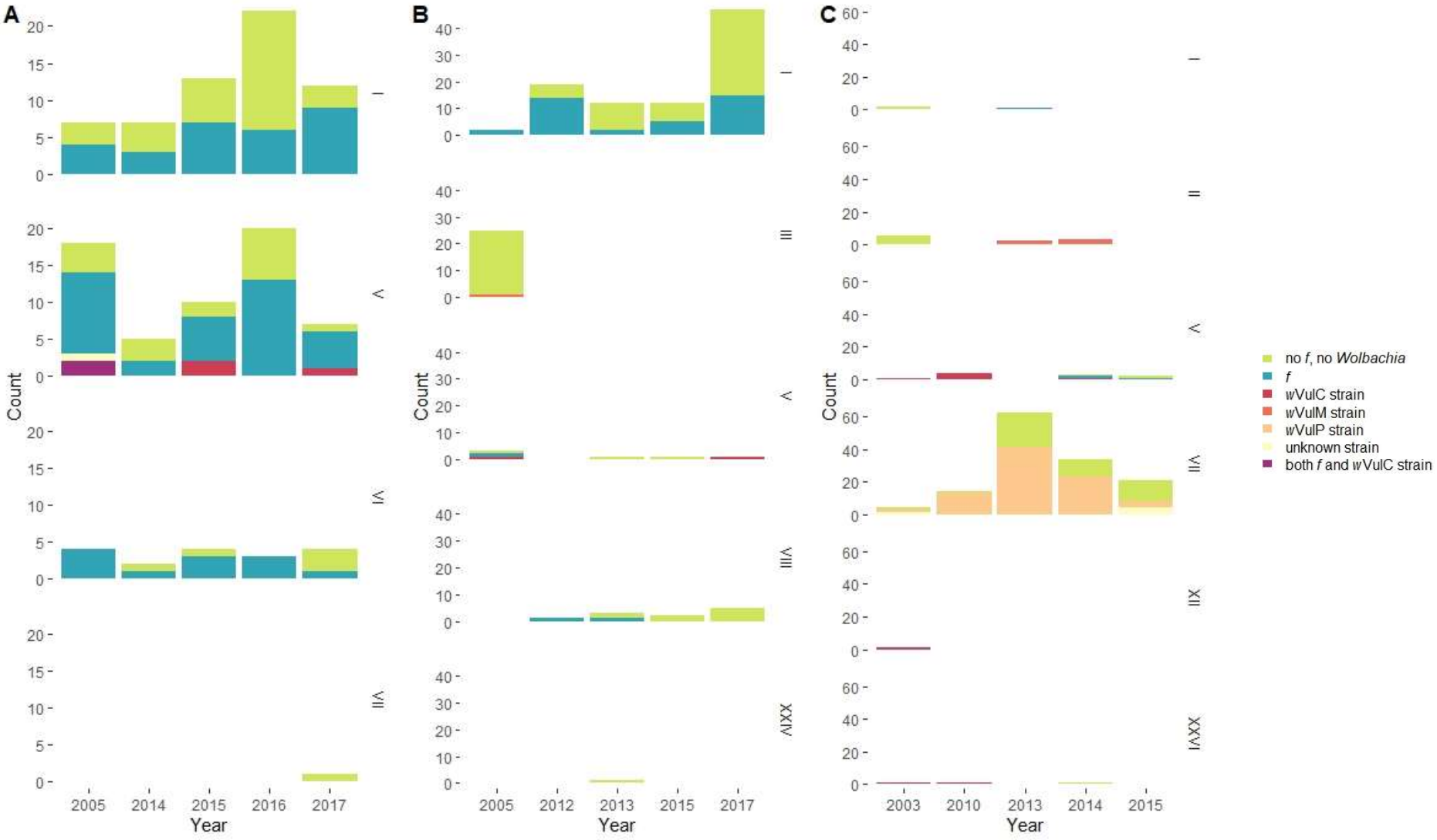
Figure 3


A
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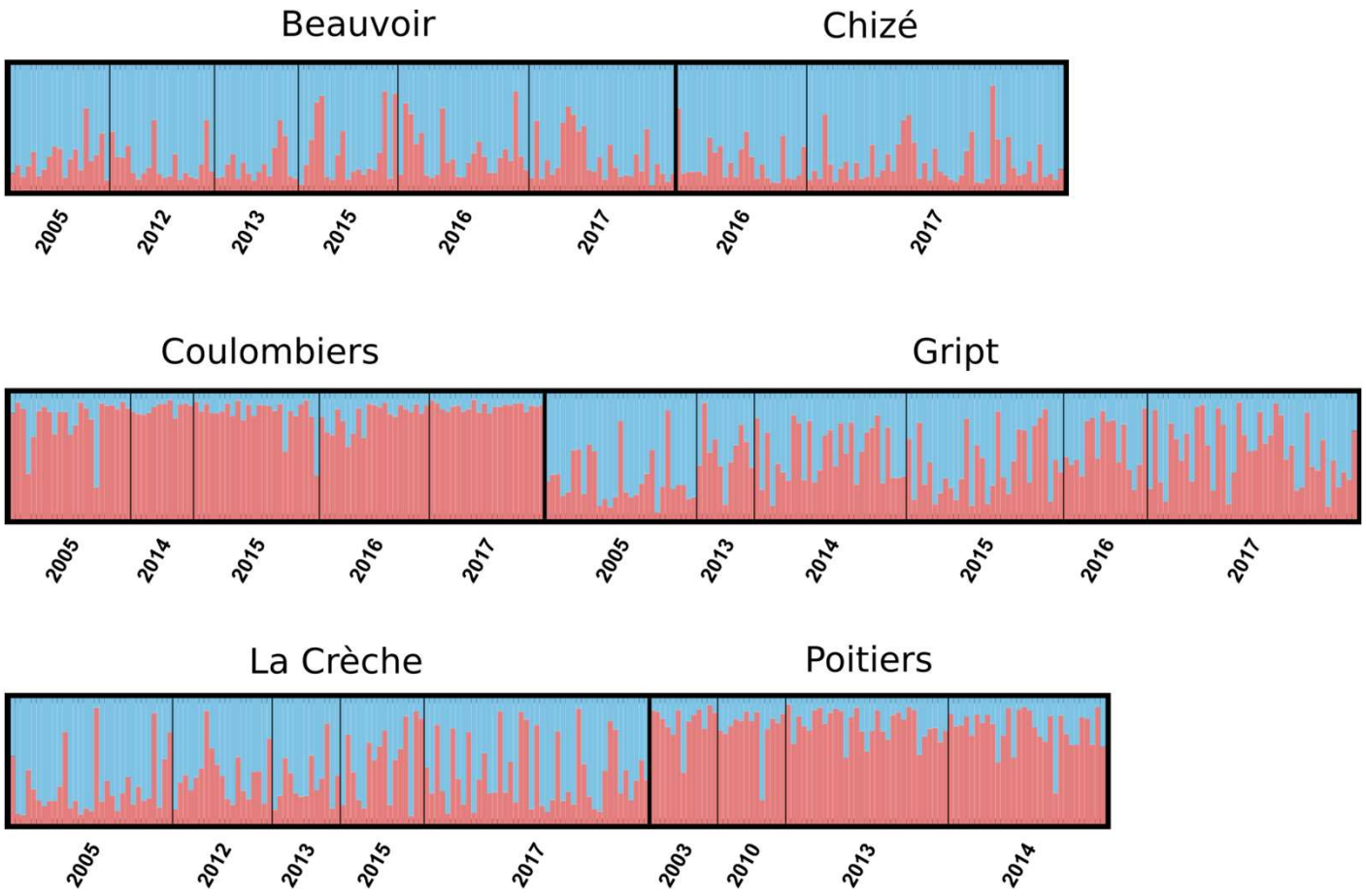
no f, no Wolbachia
f
wVulC strain
wVulM strain
wVulP strain
unknown strain
both f and wVulC strain

Figure 4


Beauvoir
Chizé
2005
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La Crèche
Poitiers
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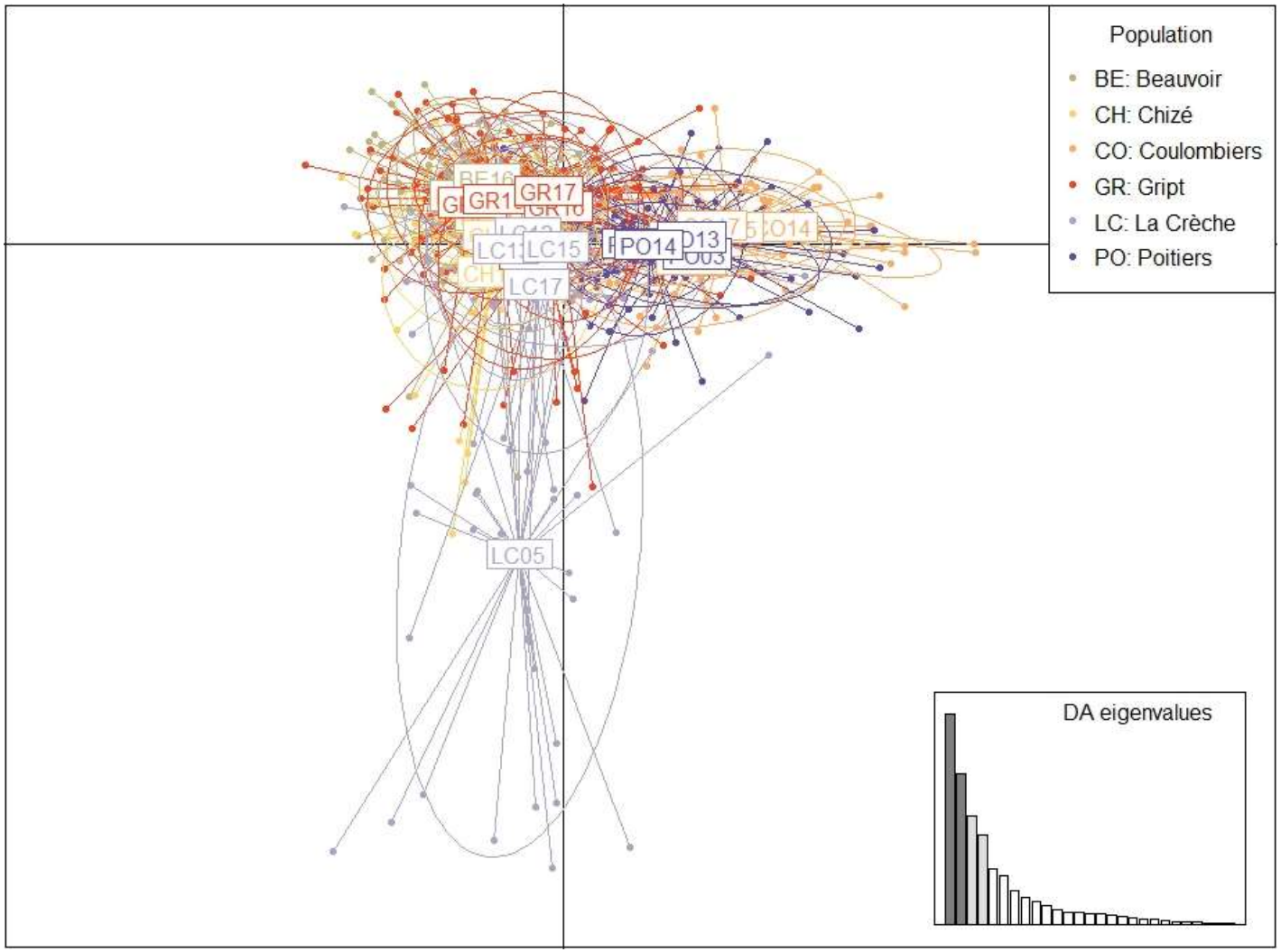
Figure 5


Population
BE: Beauvoir
CH: Chizé
CO: Coulombiers
GR: Gript
LC: La Crèche
PO: Poitiers
GR17
LC11
LC15
PO14
LC17
LC05
DA eigenvalues