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Wolbachia effects in natural populations of *Chorthippus parallelus* from the Pyrenean hybrid zone.

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Abstract

We evaluate for the first time the effect of *Wolbachia* infection, involving two different supergroups, on the structure and dynamics of the hybrid zone between two subspecies of *Chorthippus parallelus* (Orthoptera) in the Pyrenees. *Wolbachia* infection showed no effects on female fecundity or a slight increment in females infected by F supergroup although in the last case it has to be well-established. Cytoplasmic incompatibility (CI) is confirmed in crosses carried out in the field between individuals from a natural hybrid population. This CI, registered as the relative reduction in embryo production (s_h), was of $s_h = 0.355$ and $s_h = 0.286$ in unidirectional crosses involving B and F supergroups, respectively. CI also occurred in bidirectional crosses ($s_h = 0.147$) but with a weaker intensity. The transmission rates of the two *Wolbachia* strains (B and F) were estimated by the optimization of a theoretical model in order to reach the infection frequencies observed in certain population. To fit this scenario both supergroups should present transmission rates close to 1. Further, we have simulated the infection dynamics, and hence the capacity of *Wolbachia* to structure the population of the host insects, and to affect to reproduction and genetic introgression in the hybrid zone. This represents a first example of the influence of *Wolbachia* in an insect natural hybrid zone.

Key words: *Wolbachia*, *Chorthippus*, hybrid zones, cytoplasmic incompatibility.

Introduction

The Pyrenean hybrid zone of the meadow grasshopper *Chorthippus parallelus* has been the focus of detailed study, providing an important model system for the study of divergence and speciation (Hewitt, 1996; Butlin, 1998). This work has concluded that the hybrid zone was formed by secondary contact between two allopatric subspecies, *C. p. parallelus* (Cpp) and *C. p. erythropus* (Cpe) after the last Ice Age around 10,000 years ago (see Hewitt, 1993; 2012; Shuker *et al.*, 2005a). Cpp is widely distributed through most of continental Europe but, in the Iberian Peninsula, it is replaced by the endemic Cpe. The subspecies have diverged in their morphological, ethological, chromosomal and molecular traits (Butlin & Hewitt, 1985a, b; Gosálvez *et al.*, 1988; Ritchie, 1990; Cooper *et al.*, 1995; Lunt *et al.*, 1998; Ibrahim *et al.*, 2002; Bella *et al.*, 2007). Cpp and Cpe meet each other and hybridize in transverse valleys in the Pyrenees, such as the Vall d'Ossau-Valle de Tena. Such hybrid zones provide “natural laboratories” to study evolutionary agents, gene-flow barriers and genetic divergence (Barton & Hewitt, 1985; Hewitt, 1988).

Recently, the presence of *Wolbachia* has been described in *C. parallelus* populations (Dillon *et al.*, 2008; Martínez *et al.*, 2009; Zabal-Aguirre *et al.*, 2010; Bella *et al.*, 2010; Martínez-Rodríguez, 2013; Sarasa *et al.*, 2013). The genus *Wolbachia* (Hertig & Wolbach, 1924) includes a diverse group of alpha proteobacteria belonging to the order Rickettsiales. They have a strictly intracellular lifecycle and have been detected in a wide range of arthropods and nematodes (Jeyaparakash & Hoy, 2000; Hilgenboecker *et al.*, 2008). They infect the germinal cell line and are mainly transmitted by maternal inheritance (reviewed in Werren, 1997; Werren *et al.*, 2008; Saridaki & Bourtzis, 2010). The genus may be divided into different supergroups based on 16S ribosomal gene and other sequences (Werren *et al.*, 1995; Baldo *et al.*, 2006; Ros *et al.*, 2009; Zug *et al.*, 2012).

Strains belonging to the B and F supergroups have been found in *C. parallelus* (Zabal-Aguirre *et al.*, 2010; Martínez-Rodríguez *et al.*, 2013). As with many previously studied characters, the *Wolbachia* infection found in Cpp is different from that in Cpe populations (figure 1): Cpp populations, to the north of the Pyrenees, have a pattern of low-level infection, predominantly by the B supergroup; Cpe populations, to the south, have a high level of infection in which the F supergroup is widespread (Zabal-Aguirre *et al.*, 2010; Martínez-Rodríguez, 2013).

A third pattern has been found in a single population (Sallent de Gállego): it had an extremely high level of co-infection by the two types of *Wolbachia*. This population is located within a transect traversing the hybrid zone near Col de Portalet at the transition between the two other infection patterns (Zabal-Aguirre *et al.*, 2010).

Wolbachia is of considerable interest due to the reproductive manipulation of its hosts that allows it to spread and maintain itself in a population (reviewed in Werren *et al.*, 2008). These effects on its host can include parthenogenesis, feminization, male killing and sperm-egg cytoplasmic incompatibility (CI). Further, a mutualistic relationship between *Wolbachia* and some host species has also been described.

CI is the most common *Wolbachia*-induced disorder. It consists of a reduction in the viable descendants from crosses between infected males and uninfected females (unidirectional CI) or between individuals infected with different strains (bidirectional CI). Though the mechanism leading to CI remains unknown, there is an accepted model in which modified sperm, produced by *Wolbachia*-infected males, can be ‘rescued’ if they fertilize eggs from females infected with the suitable strain (reviewed in Serbus *et al.*, 2008). If there is no rescue of the infected sperm, embryonic development is disrupted. Asynchrony of male and female pronuclei at the initial stages of mitosis has been observed in the first embryonic divisions of several host species (Lassy & Karr, 1996; Reed & Werren, 1995;

Tram & Sullivan, 2002). Indirect support for this model has been obtained in the *Chorthippus parallelus* system (Sarasa *et al.*, 2013).

A final matter of interest is the potential implication of *Wolbachia*-induced effects on the evolution of their hosts (reviewed in Werren, 1997; and Werren *et al.*, 2008). It has been demonstrated that *Wolbachia*-induced CI is a major contributor to the reproductive isolation between several *Nasonia* species (Bordenstein & O'Hara, 2001). Other studies have shown the capacity of *Wolbachia*-induced effects to affect the genetic structure of an infected population. For example, a reduction of the mitochondrial DNA variability has been found in infected populations due to its shared maternal inheritance with *Wolbachia* (Turelli *et al.*, 1992; Dean *et al.*, 2003; Narita *et al.*, 2006). Our goal is to determine the role of *Wolbachia* in *C. parallelus* populations, with a special interest on its influence on the Pyrenean hybrid zone.

This study has been focused on the detection of *Wolbachia*-induced CI rather than other possible effects for the following reasons. Firstly, CI is the most common effect induced by this microorganism on its hosts (reviewed in Werren *et al.*, 2008); and secondly, there is no evidence of *Wolbachia*-induced feminization in this system despite extensive cytogenetic surveys of *Chorthippus parallelus* (Gosálvez *et al.*, 1988; Bella *et al.*, 1990; 1992; 2007; Serrano *et al.*, 1996). Besides, when a biased sex-ratio has been found it is toward male progeny (Bella *et al.*, 1992) and, therefore, male killing can be also dismissed. On the other hand, an interesting 'asymmetric homogamy' has been reported (Bella *et al.*, 1992) that could be explained by the existence of *Wolbachia*-induced CI (Zabal-Aguirre *et al.*, 2010).

Different studies have dealt with the theory of *Wolbachia* infection dynamics (Turelli, 1994; Frank, 1998; Vautrin *et al.*, 2007). They identify three key parameters: the level of CI induced by the particular strain of *Wolbachia*, the cost on their hosts and the rate

108 of the bacterial transmission from mothers to their progeny. Here, we have estimated these
109 parameters from crosses between individuals belonging to a natural population from within
110 the *Chorthippus parallelus* hybrid zone. Then, we have simulated the dynamics of the
111 infection in order to examine the ability of *Wolbachia* to spread and its possible
112 consequences in the hybrid zone of this grasshopper.

Material & Methods

Crossing design.

The individuals used for this study were collected in Portalet (Pyrenees: 42°48'03"N, 0°24'54"O; 1708m). This is a Pyrenean hybrid population located in the middle of the transect Vall d'Ossau-Valle de Tena (figure 1). Since *Wolbachia*-induced effects could be affecting gene flow in the hybrid zone, we have mainly studied hybrid individuals from a natural population. In a first approach, we tried to categorize in advance (in terms of the type of *Wolbachia* infection) the individuals to be used in the crossing programme. However, this requires mutilating the individuals to obtain the necessary DNA (probably affecting their fitness) and, besides, we did not find a good correlation between the gonads (necessary vehicle for *Wolbachia* vertical transmission) and the other body parts assayed (mainly legs; data not shown). For this reason, we concluded that it was not practicable to know *a priori* the infection status of the parental individuals used in the crosses. In previous studies, the Portalet population showed the four possible *Wolbachia* infection types: individuals infected by the F or B supergroups, BF coinfecting and uninfected individuals (Zabal-Aguirre *et al.*, 2010). Portalet is in the geographical centre of the hybrid zone; a principle factor in selecting this population for the study was the finding, from previous studies, that its infection frequencies were more even than in other populations. This maximized the probability of finding all the mating combinations with respect to *Wolbachia* infection when conducting a blind experiment like this.

We set up a total of 163 single crosses between individuals from Portalet starting in July and ending in September. In early summer, nymphs from this hybrid population were sampled, sexes segregated and the individuals kept in captivity until maturity. Each female was crossed with a single male in single-pair cages, which were kept outdoors in order to maintain the conditions of temperature, humidity, light, etc. as natural as possible.

Grasshoppers were fed with local grass. The number of effective crosses was reduced to 123 because of different causes beyond our control (parasitism, disease, etc.). For the sake of consistency, an exploratory data analysis was performed in order to exclude poor crosses with a reduced number of eggs and pods. Those crosses producing less than 20 eggs and 3 pods were discarded (these represented a 10% of the effective crosses and were distributed randomly among types of cross). Thus, a total of 110 single productive crosses were analyzed.

Pure *C. p. parallelus* and *C. p. erythropus* individuals were also sampled in Gabas (French Pyrenees; 42°53'60"N, 0°25'60"O; 1020m) and Escarrilla (Spanish Pyrenees; 42°43'54.1"N, 0°18'39.3"O; 1130m), respectively. These populations are from both ends of the hybrid zone in the transect Vall d'Ossau-Valle de Tena (figure 1). Some 40 crosses between pure individuals were set up as a control for genetic composition differences (10 of each type: ♀C_{pp} x C_{pp}♂, ♀C_{pp} x C_{pe}♂, ♀C_{pe} x C_{pp}♂ and ♀C_{pe} x C_{pe}♂). These yielded a total of 31 effective crosses (N = 7, N = 8, N = 6 and N = 10, respectively).

Crosses were performed in the field, at a site near Portalet and kindly provided by the Town Hall of Sallent de Gállego (Pyrenees; 42°45'57.5"N, 0°20'33.9"O; 1343m). *C. parallelus* is common in this area being the last place where these grasshoppers disappear in autumn. This choice of location therefore maximized the number of pods obtained by the end of the summer season.

Parents were dissected to obtain their gonads, which were conserved in absolute ethanol until DNA was extracted. Pods were maintained in moist sand and diapause was simulated under laboratory conditions (Kelly-Stebbins & Hewitt, 1972; Tregenza *et al.*, 2000). After breaking the diapause by leaving the pods at room temperature, development was reactivated and the eggs from each pod were separated and dissected to check the presence/absence of embryos. The number of pods, eggs and embryos was recorded for

each cross. Averages of hybrid and pure crosses were compared by parametric (Student's t-test, ANOVA) or non-parametric test (Mann-Whitney U-test, Kruskal-Wallis test) depending on the distributions of the analyzed variable, to detect any effects related to infection or provenance.

Wolbachia detection.

DNA of parental individuals was extracted from their gonads and analyzed in a nested PCR following Zabal-Aguirre *et al.* (2010). This method allows distinction between B and F *Wolbachia* supergroups and characterization of the *Wolbachia* infection of the parental individuals (uninfected, B infected, F infected and coinfecting). Each cross was classified in one of the 16 possible mating types according to parental infecting strain (table 1).

Wolbachia infection effects on females.

Compatible crosses do not suffer *Wolbachia*-induced CI. Therefore, differences in the number of embryos produced by infected and uninfected females crossed to uninfected males (compatible crosses, table 1) might be attributed to *Wolbachia*-induced effects on female fitness. Different number of embryos would be due to differences in the number of eggs produced (quantity of ova) and/or in the proportion of embryos obtained from those eggs (quality of ova). We have compared both variables among the different female infection types.

Wolbachia infection effects on female reproduction can be quantified as the ratio of embryos produced relative to that observed in uninfected females from compatible crosses. These estimates were equated to the relative fecundity values (F) described in Turelli's model (1994) for each female infection type. The cost of harbouring *Wolbachia* (s_f) on female fecundity could be quantified as $1-F$.

Wolbachia-induced cytoplasmic incompatibility.

Embryo proportions (number of embryos / number of eggs) were calculated for each cross. Crosses were grouped in 3 possible compatibility types: (a) *Wolbachia*-compatible crosses, where infection should not influence grasshopper reproduction; (b) crosses susceptible to unidirectional CI, when infected males are mated to uninfected females; and (c) those crosses potentially suffering bidirectional CI, where both parental individuals are infected by different *Wolbachia* supergroups (table 1).

Further, the CI level associated with the different *Wolbachia*-incompatible categories (uni- or bi-directional) was estimated from the ratio of their proportion of embryos relative to that observed in *Wolbachia*-compatible crosses. These ratios have been equated to the hatching rates (H) described in Turelli's model (1994). Accordingly, H_{UNI} (unidirectional hatching rate) and H_{BI} (bidirectional hatching rate) have been estimated. The cost of CI (s_h) for susceptible crosses could be calculated as $1-H$.

Simulations of infection dynamics.

Because the low numbers obtained in some types of crosses, we have introduced uncertainty in F and/or H variables to simulate the infection dynamics. We used OpenBUGS software (Lunn *et al.*, 2009) to calculate posterior distributions. It includes an 'expert system' which determines an appropriate MCMC (Markov chain Monte Carlo) scheme, based on the Gibbs sampler, for analyzing the specified model. A total of 100,000 iterations were performed, the first 1000 being discarded as burn-in and 99,000 values from which the posterior distribution and credible interval of each variable could be estimated.

The number of embryos was modelled as normally distributed. Fecundity distributions (F) of the different infected female types (F_o , F_b , F_f , F_{bf}) were calculated as the

ratio of the number of embryos relative to uninfected females (therefore for uninfected females, $F_0=1$). Embryo proportions were modelled as beta distributed. Hatching rate distributions (H) of the different cross types (H_0 , H_{UNIB} , H_{UNIF} , H_{BI}) were obtained as the ratio of the number of embryos relative to compatible crosses (consequently for compatible crosses, $H_0=1$). In all cases, uninformative priors were used.

We evaluated the ability of *Wolbachia* to maintain infections or spread in a population using the model of Turelli (1994) with minor modifications (see supplementary information, table S1) and the parameter values estimated from our experiments: cytoplasmic incompatibility ($s_h=1-H$) and *Wolbachia* infection effects on female fecundity ($s_f=1-F$). We tested the capacity of each strain to reach the infection levels observed in natural populations, assuming no horizontal or paternal transmission and no migration. The model results were compared with the infection rates at the Sallent de Gállego population. *Wolbachia* is extremely common in this location, reaching the highest coinfection levels observed to date: 4.4% of uninfected, 22.8% B-infected, 4.7% of F-infected and 68.1% of coinfecting individuals (Zabal-Aguirre *et al.* 2010). According to Frank (1998) and given the high coinfection frequency observed, it seems reasonable to think that Sallent de Gállego population would be close to the infection frequency expected at equilibrium.

We performed an optimization to fit vertical transmission rates ($1-\mu$, where μ is the proportion of uninfected ova produced by an infected female) in the infection-dynamics model, assuming that Sallent de Gállego is at equilibrium with respect to *Wolbachia* infection. Values between 0 and 1 would support the equilibrium considering only *Wolbachia*-induced effects. We then analyzed the dynamics of the infection in different scenarios in order to check the ability of *Wolbachia* strains to spread (i) in an uninfected population or (ii) in a population that was previously infected by a different strain. From these simulations, we have interpreted the relationship between strains and their ability to

238 induce selective “sweeps” that could have structured the genetic diversity in the host
239 populations.

240 Simulations were performed using the R 2.10.1 software (R Development Core
241 Team, 2011); the *optim* function included in the *stats* package of this software was used for
242 the optimization.

Results

A total of 110 informative crosses between individuals captured from the Portalet hybrid population produced 623 pods and 4792 eggs. After analyzing the infective status of the parents involved, 86 crosses were classified as not susceptible to CI, 16 crosses as susceptible to unidirectional incompatibility and 8 as susceptible to bidirectional incompatibility (table 2). The 31 informative crosses between individuals captured from pure populations produced 149 pods and 1361 eggs. After analyzing the parental infection, 23 crosses were classified as not susceptible to CI, 4 crosses as susceptible to unidirectional incompatibility and 4 as candidates for bidirectional incompatibility (table 3).

There were significant differences between hybrid and pure crosses in the average number of pods per cross (5.66 and 4.81 pods, respectively; Mann-Whitney $U = 1084$, $n_1 = 110$, $n_2 = 31$, $P = 0.001$) but a similar mean number of eggs per cross (43.56 and 43.90 eggs, respectively; $t_{139} = 0.141$, $P = 0.888$). This result is explained by hybrid females having smaller pod sizes than pure females (7.69 and 9.13 eggs per pod, respectively; Mann-Whitney $U = 624$, $n_1 = 110$, $n_2 = 31$, $P < 0.001$). There was no significant difference between females from the pure Cpp and Cpe populations in the number of pods, pod sizes or number of eggs (number of pods per cross, Mann-Whitney $U = 92$, $n_1 = 15$, $n_2 = 16$, $P = 0.281$; pod size, Mann-Whitney $U = 115.5$, $n_1 = 15$, $n_2 = 16$, $P = 0.861$; number of eggs, $t_{29} = 1.152$, $P = 0.259$). Similar results were found when those comparisons between hybrid and pure crosses were made within each *Wolbachia*-compatibility category (analyses done but not shown).

Wolbachia effects on female reproduction.

Within *Wolbachia*-compatible crosses, hybrid females showed no statistically significant differences between infection-status categories in the average of egg number and embryo

proportions when crossed to uninfected males (table 4). Therefore, no effects on embryo production were detected due to the infection with this analysis. As shown in table 2, uninfected females produced an average of 33.4 embryos per cross and B-infected females had a very similar production (33.6 embryos per cross), whereas F-infected and coinfecting females showed slightly higher averages (38.4 and 36.8 embryos per cross, respectively).

Similar results were found when comparing crosses involving pure females in *Wolbachia*-compatible crosses (table 4): there were no significant differences between uninfected and infected Cpp females in the number of eggs or embryo proportions; the same results were obtained when comparing uninfected and infected Cpe females. Moreover, there were no differences between Cpp and Cpe uninfected females nor between Cpp and Cpe infected females. When pure females were pooled irrespective of their subspecies, there were still no significant differences in the number of eggs or embryo proportion between uninfected and infected females from compatible crosses. Therefore, no effects on embryo production attributable to *Wolbachia* were found in pure genotype females either.

Finally, there were no significant differences between hybrid and pure uninfected females nor between pure and hybrid infected females from compatible crosses (table 4). When pure and hybrid females were pooled, no significant differences were found between uninfected and infected females.

Wolbachia-induced cytoplasmic incompatibility.

Crosses between hybrid individuals showed different embryo proportions depending on their *Wolbachia*-compatibility category (table 2). Compatible hybrid crosses produced an average of 0.772 embryos per egg; this was significantly reduced in hybrid crosses susceptible to unidirectional CI (uninfected females crossed to infected males with B and/or F strains) which showed a mean of 0.521. This is an average reduction of 32.5% in the

proportion of embryos per egg. Differences between compatible and unidirectional crosses were statistically well supported in hybrids (table 5) and, consequently, unidirectional CI follows from these data.

Each *Wolbachia* supergroup was also analyzed separately. For uninfected females crossed to males harbouring the B supergroup an average reduction of 33% in embryo proportions was observed (table 2). The unidirectional CI induced by the B supergroup was statistically well supported by comparisons with the reciprocal crosses (table 5). Crosses between uninfected females and F-infected males showed an average reduction of 23.7% relative to compatible crosses (table 2). Nevertheless, the unidirectional CI induced by the F supergroup was not statistically well supported (table 5); there is however low power to detect such an effect, as demonstrated by the absence of significant differences in the proportions of embryos per egg between the two types of unidirectional CI crosses (table 5).

Crosses between hybrid individuals harbouring different *Wolbachia* supergroup (crosses susceptible to bidirectional CI) showed an average of 0.654 embryos per egg and this represents a 15.3% reduction relative to compatible hybrid crosses (table 2). However, there were not statistically significant differences when comparing bidirectional crosses either with compatible crosses, or with unidirectional CI susceptible crosses (table 5).

Crosses between grasshoppers from populations beyond the hybrid zone (pure genotype individuals), showed different embryo proportions depending on *Wolbachia*-compatibility category (table 3). It was impossible to compare unidirectional CI ability between *Wolbachia* supergroups as all of these crosses were established by chance between uninfected females and males harbouring F strains. In this case, pure crosses susceptible to unidirectional CI (an average of 0.779 embryos per egg) showed a low reduction (6.2%) in embryo proportion relative to compatible pure crosses (an average of 0.830 embryos per

egg). This difference was not statistically significant (table 5). However, crosses between pure individuals harbouring different infections (bidirectional CI) showed on average 0.369 embryos per egg which is a statistically significant reduction of 55.5% relative to compatible pure crosses (table 5).

We have also checked the influence on embryo proportions due to the grasshopper genotype (Cpp, Cpe or hybrid). Within pure compatible crosses (free from *Wolbachia* induced CI) there were no significant differences between Cpp females crossed to Cpp or Cpe males (table 5). Similar results were found when comparing between Cpe females crossed to Cpe or Cpp males (table 5). Thus, compatible crosses were pooled according to female origin, and no differences in embryo proportions were found between Cpp and Cpe females after pooling (table 5).

We have tested for differences in embryo proportions between hybrid and pure crosses within each of the compatibility categories (compatible, unidirectional and bidirectional crosses). Comparisons showed no differences in any of the classes (table 5). These results allowed us to pool the data regarding the potential compatibility-types and ignoring the parental population origin. The number of analyzable crosses thus increased up to 109 compatible, 20 unidirectional incompatible and 12 bidirectional incompatible. Results were mostly congruent to that described above. As with unpooled data, B-induced unidirectional CI was statistically well supported but not F-induced unidirectional CI, in spite of both kind of crosses showing similar embryo proportions (table 5). However, bidirectional incompatible crosses showed statistically significant differences from compatible crosses after pooling (table 5) providing evidence of bidirectional incompatibility induced by different *Wolbachia* strains. Finally, no differences in pooled embryo proportion were found between bidirectional incompatible crosses and unidirectional incompatible crosses (table 5).

343
344 *Wolbachia-infection dynamics in the Chorthippus parallelus hybrid zone.*

345 The statistical analysis generated posterior distributions for the parameters F and H from
346 hybrid crosses (Sallent de Gállego is a hybrid population). Averages and credible intervals
347 were estimated from those posterior distributions (table 6).

348 The significance of these parameter values was investigated using the model of
349 transmission dynamics. Assuming equal female fecundities ($F_o = F_b = F_f = F_{bf} = 1$), we
350 obtained a probability of equilibrium equal to 100% with the required vertical transmission
351 rates of 0.984 for B-supergroup infected females ($\mu_b = 0.016$, credible interval 95% = 0.009-
352 0.025) and 0.969 for the F-supergroup ($\mu_f = 0.031$, credible interval 95% = 0.016-0.047). If
353 uncertainty is introduced into the model, the probability of equilibrium falls to 58.2% with
354 vertical transmission rates of 0.976 for B-supergroup infected females ($\mu_b = 0.024$, credible
355 interval 95% = 0.001-0.055) and 0.927 for the F-supergroup ($\mu_f = 0.073$, credible interval
356 95% = 0.016-0.139).

357 Assuming similar fecundity for all female types, and the averages estimated for the
358 other parameters (table 7), newly B-infected individuals would have to have a frequency
359 over 4.9% in an uninfected population in order for this strain to spread. Above this
360 threshold, B supergroup would go on to infect 96.8% of the individuals in 318 generations
361 approximately (figure 2a). The F strain threshold is estimated at 12.2% for invading a
362 *Wolbachia*-free population: above that threshold F-infection would reach a stable
363 equilibrium of 91.1% infected individuals after around 220 generations (figure 2b).
364 Furthermore, in a mature B-infected population 26.9% of the individuals would have to
365 acquire *ex novo* F strains in order for this strain to spread (figure 2c); whilst in an established
366 F-infected population that percentage would have to be higher than 11.3% to permit B
367 strains to spread (figure 2d). In these two last scenarios, coinfection would become the main

infection class after reaching stable equilibrium in 450 and 390 generations for F and B superinfection, respectively. Below these thresholds, the newly incorporated strains would be lost from the population in every case.

When uncertainty about the fecundity parameters was introduced into the simulation, averages and credible intervals for the parameters had to be calculated over those replicates that achieved equilibrium (58.2% of the total, table 7). An initial frequency of 10% would be enough for B strain to spread into an uninfected population; going on to infect the 95.1% of the individuals after 218 generations (figure 3a). The F-infection threshold falls to 0.1% in a *Wolbachia*-free population; the infection would go on to a final frequency of 80% F-infected individuals after 280 generations (figure 3b). For superinfection scenarios, the infection-thresholds and time required in to reach the equilibrium are much lower. Both strains would spread into a population in which the alternate strain was established, from a level of only 0.1% infected individuals. Coinfected individuals would be the frequent category at equilibrium, which would be achieved in 90 and 129 generations for F and B superinfection, respectively (figure 3c and 3d).

Discussion

The methodology used in the present study was successful in characterizing crosses. The genetic background of the host seems to have no effects in the variables measured to characterise the effects of *Wolbachia*. The numbers of eggs per cross was similar to that found in previous studies (Bella *et al.*, 1992; Reinhardt *et al.*, 1999). Crosses between hybrid individuals from Portalet produced a similar number of eggs per cross to pure individuals from Gabas or Escarrilla (pure Cpp or Cpe respectively). Portalet did have a higher number of pods per cross, but this cannot be considered an effect of heterosis, given that it is offset by smaller pod sizes (eggs per pod) so that the total numbers of eggs per cross were similar to the pure populations. No differences were found between crosses involving pure individuals captured in Gabas or Escarrilla populations. Both of these pure populations are located at the foot of the mountains and would share fairly similar conditions of temperature, duration of reproductive period, snow pattern, etc. Portalet is located at the top of the mountain pass, at much higher altitude than pure populations (1708m vs. 1020-1130m). These differences in local environmental conditions could have induced selection for the different distribution of eggs among pods at Portalet.

There was no evidence of negative heterosis (reduced number of descendants in the hybrids), neither in crosses between pure populations nor in naturally occurring wild hybrids. This observation fits with previous studies, which found no reduction of F1 offspring sizes (Hewitt *et al.*, 1987; Bella *et al.*, 1990; Virdee & Hewitt, 1992; Shuker *et al.*, 2005b); testicular dysfunction and meiotic abnormalities did occur in hybrid F1 males (Shuker *et al.*, 2005b), albeit only in crosses between distant populations.

The effect of *Wolbachia* on measures of hybrid female fitness remains uncertain. Comparisons between compatible crosses, free of *Wolbachia*-induced CI, showed no effects, or a slight increment on the fecundity of F-infected and coinfecting females (table 6;

see supplementary information for posterior distributions, figure S2). It has been proposed that, during *Wolbachia* and host coevolution, selection favours those strains that increase the production of infected progeny from infected mothers (Turelli, 1994; Vautrin *et al.*, 2007). Some studies have shown the evolution from a parasitic towards a more mutualistic association between *Wolbachia* and their hosts, as the theory predicts (Weeks *et al.*, 2007). Here, the high variability that we have found in F strains (Zabal-Aguirre *et al.*, 2010) suggests an ancient origin of infection and thus, a long time to evolve towards such mutualism in this grasshopper. An increment in host recombination rate has recently been proposed as another *Wolbachia*-induced effect in *C. parallelus* (Sarasa *et al.*, 2013). However, our evidence for any increment in fecundity is equivocal, due to the low number of crosses obtained in this study; more effort would be required to discern any *Wolbachia* effects on the females.

There is, however, convincing evidence that *Wolbachia* induces CI in this hybrid population. The observed incompatibility is unlikely to have been produced by other endosymbionts like *Spiroplasma* or *Cardinium*, in the light of studies by Martínez-Rodríguez *et al.* (2013), which looked directly for such effects. Unidirectional CI was statistically well supported for bacterial B supergroup but not for F supergroup in spite of both showing similar CI levels. This result is due to the large variance in embryo proportions found among unidirectional crosses where F strains are involved. When the analysis of this type of crosses is based on the beta distribution and, therefore, variation among crosses is not considered, hatching rate credible interval (table 6) and posterior distribution of embryo proportion (see supplementary information, figures S3 and S4) showed significant differences with respect to compatible crosses. This high variance might be due to the genetic variability of the F strains; four different isolates belonging to the F supergroup were described in *C. parallelus* (Zabal-Aguirre *et al.*, 2010). Studies carried out on the

Nasonia-complex have shown the existence of insect genotype-bacterial interactions, with incompatibility levels being dependent on bacterial strain (Bordenstein *et al.*, 2003). It is expected that selection on host genomes would lead to reduced CI-levels (Turelli, 1994). The different outcomes of such selection, or different time-courses could explain why the difference in B and F strain effects detected in this study. More experiments would be required to obtain more convincing evidence of differences among strains within F supergroup in this grasshopper.

Wolbachia also induces bidirectional CI but the reduction in embryo production is lower than that found in unidirectional crosses. This bidirectional incompatibility involved a statistically robust reduction in embryo proportions in pure crosses, and when pure and hybrid crosses were pooled. Such effects were not well supported in the case of hybrid crosses alone. Again, the weaker evidence could be due to the variance among crosses, and the large number of combinations that have to be considered: besides variability within the F supergroup (BxF₁, BxF₂, BxF₃, BxF₄), there are potentially 4 different bidirectional crosses (BxF, FxB, BxBF and BFxF). A much larger sized experiment would be required to study each sort of cross separately.

It appears that *Wolbachia* generally shows weaker CI-levels in natural populations than under laboratory conditions (Hoffmann *et al.*, 1998). This difference could be associated with diapause stage (Perrot-Minnot *et al.*, 1996), high temperatures (Stevens, 1989), natural antibiotics (Stevens & Wicklow, 1992) and/or male age (Hoffman *et al.*, 1986; Bressac & Rousset, 1993); all of which have been proposed to influence *Wolbachia* biology. Besides, although it has been postulated that most of the mortality occurs during the embryonic stage (Bordenstein *et al.*, 2003; see Serbus *et al.*, 2008 for a review) we can argue that, in our case, we might have detected higher mortality in incompatible crosses if we had studied the offspring after hatching or at the adult stage. Nevertheless, the CI levels

detected in this study are non-negligible and sufficient for *Wolbachia* to have a substantial effect on the dynamics of the hybrid zone.

Both strains showed CI levels high enough to drive the spread of *Wolbachia* into the population and to maintain the infection, as long as there were consistently high vertical transmission rates. Such high and consistent rates are plausible: there are many cases described in the literature where *Wolbachia* shows an almost perfect vertical transmission in natural populations, in studies which range across a variety of species (Hoffmann *et al.*, 1996; Rasgon and Scott, 2003; Charlat *et al.*, 2004; Sinkins, 2004; Narita *et al.*, 2007).

When the model takes into account the uncertainty over the fecundity, the required vertical transmission rates (table 7) and the minimum infection thresholds needed to establish *Wolbachia* in the population in the first place (figures 2 and 3) are reduced. However, the probability of reaching the equilibrium infection-frequencies observed in Sallent de Gállego is also reduced, although still pretty high ($P = 0.582$). In the future, it would be desirable to obtain more accurate estimates of the *Wolbachia* effect on female fecundity; these data would allow a more precise modelling of the infection dynamics in *C. parallelus*.

Given the detected CI levels and the infection frequencies observed in the wild populations (Zabal-Aguirre *et al.*, 2010), *Wolbachia* does not seem to have caused rapid speciation, nor is it the only reproductive barrier affecting the hybrid zone. However, the inferred infection dynamics suggest that the *C. parallelus* populations have undergone *Wolbachia* sweeps (rapid spread of one or both strains), which would have induced genetic structure in the infected populations (figure 2 and 3).

This supposition is reinforced by previous studies in other species, which show a reduction in mitochondrial DNA variation in infected populations, which has been attributed to *Wolbachia* infection sweeps (Turelli *et al.*, 1992; Dean *et al.*, 2003;

Raychoudhury *et al.*, 2010). Such an event may have occurred in Sallent de Gállego where a coinfection maximum is located; this, in turn, could constitute a barrier to the introgression of genetic markers interacting with the cytoplasm. Intriguingly, there is evidence of an incompatibility between Cpe cytoplasmic factors and a Cpp cytogenetic marker (Virdee & Hewitt, 1992). In addition, previous studies from our laboratory have described the coincidence between the *Wolbachia* coinfection peak, and the shift from 100% to 0% in the frequency of this cytogenetic marker in Sallent de Gállego (Gosálvez *et al.*, 1988; Serrano *et al.*, 1996; Zabal-Aguirre *et al.*, 2010). This pattern could be an example of an infection sweep affecting differentiation across the hybrid zone. The interaction of the two grasshopper taxa may also, in turn, affect the bacteria; for example, recombinant strains of *Wolbachia* have been found in the same area of the hybrid zone (Martínez-Rodríguez, 2013; Sarasa, 2013).

Our results show that *Wolbachia* plays a significant part in the dynamics of the *Chorthippus parallelus* hybrid zone, and suggest that infection status should be considered in reinterpreting previous analyses of the zone, and in new experiments involving experimental crosses in *C. parallelus*. Interactions involving *Wolbachia* may also be present in other insect hybrid zones, and appropriate studies should be conducted to detect their role. More studies are also required in *C. parallelus* itself, to elucidate more accurately the role of the different strains within supergroups, to determine empirical transmission rates of infection and to correlate mitochondrial variability with *Wolbachia* infection through this hybrid zone.

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The authors declare no conflict of interest.

Supplementary information is available at JEB's website.

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Legends to Figures

Figure 1.- Map of *Wolbachia* infection patterns found in the field. (A) Populations outside the hybrid zone showing the Northern pattern (low-level infection / B-supergroup in the majority) characteristic of *Chorthippus parallelus parallelus* (Cpp) and the Southern pattern (high-level infection / F-supergroup in the majority) characteristic of *Chorthippus parallelus erythropus* (Cpe). (B) Magnification of the hybrid zone where the Sallent de Gállego pattern (characterized by an extremely high-level of co-infection) becomes evident. GAB: Gabas; POR: Portalet; SAL: Sallent de Gállego; ESC: Escarrilla. See Zabal-Aguirre *et al.*, 2010.

Figure 2.- Infection dynamics in the *Chorthippus parallelus* hybrid zone without *Wolbachia*-induced effects on female ($F_o = F_b = F_f = F_{bf} = 1$). Averages obtained from the posterior distributions estimated in the Sallent de Gállego equilibrium were used (table 7, without F uncertainty). A and B, new infection in a *Wolbachia*-free population; C and D, superinfection in a previously infected population. Uninfected, solid grey; B-infected, dashed grey; F-infected, dashed, black; Co-infected, solid black.

Figure 3.- Infection dynamics in the *Chorthippus parallelus* hybrid zone introducing fecundity uncertainty. Averages obtained from the posterior distributions estimated in the Sallent de Gállego equilibrium were used (table 7, with F uncertainty). A and B, new infection in a *Wolbachia*-free population; C and D, superinfection in a previously infected population. Uninfected, solid grey; B-infected, dashed grey; F-infected, dashed, black; Co-infected, solid black.

728 Supplementary Information

729 Table S1. Model considerations

Cross		Frequency	Type of descendants			
♀	♂		B	F	BF	O
B	B	$p_b \cdot p_b$	$F_b \cdot (1 - \mu_b)$	-	-	$F_b \cdot \mu_b \cdot H_{UNIB}$
B	F	$p_b \cdot p_f$	$F_b \cdot (1 - \mu_b) \cdot H_{BI}$	-	-	$F_b \cdot \mu_b \cdot H_{UNIF}$
B	BF	$p_b \cdot p_{bf}$	$F_b \cdot (1 - \mu_b) \cdot H_{BI}$	-	-	$F_b \cdot \mu_b \cdot H_{UNIBf}$
B	O	$p_b \cdot q$	$F_b \cdot (1 - \mu_b)$	-	-	$F_b \cdot \mu_b$
F	B	$p_f \cdot p_b$	-	$F_f \cdot (1 - \mu_f) \cdot H_{BI}$	-	$F_f \cdot \mu_f \cdot H_{UNIB}$
F	F	$p_f \cdot p_f$	-	$F_f \cdot (1 - \mu_f)$	-	$F_f \cdot \mu_f \cdot H_{UNIF}$
F	BF	$p_f \cdot p_{bf}$	-	$F_f \cdot (1 - \mu_f) \cdot H_{BI}$	-	$F_f \cdot \mu_f \cdot H_{UNIBf}$
F	O	$p_f \cdot q$	-	$F_f \cdot (1 - \mu_f)$	-	$F_f \cdot \mu_f$
BF	B	$p_{bf} \cdot p_b$	$F_{bf} \cdot (1 - \mu_b) \cdot \mu_f$	$F_{bf} \cdot \mu_b \cdot (1 - \mu_f) \cdot H_{BI}$	$F_{bf} \cdot (1 - \mu_b) \cdot (1 - \mu_f)$	$F_{bf} \cdot \mu_b \cdot \mu_f \cdot H_{UNIB}$
BF	F	$p_{bf} \cdot p_f$	$F_{bf} \cdot (1 - \mu_b) \cdot \mu_f \cdot H_{BI}$	$F_{bf} \cdot \mu_b \cdot (1 - \mu_f)$	$F_{bf} \cdot (1 - \mu_b) \cdot (1 - \mu_f)$	$F_{bf} \cdot \mu_b \cdot \mu_f \cdot H_{UNIF}$
BF	BF	$p_{bf} \cdot p_{bf}$	$F_{bf} \cdot (1 - \mu_b) \cdot \mu_f \cdot H_{BI}$	$F_{bf} \cdot \mu_b \cdot (1 - \mu_f) \cdot H_{BI}$	$F_{bf} \cdot (1 - \mu_b) \cdot (1 - \mu_f)$	$F_{bf} \cdot \mu_b \cdot \mu_f \cdot H_{UNIBf}$
BF	O	$p_{bf} \cdot q$	$F_{bf} \cdot (1 - \mu_b) \cdot \mu_f$	$F_{bf} \cdot \mu_b \cdot (1 - \mu_f)$	$F_{bf} \cdot (1 - \mu_b) \cdot (1 - \mu_f)$	$F_{bf} \cdot \mu_b \cdot \mu_f$
O	B	$q \cdot p_b$	-	-	-	$1 \cdot H_{UNIB}$
O	F	$q \cdot p_f$	-	-	-	$1 \cdot H_{UNIF}$
O	BF	$q \cdot p_{bf}$	-	-	-	$1 \cdot H_{UNIBf}$
O	O	$q \cdot q$	-	-	-	1

730

731 The model assumes panmixia and absence of migration, horizontal transmission, as well as
732 paternal transmission.

733 p_i ; frequency of B infected (p_b), F infected (p_f) and coinfectd (p_{bf}).

734 q ; frequency of uninfected.

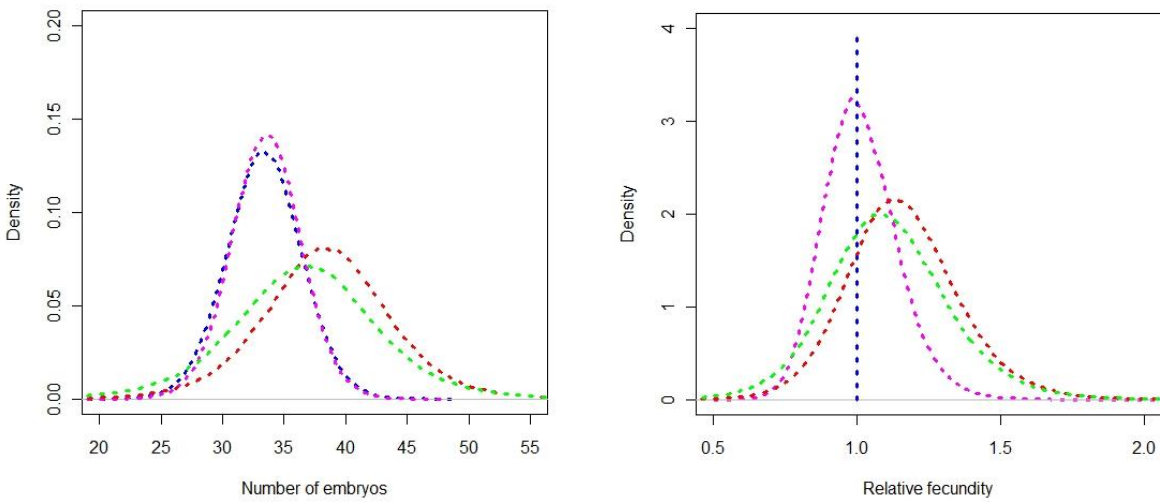
735 H_{UNI} ; average of hatching rate from unidirectional incompatible crosses with an involved B
736 infected (H_{UNIB}), F infected (H_{UNIF}) or coinfectd male ($H_{UNIBf} = H_{UNIB} \times H_{UNIF}$) relative
737 to compatible crosses ($H_0=1$).

738 H_{BI} ; average of hatching rate from bidirectional incompatible crosses relative to compatible
739 crosses ($H_0=1$).

740 F_i ; fecundity of B infected (F_b), F infected (F_f) and coinfectd females (F_{bf}) relative to
741 uninfected females ($F_0=1$).

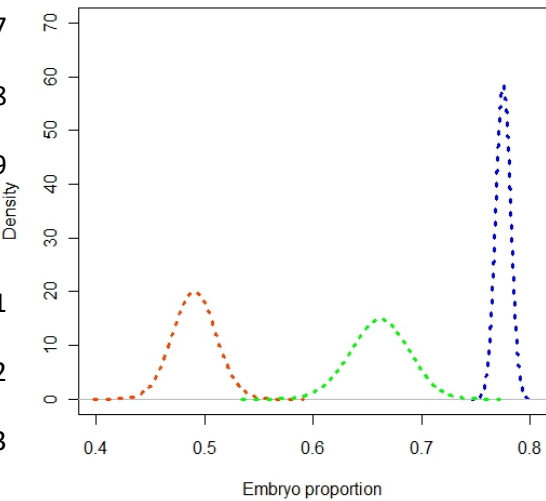
742 μ_i ; fraction of uninfected ova produced by an infected female harbouring B (μ_b) or F strain
743 (μ_f).

Figure S2. Embryo numbers and fecundity (F) in compatible crosses

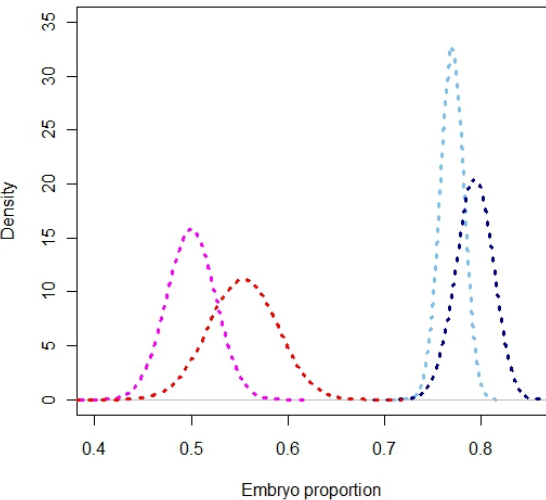


Probability density functions of posterior distributions belonging to uninfected (blue), B-infected (magenta), F-infected (red) and coinfecting (green) females crossed to uninfected males. Bayesian approach to estimate the average using the normal distribution with uninformative priors.

Figure S3. Embryo proportions in incompatible crosses

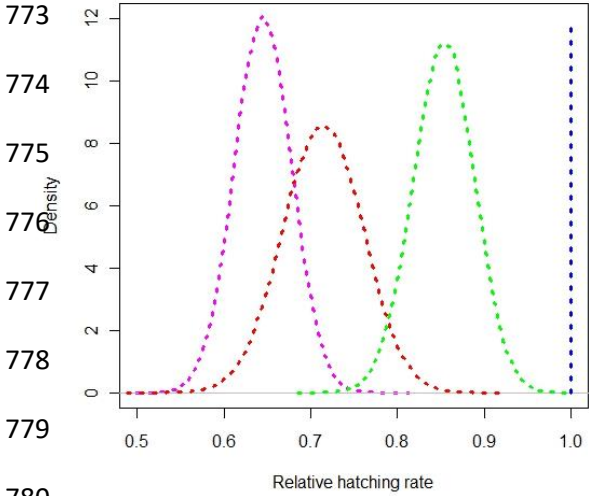


Probability density functions of embryo proportion posterior distributions belonging to compatible (blue), unidirectional (orange) and bidirectional (green) crosses attending to cytoplasmic incompatibility classification. Bayesian approach to estimate the average using the beta distribution with uninformative priors.



Probability density functions of embryo proportion posterior distributions belonging to BxO (light blue), OxB (magenta), FxO (dark blue) and OxF (red) crosses attending to *Wolbachia* strain classification. Bayesian approach to estimate the average using the beta distribution with uninformative priors.

Figure S4. Relative hatching rate (H) in incompatible crosses



Posterior distributions belonging to compatible (blue), B strain induced unidirectional CI (magenta), F strain induced unidirectional CI (red) and bidirectional CI (green) crosses. Bayesian approach to estimate the average using the beta distribution with uninformative priors.

Table 1. Classification of the different types of crosses obtained after the blind experiment conducted. Total numbers are specified according to male and female posterior characterization in relation to *Wolbachia* infection. In B) total numbers are, in turn, subdivided according to subspecific provenance of both males and females. Compatible, Bidirectional CI and Unidirectional CI crosses are differentiated by using the included gray scale legend.

A) Hybrid genotype individuals

♀ \ ♂	O	B	F	BF
O	27	9	6	1
B	27	5	4	2
F	9	-	3	2
BF	8	5	2	-

	Compatible
	Bidirectional CI
	Unidirectional CI

B) Pure genotype individuals

♀ \ ♂	O	B	F	BF
O	1 8 2 4 1	- - - - - -	- 4 3 1 - -	- - - - - -
B	- 1 - - 1 1	1 1 - - 1 -	1 1 - - - -	- 2 1 - 1 1
F	- - - - - -	- - - - - -	- 5 1 1 3	- 1 - 1 1 -
BF	2 2 - - 2 -	2 2 - - 2 -	- 1 - 1 - -	- 3 1 1 3 1

a	T	b
c		d

- a: Cpp x Cpp
- b: Cpp x Cpe
- c: Cpe x Cpe
- d: Cpe x Cpp

T: Sum of a, b, c and d

Table 2. Crosses performed between hybrid genotype individuals. Total number (110 crosses) is subdivided according to compatibility type and *Wolbachia* infection found in male and female partners. Average number of pods, eggs, embryos and embryo proportions are shown for each type (SD: standard deviation).

Crosses		Pods		Eggs		Embryos		Embryos / Eggs	
Type / Infection (♀x♂)	Counts	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Compatible	86	5.63	1.37	44.81	12.63	34.74	14.44	0.772	0.213
OxO	27	5.56	1.42	44.89	11.58	33.41	15.34	0.746	0.277
BxO	27	5.67	1.44	43.59	13.14	33.56	14.33	0.760	0.200
FxO	9	6.00	1.00	48.44	15.05	38.44	14.34	0.786	0.061
BFxO	8	5.50	1.51	45.50	12.76	36.75	15.17	0.809	0.213
Others	15	5.53	1.41	44.33	13.25	36.00	14.00	0.813	0.171
Unidirectional CI	16	5.63	1.02	39.38	8.96	19.31	11.36	0.521	0.300
OxB	9	5.67	0.71	43.11	7.82	21.56	12.75	0.517	0.281
OxF	6	5.50	1.52	32.50	7.04	18.00	9.27	0.589	0.332
OxBF	1	6.00	-	47.00	-	7.00	-	0.149	-
Bidirectional CI	8	6.13	2.10	38.50	10.74	25.50	14.84	0.654	0.269
TOTAL	110	5.66	1.38	43.56	12.19	31.83	15.07	0.727	0.246

Table 3. Crosses performed between pure genotype individuals. Total number (31 crosses) is subdivided according to compatibility type and the subspecies of male and female partners. Average number of pods, eggs, embryos and embryo proportions are shown for each type (SD: standard deviation).

Crosses		Pods		Eggs		Embryos		Embryos / Eggs	
Type / Subspecies (♀x♂)	Counts	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Compatible	23	4.91	1.08	44.87	10.09	36.65	12.07	0.830	0.209
Cpp x Cpp	6	5.50	0.55	49.67	5.39	44.00	7.40	0.885	0.104
Cpp x Cpe	4	5.25	0.96	46.50	6.95	38.50	4.65	0.839	0.128
Cpe x Cpp	5	4.60	1.34	45.60	12.90	34.00	20.09	0.802	0.420
Cpe x Cpe	8	4.50	1.20	40.00	11.61	31.88	9.98	0.803	0.134
Unidirectional CI	4	4.75	1.26	45.75	12.07	34.50	16.68	0.779	0.342
Cpp x Cpe	3	4.33	1.15	42.67	12.70	30.67	18.15	0.760	0.416
Cpe x Cpe	1	6.00	-	55.00	-	46.00	-	0.836	-
Bidirectional CI	4	4.25	0.96	36.50	11.09	14.50	16.58	0.369	0.355
Cpp x Cpp	1	5.00	-	44.00	-	39.00	-	0.886	-
Cpp x Cpe	1	4.00	-	36.00	-	3.00	-	0.083	-
Cpe x Cpp	1	5.00	-	45.00	-	10.00	-	0.222	-
Cpe x Cpe	1	3.00	-	21.00	-	6.00	-	0.286	-
TOTAL	31	4.81	1.08	43.90	10.49	33.52	14.77	0.764	0.284

Table 4. Comparisons among different types of females from compatible crosses. Several tests were performed to compare numbers of eggs and embryo proportions. Statistics (F, ANOVA; χ^2 , Kruskal-Wallis; t, t-Student; U, Mann-Whitney test), degrees of freedom, sample sizes (n_1 and n_2) and P-values are shown. When a type of cross is indicated always in the form ♀x♂.

Type of female from compatible crosses	Eggs	Embryos/Eggs
Hybrid females (OxO, BxO, FxO, BFxO)	$F_{3,67} = 0.331$, $P = 0.803$	$\chi^2_3 = 1.640$, $P = 0.650$
Pure females (Uninfected vs. Infected females)	$t_{21} = -0.210$, $P = 0.835$	$U = 58$, $n_1 = 8$, $n_2 = 15$, $P = 0.925$
Uninfected vs. Infected Cpp females	$t_8 = -0.243$, $P = 0.814$	$U = 9$, $n_1 = 3$, $n_2 = 7$, $P = 0.833$
Uninfected vs. Infected Cpe females	$t_{11} = 0.011$, $P = 0.922$	$U = 17$, $n_1 = 5$, $n_2 = 8$, $P = 0.724$
Cpp vs. Cpe Uninfected females	$t_6 = 0.949$, $P = 0.379$	$U = 7$, $n_1 = 3$, $n_2 = 5$, $P = 1.000$
Cpp vs. Cpe Infected females	$t_{13} = 1.132$, $P = 0.278$	$U = 27$, $n_1 = 7$, $n_2 = 8$, $P = 0.955$
Total females (Uninfected vs. Infected females)	$t_{107} = -0.049$, $P = 0.961$	$U = 1235$, $n_1 = 35$, $n_2 = 74$, $P = 0.697$
Hybrid vs. Pure Uninfected females	$t_{33} = -0.146$, $P = 0.885$	$U = 87.5$, $n_1 = 27$, $n_2 = 8$, $P = 0.428$
Hybrid vs. Pure Infected females	$t_{72} = 0.113$, $P = 0.910$	$U = 335.5$, $n_1 = 59$, $n_2 = 15$, $P = 0.150$

Table 5. Comparisons among different types of crosses. Mann-Whitney U-tests were performed between pairs of crosses to compare embryo proportions. U statistic, sample sizes (n_1 and n_2) and P-values are shown.

Type of cross ($\text{♀} \times \text{♂}$)	Embryos/Eggs
Hybrid crosses	
Compatible vs. Unidirectional crosses	$U = 316.5, n_1 = 86, n_2 = 16, P = 0.001$
Unidirectional crosses (OxB vs. OxF)	$U = 22, n_1 = 9, n_2 = 6, P = 0.607$
CI induced by B supergroup (BxO vs. OxB)	$U = 52, n_1 = 27, n_2 = 9, P = 0.010$
CI induced by F supergroup (FxO vs. OxF)	$U = 15, n_1 = 9, n_2 = 6, P = 0.181$
Bidirectional vs. Compatible crosses	$U = 228.5, n_1 = 8, n_2 = 86, P = 0.117$
Bidirectional vs. Unidirectional crosses	$U = 46.5, n_1 = 8, n_2 = 16, P = 0.291$
Pure crosses	
Compatible vs. Unidirectional crosses (all of them OxF)	$U = 38, n_1 = 23, n_2 = 4, P = 0.622$
Compatible crosses (Cpp females vs. Cpe females)	$U = 63, n_1 = 10, n_2 = 13, P = 0.927$
CppxCpp vs. CppxCpe	$U = 9, n_1 = 6, n_2 = 4, P = 0.610$
CpexCpe vs. CpexCpp	$U = 8, n_1 = 8, n_2 = 5, P = 0.093$
Bidirectional vs. Compatible crosses	$U = 14, n_1 = 4, n_2 = 23, P = 0.027$
Bidirectional vs. Unidirectional crosses	$U = 3, n_1 = 4, n_2 = 4, P = 0.200$
Total crosses	
Compatible crosses (hybrid vs. pure crosses)	$U = 774, n_1 = 86, n_2 = 23, P = 0.110$
Unidirectional crosses (hybrid vs. pure crosses)	$U = 15, n_1 = 16, n_2 = 4, P = 0.122$
CI induced by B supergroup (BxO vs. OxB)	$U = 53, n_1 = 28, n_2 = 9, P = 0.008$
CI induced by F supergroup (FxO vs. OxF)	$U = 40, n_1 = 9, n_2 = 10, P = 0.720$
Bidirectional crosses (hybrid vs. pure crosses)	$U = 8, n_1 = 8, n_2 = 4, P = 0.214$
Compatible vs. Unidirectional crosses	$U = 650, n_1 = 109, n_2 = 20, P = 0.004$
Bidirectional vs. Compatible crosses	$U = 358.5, n_1 = 12, n_2 = 109, P = 0.010$
Bidirectional vs. Unidirectional crosses	$U = 118.5, n_1 = 12, n_2 = 20, P = 0.954$

822 Table 6. Averages and credible intervals calculated from posterior distributions of hybrid
 823 crosses. F_b , F_f , F_{bf} : relative fecundities of B, F and BF infected females. H_{UNib} , H_{UNif} , H_{BI} :
 824 relative hatching rates of B/F unidirectional and bidirectional crosses.

825

	Median	Credible Interval 95%
F_b	1.005	0.783 - 1.295
F_f	1.151	0.792 - 1.589
F_{bf}	1.100	0.695 - 1.576
H_{UNib}	0.645	0.580 - 0.710
H_{UNif}	0.714	0.624 - 0.804
H_{BI}	0.853	0.783 - 0.922

826

827 Table 7. Averages and credible intervals required to reach the equilibrium in Sallent de Gállego.
828 F_b , F_f , F_{bf} : relative fecundities of B, F and BF infected females. H_{UNib} , H_{UNif} , H_{BI} : relative
829 hatching rates of B/F unidirectional and bidirectional crosses. μ_b , μ_f : proportion of uninfected
830 descendants produced by B and F infected females.
831

	without F uncertainty		with F uncertainty	
	Median	Credible Interval 95%	Median	Credible Interval 95%
F_b	1	-	0.991	0.776 - 1.241
F_f	1	-	1.100	0.766 - 1.426
F_{bf}	1	-	1.203	0.944 - 1.652
H_{UNib}	0.645	0.580 - 0.710	0.645	0.580 - 0.709
H_{UNif}	0.714	0.624 - 0.804	0.714	0.624 - 0.803
H_{BI}	0.853	0.783 - 0.922	0.850	0.780 - 0.918
μ_b	0.016	0.009 - 0.025	0.024	0.001 - 0.055
μ_f	0.031	0.016 - 0.047	0.073	0.016 - 0.139

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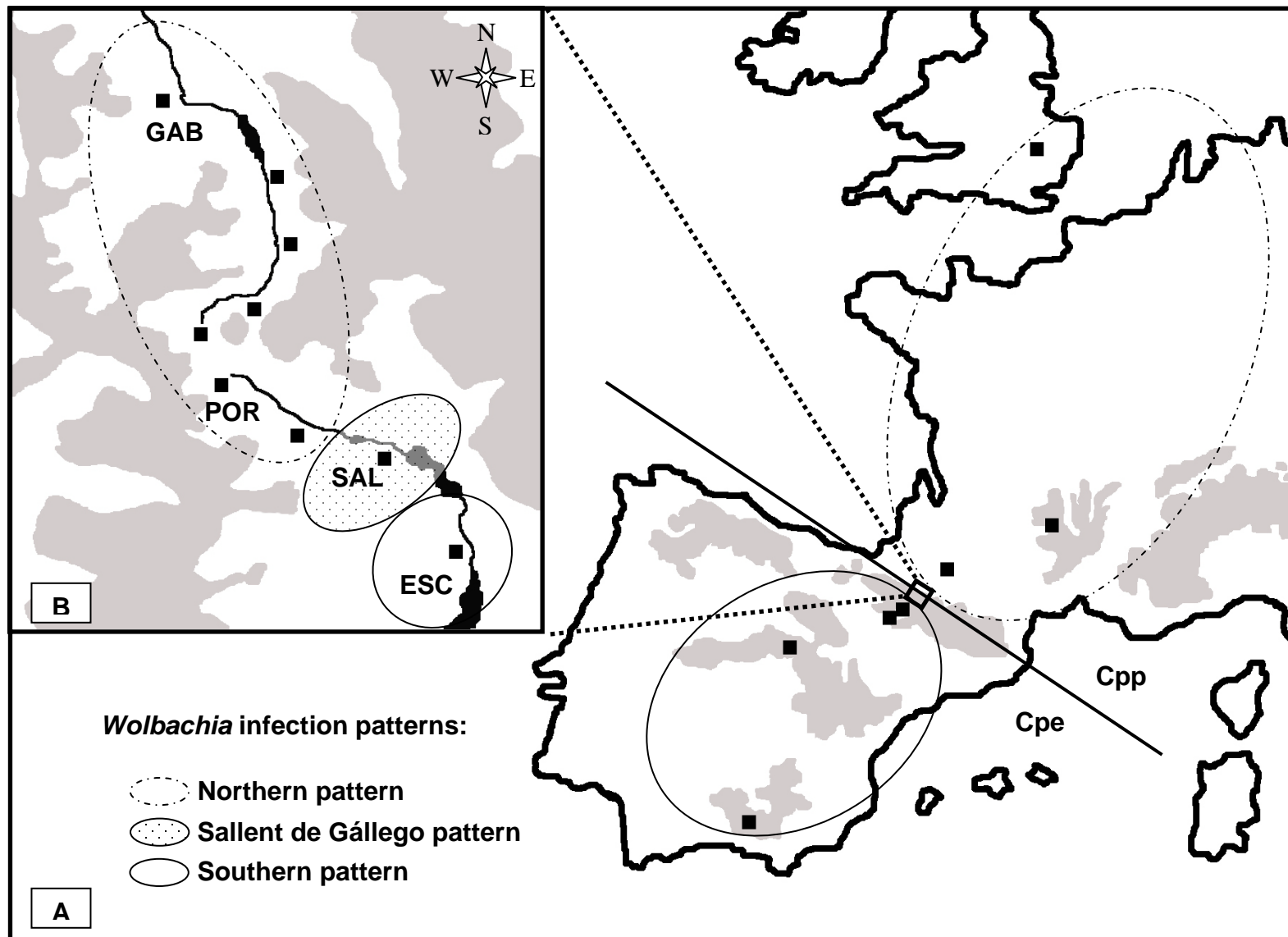


Figure 1

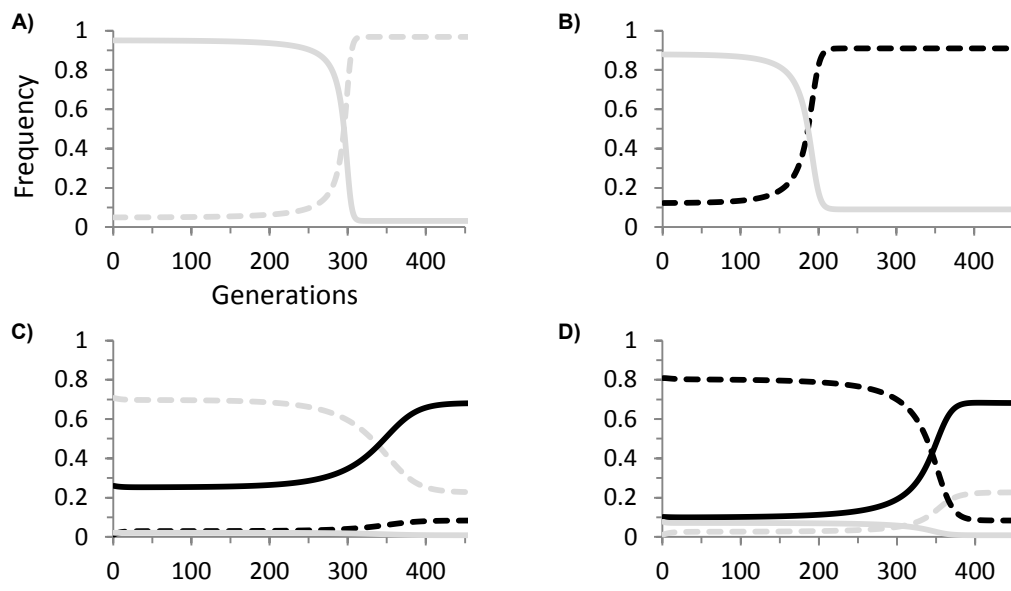


Figure 2

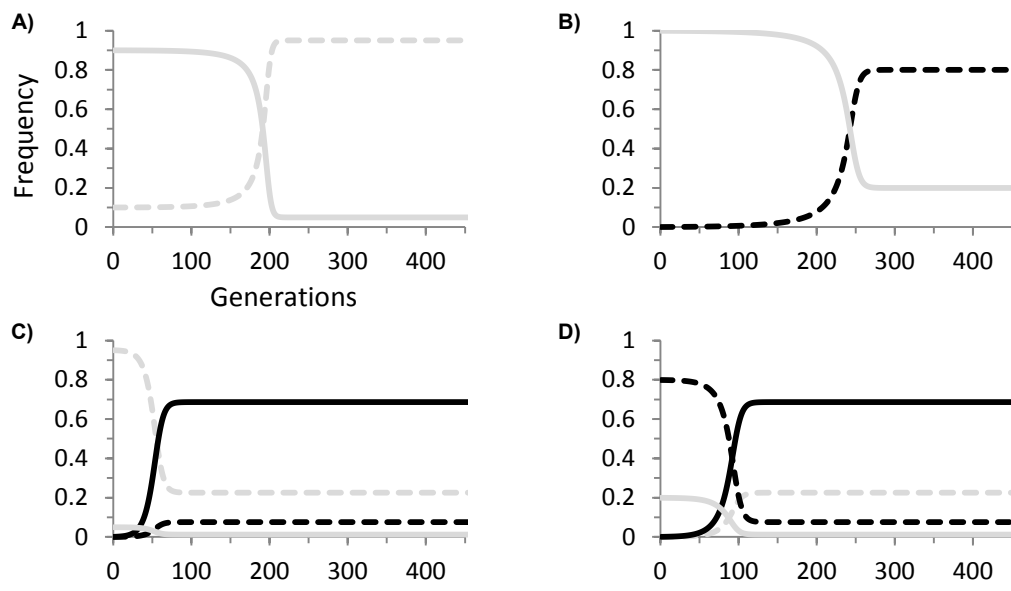


Figure 3