

A model for the study of *Wolbachia pipientis* Hertig (Rickettsiales: Rickettsiaceae)- induced cytoplasmic incompatibility in arrhenotokous haplodiploid populations: consequences for biological control

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Abstract. *Wolbachia* is an endocyttoplasmic bacterium responsible for various reproductive modifications in arthropods. In several species, *Wolbachia* induces a phenomenon called cytoplasmic incompatibility (CI), whereby crosses between a *Wolbachia*-infected male and a healthy female are incompatible. In haplodiploid species reproducing with arrhenotokous parthenogenesis, CI crosses produce only parthenogenetic males, inducing a male-biased sex ratio in the population. Here, we used two modeling approaches to evaluate the respective influences of demographic and biological parameters on *Wolbachia* fixation probability and on the sex ratio peak occurring during a *Wolbachia* invasion, and compared these parameters to values reported in the literature. Results suggest that the impact of *Wolbachia* invasion on population dynamics remains relatively limited, especially for parasitoids with high rates of sib-mating. The consequences for introduction of the parasitoids for biological control are discussed.

Résumé. Un modèle d'étude de l'incompatibilité cytoplasmique induite par *Wolbachia pipientis* Hertig 1936 (Rickettsiales : Rickettsiaceae) induisant une incompatibilité cytoplasmique chez les populations arrhénotoques haplodiploïdes : les conséquences pour la lutte biologique.

Wolbachia est une bactérie endocytoplasmique responsable de plusieurs phénomènes de modification de la reproduction. Chez plusieurs espèces, *Wolbachia* induit un phénomène appelé Incompatibilité Cytoplasmique (IC) : les croisements entre mâle infecté par *Wolbachia* et femelle non-infectée sont incompatibles. Chez les espèces incompatibles se reproduisant par parthénogenèse arrhénotoque, du fait que les croisements incompatibles donnent uniquement des mâles, l'IC entraîne un biais de sex-ratio dans la population. Dans cette étude, nous avons utilisé deux approches de modélisation pour évaluer les influences respectives de paramètres démographiques et biologiques sur la probabilité de maintenir *Wolbachia* et sur l'augmentation de la sex-ratio durant la phase d'invasion. Ces paramètres ont été comparés aux valeurs observées dans la littérature. Les résultats suggèrent que l'impact de l'invasion de *Wolbachia* sur la dynamique des populations est relativement limité, particulièrement pour les parasitoïdes avec un fort taux de croisement frères-soeurs. Les conséquences pour les stratégies d'introduction de parasitoïdes dans le cadre de la lutte biologique sont discutées.

Keywords: *Wolbachia*, stochastic modeling, biological control, stem borer, Africa.

Many microorganisms, including bacteria (Hunter 1999) and viruses (Varaldi *et al.* 2003), can modify the reproductive strategies of insects. Such effects can have demographic consequences on beneficial insects including the parasitoid wasps commonly used as classical biological control agents. Understanding these constraints may allow a better biological control strategy for controlling pests.

Wolbachia pipientis Hertig 1936 (Rickettsiales: Rickettsiaceae) is an endocyttoplasmic symbiotic bacterium responsible for many reproductive modifications in arthropods and other phyla (Werren 1997). In several species, these bacteria cause

cytoplasmic incompatibility (CI) (reviewed in Stouthamer *et al.* 1999) which affects population dynamics by preventing crosses between infected and healthy individuals. In haplodiploid species reproducing with arrhenotokous parthenogenesis, such as parasitoid wasps, incompatible crosses occurring between infected males and healthy females leads to male progeny only. This can result from two different CI phenotypes affecting diploid eggs. In the case of the Male Development (MD) phenotype, diploid eggs develop as males, whereas in the case of the Female Mortality (FM) phenotype, diploid eggs die (Vavre *et al.* 2000). Both MD and FM phenotypes lead to a male-biased sex ratio in the population. Since only females lay eggs, population growth is particularly correlated to female frequency.

Population genetic diversity may also be affected by CI: in gregarious parasitoids, siblings often mate

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together after hatching from cocoon masses, but cocoons affected by CI will produce only males and will not be able to sib-mate, thereby reducing population inbreeding. Thus, *Wolbachia* could potentially affect population genetics and dynamics during an invasion by reducing the number of females. The theoretical model proposed by Mochiah and co-workers (2002) showed that introducing a biocontrol agent infected by *Wolbachia* into a non-infected population results in a transient reduction in population growth rate due to *Wolbachia* invasion. This model considered the effect of sib-mating, but did not consider the effect of other parameters varying between species and/or the *Wolbachia* strains and density (Guillemaud *et al.* 1997) as penetrance of CI (the proportion of crosses predicted to be incompatible that are really incompatible), and the rates of vertical transmission of *Wolbachia* (the probability for a female to transmit *Wolbachia* to its offspring) (Turelli & Hoffmann 1995; Rasgon & Scott 2003); such parameters may affect the impact of *Wolbachia* on population growth rate. Furthermore, this model is determinist and does not consider the effect of population size.

Here, we evaluate the robustness of the model developed by Mochiah *et al.* (2002) on the demographic impact of *Wolbachia* invasion in a resident population of a parasitoid over a wider range of parameters. Some effects on genetic diversity are also evaluated. Hypotheses of stochastic dynamics and finite population size, which are likely to be more realistic, are considered. The effects of variations in CI penetrance and rates of vertical transmission on the sex ratio modification and on the probability of *Wolbachia* fixation are also evaluated.

First the discrete determinist equations are used as in Mochiah *et al.* (2002) and Stouthamer *et al.* (2000) to show the impact of several life history traits on *Wolbachia*-parasitoid dynamics. Then the results are compared to the stochastic model with finite population size.

Material and methods

Equations

Equations were developed based on Mochiah *et al.* (2002) and Stouthamer *et al.* (2000) to determine the evolution of sex ratio (proportion of males) during *Wolbachia* invasion. We considered two additional parameters: penetrance of CI and *Wolbachia* vertical transmission rate (see above).

Assuming infinite population size and no migration. $F_{I,T}$ and $M_{I,T}$ are the proportion of infected females and males at time t , respectively. Other parameters were x , the proportion of females in the offspring; tCI , the penetrance of CI; μ the vertical transmission rate; and s , the sib-mating frequency.

At $t = 0$, 10 percent of the population is infected. The sex ratio (males:females) is given a value of $x = 0.25$ in the absence of a *Wolbachia* effect. Local mate competition leads to female-biased sex ratios, because it is advantageous for a female to produce more females when the number of competitor females is low (Hamilton 1967). Furthermore, in arrhenotokous haplodiploids species, females can easily control the progeny sex ratio because males result from unfertilized eggs (Hardy *et al.* 1999). We chose to apply a female-biased sex ratio (i.e. 0.25) as observed, for instance, in *Cotesia sesamiae* (Cameron 1891) (Hymenoptera: Braconidae) (Le Rü *pers. com.*), a biological control agent used against stemborers in Africa.

The proportion of infected females from one generation to the next is given by the equation below; note that it does not depend on the nature of the CI phenotype, MD or FM:

$$F_{I,T+1} = \frac{\mu F_{I,T}}{s + (1-s)} \times \frac{1}{[F_{I,T} + (1-F_{I,T})(1-M_{I,T}) + (1-tCI)(1-F_{I,T})M_{I,T}]}$$

The number of infected males in the next generation depends on the CI phenotype. Under FM, it is:

$$M_{I,T+1} = \mu F_{I,T}$$

Under the MD phenotype, incompatible crosses produce fewer infected males:

$$M_{I,T+1} = \frac{\mu F_{I,T}(1-x)}{(1-x) + x(1-s)(1-F_{I,T})M_{I,T}tCI} \quad (3)$$

Assuming x is constant between generations, the sex ratio in the population at each generation is given for the FM phenotype by:

$$SR_{T+1} = x \frac{1 - (1-s)(1-F_{I,T})M_{I,T}tCI}{1 - x(1-s)(1-F_{I,T})M_{I,T}tCI} \quad (4a),$$

and for the MD phenotype by:

$$SR_{T+1} = x[1 - (1-s)(1-F_{I,T})M_{I,T}tCI] \quad (4b).$$

Sex ratio variations were calculated among generations assuming equation 4 for sets of values of s , μ and tCI .

Stochastic model

The stochastic model was implemented under Scilab 3.1 (INRIA 2005). Individuals were identified by infection status (TRUE for infected and FALSE for uninfected). We assumed 10 percent of the population was infected at $t = 0$, and a population size that was constant among generations. The number of females ($Nf_{t,t}$) and males ($Nm_{t,t}$) were calculated as a function of the sex ratio. $Nf_{i,t}$ and $Nm_{i,t}$ are the number of infected females and males, respectively, at time t . Within the total populations, the infected effective (reproductive) females and males (Nf'_t and Nm'_t , respectively) are randomly sampled, assuming the number of infected females and males is given by:

$$Nf_I' \rightarrow B(Nf_I', \frac{Nf_{I,t}}{Nf_{T,t}}) \quad \text{(Ia), and}$$

$$Nm_I' \rightarrow B(Nm_I', \frac{Nm_{I,t}}{Nm_{T,t}}) \quad \text{(Ib),}$$

where $B(N,p)$ is the binomial probability distribution of probability p and number of trials N .

Infected male and female reproducers (Nf_I'' and Nm_I'' , respectively) are sampled with a fitness-cost probability $1-c$, where c represents the fitness cost of carrying *Wolbachia*:

$$Nf_I'' \rightarrow B(Nf_I'', 1-c) \quad \text{(IIa)}$$

$$Nm_I'' \rightarrow B(Nm_I'', 1-c) \quad \text{(IIb)}$$

Mating was independent of infection status. Sib-mating females ($Nf_{I,S}$) were randomly sampled with a probability s :

$$Nf_{I,S} \rightarrow B(Nf_{I,S}, s) \quad \text{(III).}$$

Infected females after reproduction (Nf_I''') are sampled assuming a vertical transmission probability μ :

$$Nf_I''' \rightarrow B(Nf_{I,S}, \mu) + B(Nf_I'' - Nf_{I,S}, \mu) \quad \text{(IV).}$$

The number of healthy females that do not sib-mate ($Nf_{H,1-S}$) that mate of them mating with infected males (N_{CI}) were sampled assuming a sib-mating probability s and proportion of infected males sampled above:

$$Nf_{H,1-S} \rightarrow B(1 - Nf_I''', 1-s) \quad \text{(Va), and}$$

$$N_{CI} \rightarrow B(Nf_{H,1-S}, \frac{Nm_I'''}{Nm_I + Nm_H}) \quad \text{(Vb).}$$

Effective CI matings ($N_{CI,t}$) are sampled from N_{CI} with a

$$N_{CI,t} \rightarrow B(N_{CI,t}, tCI) \quad \text{(VI).}$$

penetrance tCI (CI penetrance):

The total number of females and males at $t+1$ was calculated as:

$$Nf_{T,t+1} = Nf_{T,t} - N_{CI,t} \quad \text{(VIIa)}$$

$$Nm_{T,t+1} = Nm_T \quad \text{(VIIb)}$$

under the FM phenotype, or

$$Nm_{T,t+1} = Nm_T + N_{CI,t} \quad \text{(VIIc)}$$

under the MD phenotype.

The sex ratio of the next generation is given by:

$$SR_{t+1} = \frac{Nm_{T,t+1}}{Nf_{T,t+1} + Nm_{T,t+1}} \quad \text{(VIII).}$$

When a parameter varies, sib-mating is fixed at 0.5, tCI and μ are fixed at 1, physiological cost is fixed at 0 and population size is fixed at 200 individuals.

The sex ratio (proportion of males) and the probability that *Wolbachia* is maintained in the population after 40 generations, Pm , (number of infected populations after 40 generations divided by number of repetitions) were recorded. Statistics were performed with R Development Core Team (2005) (version 2.2.0).

Results

The impact of the FM phenotype on sex ratio is weaker and shorter in duration than that of MD (fig. 1), but their response curve to variations in parameter values have the same general shape. The results are presented below for the MD phenotype only.

Comparison of stochastic and determinist models (fig. 2)

Almost the same results were found between stochastic and determinist models for the effect of invasion on sex ratio. The major difference was that *Wolbachia* invasion was faster in the stochastic case because of the drift effect. Between different stochastic models, the smaller the population, the faster is the invasion. Another difference between the stochastic and determinist models are estimates of a population's ability to maintain a *Wolbachia* infection. In the case of the determinist model, the population cannot maintain *Wolbachia* below a transmission rate of 0.97, while in the stochastic model *Wolbachia* can be maintained for transmission rates less than 0.9, due to drift.

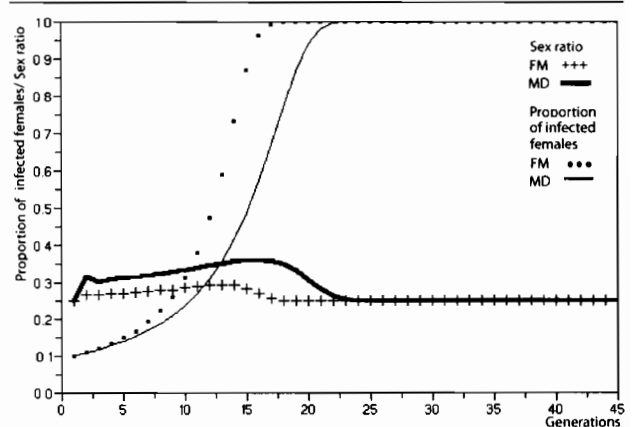


Figure 1
Evolution of sex-ratio and proportion of parasitoid females infected with *Wolbachia* in Female Mortality phenotype (FM) and Male Development phenotype (MD) ($s = 0.5$, $tCI = 1$, $\mu = 1$).

Sib-mating (fig. 2)

As in Mochiah *et al.* (2002), sib-mating strongly reduces the sex ratio peak during invasion. This is due to a decrease in the proportion of mating between infected and non-infected individuals. We also found that sib-mating at high rates reduces the probability of maintaining *Wolbachia*. This is due to an increase in the drift effect, which reduces the effect of *Wolbachia* fitness advantage (only infected females reproduce) during invasion.

CI penetrance (fig. 2)

A lower CI penetrance has a similar but less important impact on the sex ratio than a higher rate of sib-mating. As for sib-mating, a lower CI penetrance reduces the effect on the sex ratio, but by slowing down the invasion process, it distributes the sex ratio impact among more generations.

Transmission rate (fig. 2)

The transmission rate does not affect the sex ratio disturbance but greatly affects the probability of maintaining *Wolbachia*. For instance, in a population size of 200 individuals, if the probability of vertical transmission is 87%, the Pm drops to 0.040 ± 0.058 (95% confidence interval). *Wolbachia* needs a strong vertical transmission rate in CI to be maintained in the population.

Physiological cost (fig. 3)

As for the transmission rate, the physiological cost does not affect the sex ratio disturbance but it does affect the probability of maintaining *Wolbachia*. This means that the selective advantage of infected females in comparison to healthy ones is rapidly balanced by the physiological cost of carrying the bacteria.

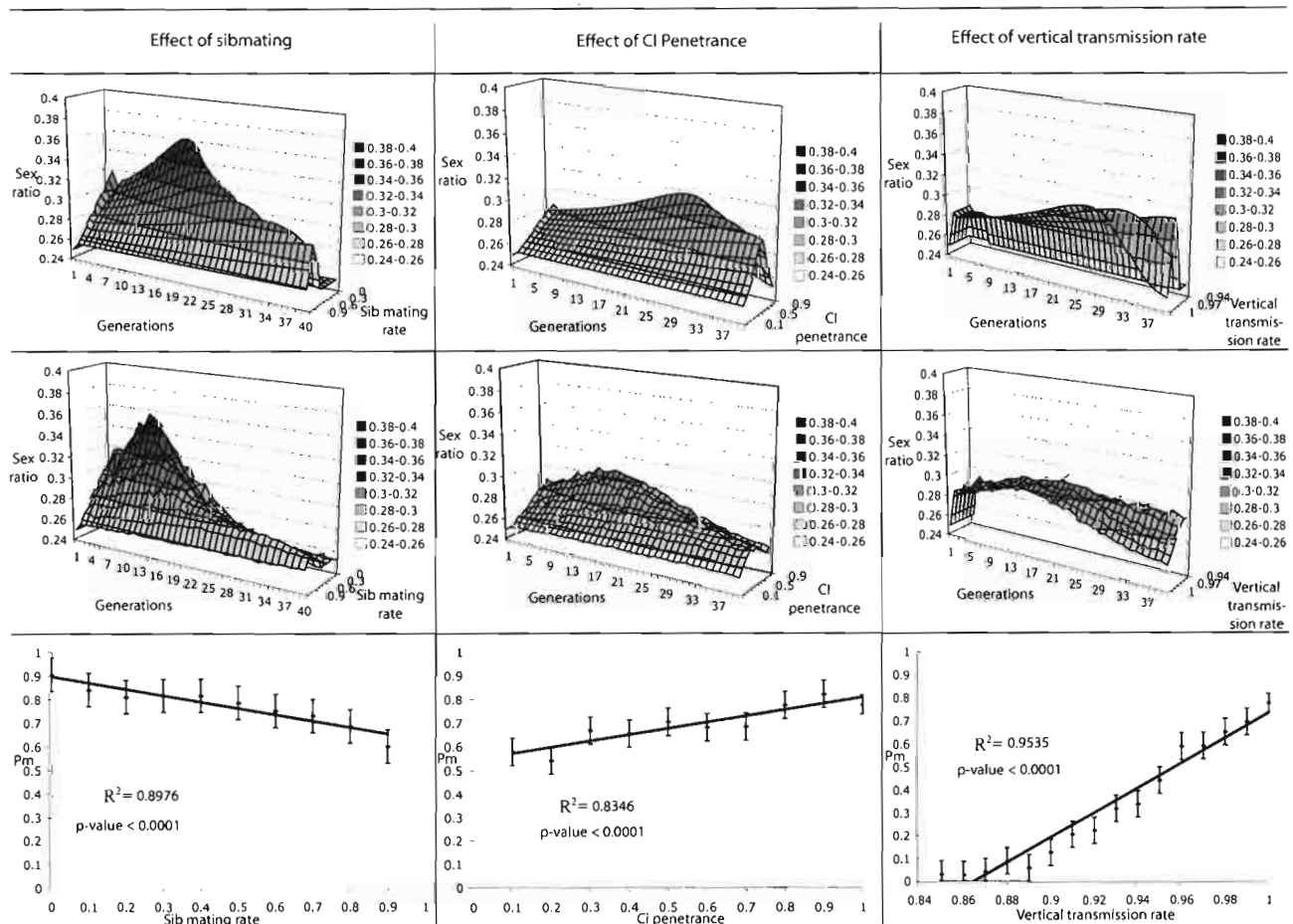


Figure 2 Effects of parameters on sex ratio and probability of maintaining *Wolbachia* infection in a population of parasitoids: (a) sex ratio results (proportion of males) in the stochastic model, (b) sex ratio results in the determinist model, (c) probability of maintaining *Wolbachia*. In 3D graphics (a and b), X-axis represents time in generations, Y-axis represents sex ratio value and Z-axis correspond to values of the variables (sib-mating, CI (cytoplasmic incompatibility) penetrance or transmission rate). In 2D-graphics (c), Y-axis corresponds to probability of maintaining *Wolbachia* in the population and X-axis to values of the variables.

Population size (fig. 3)

Population size has an effect on P_m up to $N = 500$ ($P_m = 0.95 \pm 0.07$). Invasion time is longer when the population is larger.

Discussion

The two *Wolbachia* invasion models developed in this work show that the sex ratio during invasion is always less than 50%, suggesting that a viable population could overcome the detrimental effects of *Wolbachia* invasion. In addition, it is observed that several life history traits can reduce the effect of *Wolbachia* invasion on population dynamics. A high sib-mating rate or a low CI penetrance limits the impact of *Wolbachia*. However, for transmission rates less than 1, infected and healthy individuals may coexist in the population, thereby leading to **invasion/loss** *Wolbachia* dynamics due to migration/drift processes with their associated detrimental effect on population growth rate.

In gregarious parasitoids, sib-mating occurs frequently. In *Cotesia glomerata* (L. 1758) (Hymenoptera: Braconidae), approximately 60% of females breed with their brothers (Kitano & Tagawa 1981). Thus, sib-mating could be an important limiting factor to the impact of *Wolbachia* invasion on population growth rate.

Previous investigations have shown that *Wolbachia* is transmitted at variable rates among different host species. In *Drosophila simulans* Sturtevant 1919 (Diptera: Drosophilidae), field data show a transmission rate of 96–97% (Turelli & Hoffmann 1995) and in the mosquito *Culex pipiens* L. 1758 (Diptera: Culicidae), a 98.6% transmission rate was reported (Rasgon & Scott 2003). Such high rates of transmission of *Wolbachia* in populations harbouring the bacterium are consistent with the prediction of the models presented in this work: when the bacterium induces CI, only a high rate of transmission will allow *Wolbachia* to be maintained within a population.

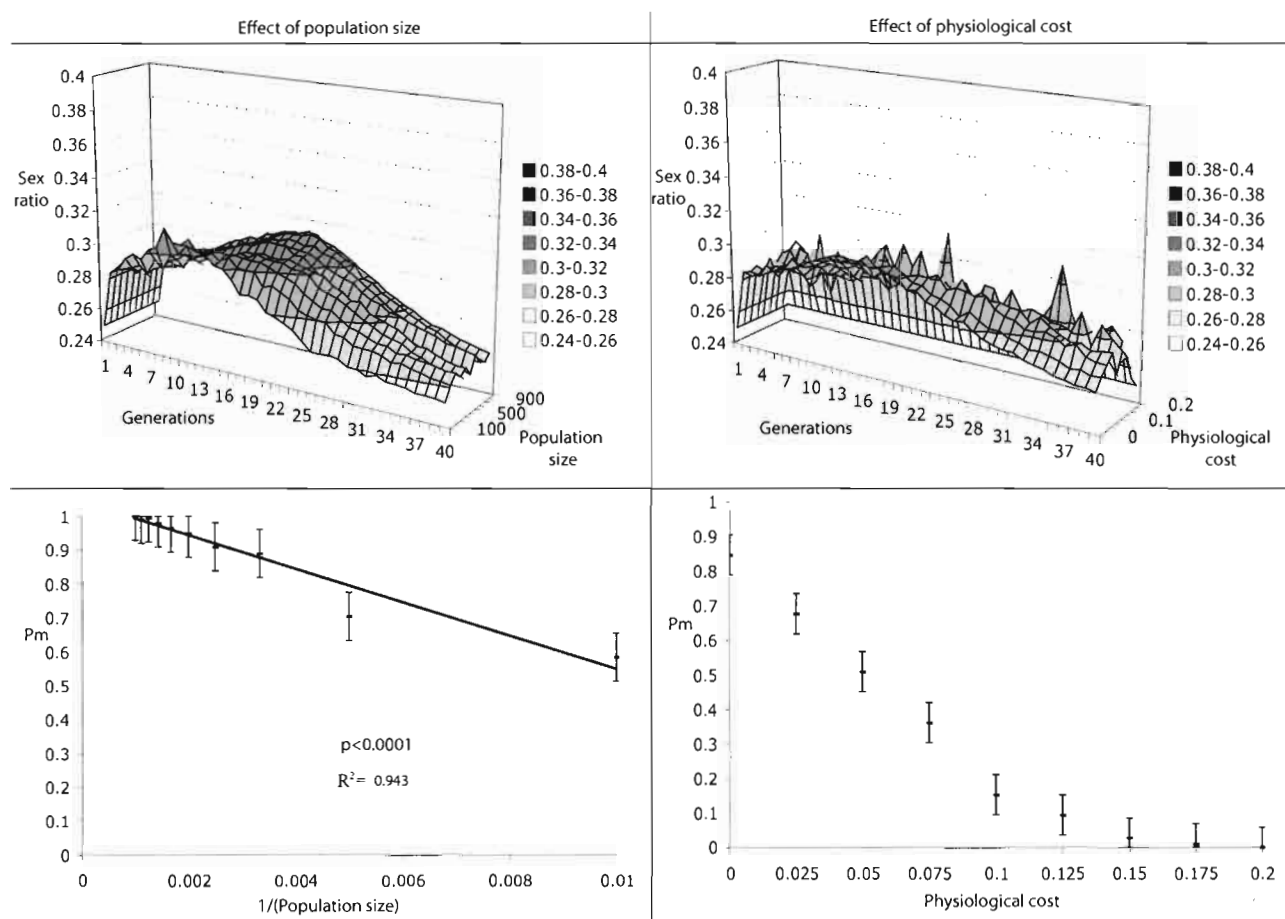


Figure 3 Effect of population size (a) and physiological cost (b) on sex ratio and probability of maintaining *Wolbachia* in parasitoid populations of different sizes (c) and physiological cost (d). For axes, see fig. 1.

Concerning the penetrance of CI, its value depends on several factors: the strain of *Wolbachia*, the genotype of the host and the density of bacteria in the eggs (Hunter 1999). Consequently, although it is difficult to estimate this parameter, we observed that it can have a strong effect on limiting invasion impact. We also found that *Wolbachia* must have a very limited or no fitness cost to be maintained in the population. The impact of *Wolbachia* on its host's fitness has been found to be very limited so far (Poinsot & Merçot 1997; Werren 1997; Stouthamer *et al.* 1999). Thus, it seems to be essential for this reproductive parasite to avoid any physiological cost to its host.

Populations of parasitoids may suffer important seasonal reductions in population size due to climatic factors or variations in host occurrence. Our model predicts that this situation would accelerate invasion.

In biological control, *Wolbachia* could play a major role if an infected population is introduced into a healthy one or the reverse. Since only the non-infected females suffer reproductive depression, the implications for introduction success differ between these two cases. The *Wolbachia*-free population will have a reduced growth rate, especially when less abundant. If the *Wolbachia*-free population is local, it may be endangered when the introduced population reaches high levels. If a *Wolbachia*-free parasitoid population is introduced into an infected population, it may have a reduced growth rate during the early stages of invasion when its population is low, which may compromise introduction success. Nevertheless, high sib-mating, as well as low CI penetrance, reduces this impact.

From the results of their model, Mochiah and co-workers (2002) have suggested that releasing individuals with *Wolbachia* status different from that of the native population may reduce the chance of introduction success, due to the impact of cytoplasmic incompatibility on the sex ratio. Our results suggest, however, that the effect of the sex ratio on overall reproductive rate (of introduced and local populations) may be limited. Nevertheless, *Wolbachia* can still have a strong effect on biological control introduction success in a situation in which the two populations are infected by *Wolbachia* from different strains which are reciprocally incompatible, thereby leading to bi-directional cytoplasmic incompatibility (BCI) (Bordenstein *et al.* 2001). In this case, CI is expressed in both directions of the cross. Therefore, an introduction of a population carrying a strain different from the native population could potentially affect the success of biological control more than in the other cases described previously. In such a case, the best solution may be to breed native populations in the laboratory and release them in their endemic location (augmentative biological control).

In conclusion, it is clear that before any use of a

biological control agent infected with *Wolbachia*, the *Wolbachia* status of the local population must be known. While the predictive effect of *Wolbachia* is very difficult to estimate because of the difficulty associated with estimating some of the model parameters, our results show that in the presence of only one strain of *Wolbachia*, its effect on the success of a biological control program can be overcome.

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