



HAL
open science

Consequences of Wolbachia transmission process on the infection dynamics

Thomas Guillemaud, François Rousset

► **To cite this version:**

Thomas Guillemaud, François Rousset. Consequences of Wolbachia transmission process on the infection dynamics. *Journal of Evolutionary Biology*, 1997, 10 (4), pp.601 - 612. 10.1046/j.1420-9101.1997.10040601.x . hal-02940577

HAL Id: hal-02940577

<https://hal.science/hal-02940577>

Submitted on 16 Sep 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Consequences of *Wolbachia* transmission process on the infection dynamics

T. Guillemaud* and F. Rousset

Institut des Sciences de l'Evolution (UMR 5554), Laboratoire Génétique et Environnement, Université de Montpellier II, CC 065, F-34095 Montpellier Cedex 05, France, e-mail: Guillema@isem.univ-montp2.fr

Key words: *Wolbachia*; cytoplasmic incompatibility; bacterial load; evolution.

Abstract

In some species displaying *Wolbachia*-induced cytoplasmic incompatibility, the intensity of incompatibility depends on the density of symbionts in both parents. Although modalities of the transmission process are poorly known, it appears that the density of *Wolbachia* within the offspring of a female is variable and is correlated with that of the mother. Assuming that the infection level of an host is a continuous trait, we examine some theoretical consequences of the *Wolbachia* transmission process on the evolution of the infection level within a population. The hypotheses of this model concern two main points: the transmission of *Wolbachia* is affected by stochastic processes and a deterministic bias, and the bacterial load of the parents of a cross affects their compatibility relationships. It is shown that the variance of the number of bacteria transmitted induced by the stochastic processes tends to counteract the effect of bacterial curing on the dynamics of infection. A general consequence of the model is that the extinction of *Wolbachia* is possible even if there is strong incompatibility and no selective disadvantage for the host to bear the bacteria. The model indicates that the evolution of bacterial mutants does not depend on the level of incompatibility they induce, but that mutants with higher transmission variance can be selected for. Moreover, the mean infection level of the host population increases in the presence of such bacteria.

* Author for correspondence.

Introduction

Wolbachia, a *Rickettsia*-like endocellular microorganism, is present in many species of Arthropods (O'Neill et al., 1992; Juchault and Mocquard, 1993; Werren et al., 1995). This microorganism, which is maternally transmitted through the egg cytoplasm, is known to alter the sexuality of its host by different means, including parthenogenesis (Stouthamer, 1990), feminisation (Rigaud et al., 1991) and cytoplasmic incompatibility, an inability for two different strains of the host to produce viable progeny. In the simplest case, this phenomenon is observed when an infected male mates with an uninfected female, resulting in the failure of egg hatching (Yen and Barr, 1973).

Theoretical analyses have focused on the dynamics of cytoplasmic incompatibility and particularly on the conditions required for loss, fixation or polymorphism of the infection in natural populations. These models distinguished the presence or absence of *Wolbachia* in individuals (Turelli, 1994; Hurst and McVean, 1996 and references therein). In several species where cytoplasmic incompatibility is known to occur, natural populations of the host are polymorphic for the infection, i.e. infected individuals coexist with uninfected ones (e.g., Turelli and Hoffmann, 1995). This phenomenon is of interest because females carrying a *Wolbachia*-free cytoplasm are at a disadvantage in a population with infected males. However, for *Drosophila simulans*, *D. melanogaster* and *Nasonia vitripennis*, the level of the infection is variable between individuals (Boyle et al., 1993; Breeuwer and Werren, 1993; Bressac and Rousset, 1993; Solignac et al., 1994; Rousset and De Stordeur, 1994; Merçot et al., 1995; Hoffmann et al., 1996) and the incompatibility has been found to depend, at least in part, on the level of infection of the insects i.e. the bacterial densities in the hosts (Boyle et al., 1993; Breeuwer and Werren, 1993; Solignac et al., 1994; Merçot et al., 1995; Sinkins et al., 1995; Bourtzis et al., 1996).

The bacterial densities of an offspring and its mother may be different (Boyle et al., 1993; Breeuwer and Werren, 1993; Rousset and De Stordeur, 1994; Solignac et al., 1994; Turelli and Hoffmann, 1995). However, they are correlated, as illustrated by the relative stability of infection levels within strains or the gradual decrease of infection in crosses with infected females and uninfected males (Rousset and De Stordeur, 1994). This peculiar inheritance of infection level may be due to stochastic processes during oogenesis and maternal transmission by which the segregation of a limited number of microorganisms is not equal among a finite pool of cells. According to this hypothesis, the variability of infection level may be a more general phenomenon. Breeuwer and Werren (1993) have proposed a simple model to explain the effect of parental bacterial densities on cytoplasmic incompatibility in *Nasonia* wasps. Following their "bacterial dosage" model, sperm would be incompatible with an egg when the number of bacteria in the male strain is greater than in the female strain. In *D. simulans* and *D. melanogaster*, the fraction of incompatible zygotes seems to be a more continuous function of densities in parental strains at least in paternal strains (Solignac et al., 1994; Merçot et al., 1995).

The possible evolutionary consequences of such a mechanism of inheritance of bacterial density and its effect on incompatibility are investigated in the model

Dynamics of *Wolbachia* infection

presented here, that incorporates a potentially continuous range of infection density (Fig. 1), and assumes that the fraction of incompatible zygotes is a continuous function of densities in parental strains.

Analytical model and simulations

This analysis is focused on the dynamics of the *Wolbachia* infection in a panmictic population. We assumed that the individual infection is a continuous variable and is characterised by a probability density. We defined three identically distributed variables, M and F for the distribution of infection level m in males and f in females and Z as a third auxiliary variable. In this model, the bacterial density may evolve under different constraints of selection, i.e. the incompatibility phenomenon, and maternal transmission. In order to simplify the analytical part of the model, we will assume that the survival probability of the offspring depends on the infection level of the parents, that the effects of the maternal transmission on the bacterial density take place during the development of the individuals, and finally we will neglect the boundary effect of the limiting values 0 (no infection) and 1 (maximum infection level). Such assumptions have been made to keep the analysis

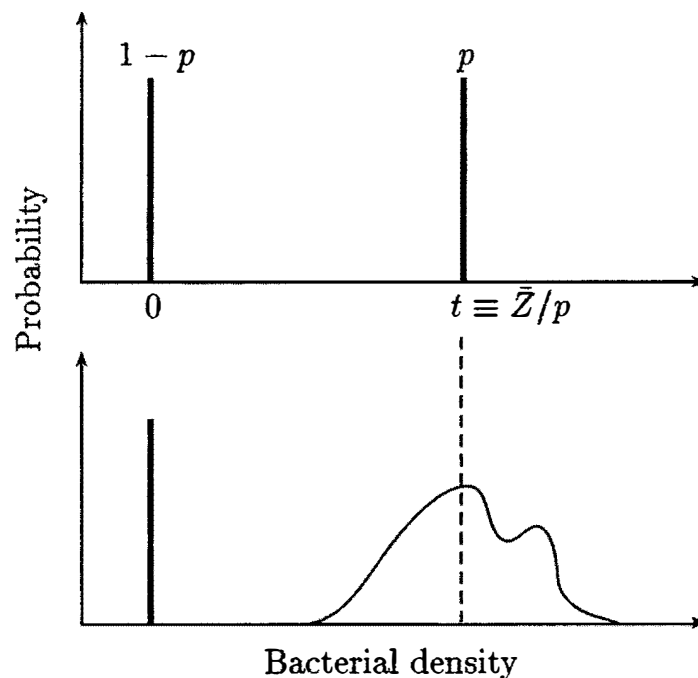


Fig. 1. General patterns of distributions of *Wolbachia* infection level of an host population showing the main differences between the present model (continuous) and the discrete model. The two distributions have the same average \bar{Z} .

as simple as possible. Simulations where it is assumed first, that stochastic factors affect the infection level of the gametes, second, that survival is a function of infection levels of the gametes and where boundary effects are taken into account, will be given.

1. Selection

We will consider that the survival probability of an embryo depends linearly on the infection levels m of the male and f of the female.

$$s(f, m) = 1 - \alpha m(1 - f) \quad (1)$$

where the constant α is the parameter describing the strength of the incompatibility of an incompatible cross. For example, in the case of an incompatible cross, if the male has a bacterial density of 1 ($m = 1$, in practice the maximum observed) and the female is *Wolbachia*-free ($f = 0$), incompatibility will be α . Following Eq. (1) and under the hypothesis of panmixia, the average survival is $\bar{s} = 1 - \alpha \bar{Z}(1 - \bar{Z})$ where $\bar{M} = \bar{F} = \bar{Z}$ is the average infection level of parents. Let us define the expected survival of embryo with a maternal infection level of f as $s(f) = 1 - \alpha \bar{Z}(1 - f)$. Then, the product of embryo's infection from f infected mother by embryo's relative fitness is $f s(f)/\bar{s}$. Thus, integrating this expression over the distribution of f , the mean infection level of the embryos after selection can be written

$$I = Z \left(1 + \frac{\alpha V}{\bar{s}} \right) \quad (2)$$

where V is the variance of Z .

2. Transmission

The hypothesis assumes the existence of two factors affecting the maternal transmission: some stochastic events and a transmission bias. First, the stochastic variation corresponds to a drift during transmission since the segregation of a limited number of microorganisms is not equal among a finite pool of cells. Therefore, a variance in the number of bacteria transmitted by a female exists (this variance v is called transmission variance in the following). Second, a number of works supposed the existence of transmission bias that can be explained, for example, by larval curing (Hoffmann et al., 1986; Hoffmann and Turelli, 1988; Hoffmann et al., 1990; Turelli et al., 1992; Wade and Stevens, 1994) by which an infected female produces *Wolbachia*-free offspring. In our continuous model, such a transmission bias is assumed to act on the expected average density of symbionts during the development of the larva. Therefore, if we assume that the decrease of bacterial density in larva is proportional to the parasitic load, the mean infection level of the larvae \bar{I}_L is

$$\bar{I}_L = (1 - \mu)\bar{I}. \quad (3)$$

In the following, we will be interested in the cases where a female generally transmits to the next generation fewer *Wolbachia* than its initial load (e.g., larval curing), so that $0 \leq \mu \leq 1$.

3. Synthesis

The infection dynamics of a population is subject to two opposite forces. The transmission bias tends to decrease the mean bacterial density while the infection variance among hosts increases the mean injection level of the population. Indeed, the variation $\Delta\bar{Z}$ of the mean bacterial density between \bar{I}_L , the larval population and \bar{Z} , the parental population can be obtained by computing Eq. (2) and Eq. (3) after simplifications:

$$\Delta\bar{Z} \sim \bar{Z}(\alpha V - \mu). \quad (4)$$

We can compare the two different biological cases of discrete and continuous infection distributions presented in Figure 1, and we can show that the selection due to the incompatibility relationship has a stronger effect on the infection distribution in the case of variably infected individuals than in the discrete case of presence/absence of bacteria. As a matter of fact, the two infection distributions with the same mean \bar{Z} have different variances (Fig. 1) and this difference is proportional to the variance of the infection distribution among infected individuals of the continuous distribution:

$$V_{\text{continuous}} - V_{\text{discrete}} = p \text{Var}(Z \mid \text{infection}) \quad (5)$$

where p is the frequency of infected individuals. It results in a difference in the mean variation of infection:

$$\Delta\bar{Z}_{\text{continuous}} = \Delta\bar{Z}_{\text{discrete}} + \bar{Z} \alpha p \text{Var}(Z \mid \text{infection}). \quad (6)$$

Moreover, the relative effect of the presence of variably infected individuals will be more important when the mean of infection is low because $V_{\text{discrete}} = \bar{Z}^2 p(1-p)$.

Additionally, the model suggests that the conditions of persistence of the symbionts are less restrictive in the continuous than in the discrete model. Indeed, if the transmission variance goes to zero (i.e. discrete model) the infection variance will go to a minimum ($V_{\text{discrete}} < V_{\text{continuous}}$ for equal mean infection levels) and persistence of the symbionts at equilibrium will be possible for a lower range of values of μ .

As assumptions were made to simplify the analytical development and since no exact analytical relation between the evolution of infection and the transmission bias and variance is available, simulations of the infection dynamics in a population of hosts have been made. We considered a panmictic population of constant size N with non-overlapping generations, and an unbiased sex-ratio. We assumed that the infection level of the female gamete (and therefore of the offspring) is randomly

sampled in a normal probability distribution with a mean $(1 - \mu)f$ where f is the infection of the mother, and a variance v (the transmission variance). The survival probability of the offspring was generated as in Eq. (1) of the analytical model with the bacterial loads of the gametes instead of those of the parents. Moreover, the values 0 and 1 of infection level are limiting values, i.e. offspring of non infected females will be non infected and females with maximum bacterial density will produce only offspring with less or equal amounts of *Wolbachia*.

Simulations show that the transmission variance v has the same influence on the dynamics of the mean infection as that found in the mathematical model (Fig. 2a) since it partially determines total variance of the infection level of the population V . For a population at equilibrium for an intermediate mean infection level, simulations show that $\mu \sim \alpha V$ (Fig. 2b), which is a sufficient condition to obtain $\Delta Z = 0$ in Eq. (4). We have studied the relationship between transmission variance and the probability of losing the bacteria during a fixed generation number (Fig. 2c). As expected, increased transmission variance favours the persistence of the symbionts. For large values of transmission variance v , the bacterial loss is due to stochastic effects, and for low values of v , bacterial loss is deterministic. Figure 2a illustrates this point, where *Wolbachia* is maintained in a large population (2500 individuals) for large values of v , and is lost for low values of v .

4. Alternative survival functions

We have assumed that the survival of an offspring is linearly correlated to parental bacterial densities. Our model supposes that an intrasrain cross can lead to a maximum incompatibility rate of 0.25 when the insects are slightly infected. This is roughly in agreement with the results of Rousset and De Stordeur (1994) for crosses between *Drosophila simulans* strains. The data available are too scarce for a good approximation, but the form

$$s(f, m) = 1 - \alpha m^n (1 - f)^k \quad (7)$$

has been investigated and we found that

$$s(f, m) = 1 - \sqrt{m}(1 - f) \quad (8)$$

is a better fit for the results of Rousset and De Stordeur (1994). This function describes more exactly the intensity of partial bidirectional incompatibility encountered between moderately infected parents. In this case, considering a higher intensity of incompatibility, thus harder selection, the weight of the infection variance on the dynamics of *Wolbachia* is increased. Simulations give the same result and, for example, equilibrium at intermediate mean infection level can be maintained when $\mu > V$ (Fig. 2b). However, Merçot et al. (1995) found a linear relationship between male infection level and incompatibility in crosses with infected males and uninfected females in *Drosophila simulans*. The situation seems to be variable when considering different host species. For example, Solignac et al.

Dynamics of *Wolbachia* infection

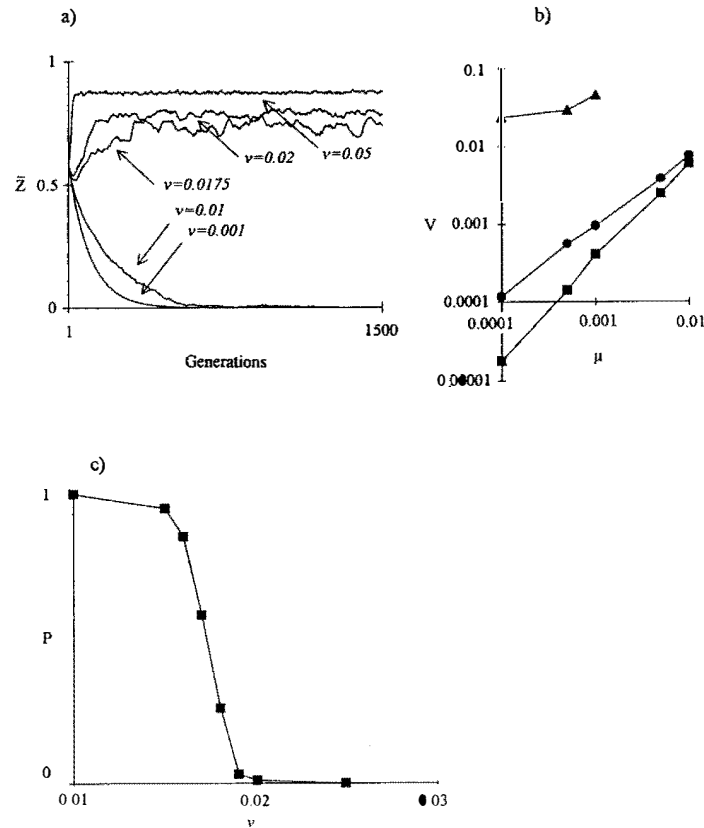


Fig. 2. (a) Dynamics of the mean infection level \bar{Z} of a population. Simulations use $n = 2500$ individuals and $\mu = 0.01$. (b) Relation between the transmission bias μ and the mean infection variance V over 2000 generations of quasi-stable populations for their infection level ($\Delta\bar{Z} \sim 0$) with $s(f, m) = 1 - m(1 - f)$ (circles), $s(f, m) = 1 - \sqrt{m}(1 - f)$ (squares) and $s(f, m) = 1 - m(1 - f)^k$ with $k = 72$ (triangles); $n = 2500$ individuals. (c) Probability P of the bacterial loss as a function of the transmission variance v . Simulations use $n = 100$ individuals, 100 repetitions, 2000 generations and $\mu = 0.01$.

(1994) have compared the results of crosses between females of *Drosophila melanogaster* from various strains and males from a highly infected strain, and crosses between females from an uninfected strain and males from the various other strains. A correlation was found between infection level of males and strength of incompatibility when males were crossed with uninfected females while females from the weakly infected strains were as compatible with heavily infected males and females from heavily infected strains (uninfected females were incompatible). Thus, the results of Solignac et al. (1994) are compatible with the limit situation where $m = 1$ and k is large in Eq. (7). In this case, we may expect from the model that selection will be negligible and that the maternal transmission bias would efficiently lead to the decrease of bacterial load. Indeed, simulations show that the equilibrium

variance must be far larger than μ (Fig. 2b). When female infection is low, $s(f, m) \sim 1 - \alpha m(1 - kf)$, so that the probability of bacterial loss is not increased compared to that of the first model when initial infection is low. However, we expect from this alternative situation that *Wolbachia* would rapidly reach a low density in the host population and therefore are susceptible to be lost by stochastic effects. This may be the case in *Drosophila melanogaster* where only 34% of the flies are infected and where the mean infection level in males is only about 6% of infected cysts (Solignac et al., 1994).

5. Two clones

It is possible to generalize the model by considering two different clones of *Wolbachia* in a single host population: clone S (sterilising) and L (less sterilising). We assume that multiple infections do not occur. The difference between bacteria S and L is their capacity to make a cross more or less incompatible when S or L *Wolbachia* are present in a male whatever the bacteria present in the female. This difference is characterised by the incompatibility parameters α_S and α_L with $\alpha_S \geq \alpha_L$. We will consider the two continuous variables Z_S and Z_L describing the infection levels of individuals carrying S and L *Wolbachia*. In this model, the constraints are the same as those for single-bacteria model.

An interesting point is the case of a host carrying a L mutant bacteria in a population of S infected individuals. It can be shown that the evolution of the mutant does not depend on its capacity to sterilise females of the population but on the infection level at which it appears (Eq. (9)). This confirms the results of Turelli (1994) in the cases where no kin selection is included in the models (see Frank, 1997).

Using the same argument as in the single clone model and considering the most general conditions for the appearance of an individual carrying L mutant *Wolbachia*, we can write the mean of the infection variation due to incompatibility and bacterial curing over all individuals with L bacteria as

$$\Delta \bar{Z}_L \sim [-\mu + \alpha_S(ptg - p^2t^2)]g \quad (9)$$

where p is the proportion of infected individuals, $t \equiv \bar{Z}_S/p$, and g the infection level at which the mutant appears in the population.

Another interesting consequence of this model is that mutant bacteria are generally neutral and that the maintenance of *Wolbachia* infection in a population is due to the fraction of individuals with a high bacterial load.

Indeed, given that the roots of Eq. (9) are

$$g = 0 \quad \text{and} \quad g = \frac{\mu}{\alpha_S p t} + pt \approx pt \quad (10)$$

and that the mean of infection level at which the mutant appears is $\bar{G} = pt$, mutant

Dynamics of *Wolbachia* infection

will be generally neutral. The only cases of maintenance involve mutants with a high infection level ($g > pt$).

However, mutants associated with higher transmission variance can be selected for. Indeed, if the transmission variance of a mutant can be different from that of the prevalent clone, a mutant with a high transmission variance is more likely to reach the lowest as well as the highest infection loads, and if the most highly infected hosts are the future ancestors of the population, a mutant *Wolbachia* with unchanged transmission efficiency but higher transmission variance should be selected for. Simulations (Fig. 3) confirm that this is so, the probability of fixation of the mutant in this case being equivalent to that of a mendelian mutant with selective advantage:

$$s \sim \frac{0.03}{5} \left(\frac{v_{\text{mutant}} - v_{\text{wild}}}{v_{\text{wild}}} \right). \quad (11)$$

Discussion

This model shows that selection due to incompatibility depends on the distribution of the infection level in the population. For example, consider the extreme case

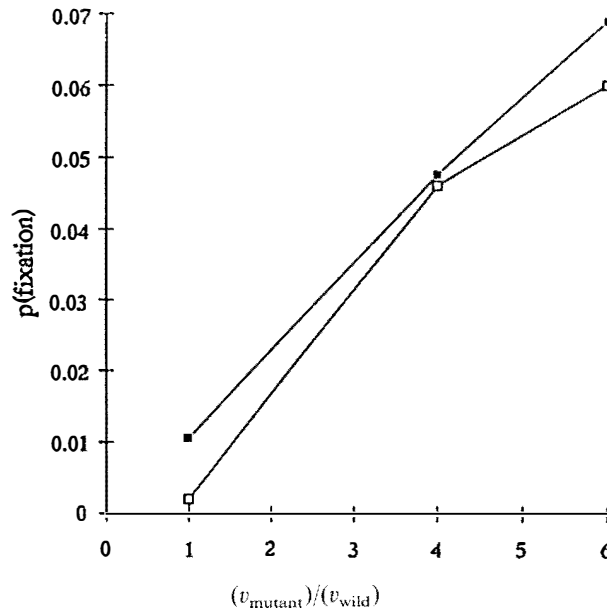


Fig. 3. Probability of fixation of a mutant as a function of its transmission variance v_{mutant} . Simulations use $n = 100$ (closed squares) or 500 (open squares) individuals, 2000 repetitions, 3000 generations, $\mu = 0.0015$ and the wild *Wolbachia* transmission variance $v_{\text{wild}} = 0.005$.

of an infection with null variance in the population, selection due to cytoplasmic incompatibility will not act because all individuals have the same bacterial load, so that the infection can be lost if the transmission bias is positive ($1 - \mu < 1$). Conversely, the selection will have maximum effect when there is maximal variance in the population.

The significance of infection distribution variance suggests that the stochastic effects affecting the transmission of the endosymbionts during the oogenesis are important to explain the evolution of the infection because it partially determines the total variance of infection in the next generations. Simulations confirm the importance of the transmission variance, v . With a fixed parameter of bacterial curing μ , a population can lose the symbionts if v is small. On the other hand, the infection can be maintained at a high level when the variance of transmission is large in spite of the recurrent curing of the microorganisms in females. Previous models have shown that the *Wolbachia* infection can be lost in the case of cost inducing bacteria; see for example Hurst and McVean (1996) who presented a general model. Here, we have shown that the extinction of *Wolbachia* is possible even if there is strong incompatibility and no selective disadvantage for the host to bear the bacteria.

Influence of transmission bias μ cannot be easily compared to the curing parameter of previous works. In discrete models, this parameter is the proportion of individuals losing *Wolbachia* in one generation (e.g. Turelli et al., 1992). In the continuous model, total curing mainly concerns individuals whose mothers were very slightly infected. It will depend on the distribution of infection in females and on the distribution of differences between mothers and offspring.

In the case of bacterial mutation in an initially infected population, the evolution of the mutant does not depend initially on the level of incompatibility they induce. The spread or loss of the new bacteria depends on the infection level of its host compared with the distribution of infection of the original population. A mutant bacteria will spread initially in the population if its density in the host is high enough (higher than the mean infection of the population). The study of a mutant is a way to compare the dynamics of individual infection with the dynamics of population infection: it shows that the ancestors of the whole bacterial population may be the bacteria in individuals with a bacterial load higher than the mean infection of the population, and that mutants with a higher transmission variance will be selected for if there is variation for this trait.

We still know very little of both the distribution of infection in the offspring of a female and of potential variation of the characters considered here that could help predict the fate of an infection in natural populations. However, these models show that the transmission variance may be an important factor that deserves attention in experimental and population studies. Indeed, different experimental lines may differ in their incompatibility levels due to purely stochastic factors (i.e. neither host nor symbionts differences). This will be particularly relevant when the transmission bias is positive (Rousset and De Stordeur, 1994), the infection frequency of the host population is intermediate and the strength of incompatibility is intermediate (Hoffmann et al., 1994; Solignac et al., 1994).

Acknowledgements

We are very grateful to S. Frank, S. L. O'Neill, D. Bourguet, P. Parker and M. Raymond for helpful comments and discussions. This work was financed in part by a GDR 1105 du programme Environnement, Vie & Sociétés du CNRS, an ACC (No SV39503017) and a CEE grant (No ERBCHRXCT930172). T.G. benefited from a MESR fellowship (n° 94137). This is contribution ISEM 96.162 of the Institut des Sciences de l'Evolution (UMR CNRS 5554).

References

- Bourtzis, K., A. Nirgianaki, G. Markakis and C. Savakis. 1996. *Wolbachia* infection and cytoplasmic incompatibility in *Drosophila* species. *Genetics* 144: 1063–1073.
- Boyle, L., S. L. O'Neill, H. M. Robertson and T. L. Karr. 1993. Interspecific and intraspecific horizontal transfer of *Wolbachia* in *Drosophila*. *Science* 260: 1796–1799.
- Breeuwer, J. A. J. and J. H. Werren. 1993. Cytoplasmic incompatibility and bacterial density in *Nasonia vitripennis*. *Genetics* 135: 565–574.
- Bressac, C. and F. Rousset. 1993. The reproductive incompatibility system in *Drosophila simulans*: Dapi-staining analysis of the *Wolbachia* symbionts in sperm cysts. *J. Inv. Pathol.* 31: 226–230.
- Frank, S. A. 1997. Cytoplasmic incompatibility and population structure. *J. Theor. Biol.* 183: 327–330.
- Hoffmann, A. A., D. Clancy and J. Duncan. 1996. Naturally-occurring *Wolbachia* infection in *Drosophila simulans* that does not cause cytoplasmic incompatibility. *Heredity* 76: 1–8.
- Hoffmann, A. A., D. J. Clancy and E. Merton. 1994. Cytoplasmic incompatibility in Australian populations of *Drosophila melanogaster*. *Genetics* 136: 993–999.
- Hoffmann, A. A. and M. Turelli. 1988. Unidirectional incompatibility in *Drosophila simulans*: Inheritance, geographic variation and fitness effects. *Genetics* 119: 435–444.
- Hoffmann, A. A., M. Turelli and L. G. Harshman. 1990. Factors affecting the distribution of cytoplasmic incompatibility in *Drosophila simulans*. *Genetics* 126: 933–948.
- Hoffmann, A. A., M. Turelli and G. M. Simmons. 1986. Unidirectional incompatibility between populations of *Drosophila simulans*. *Evolution* 40: 692–701.
- Hurst, L. D. and G. T. McVean. 1996. Clade selection, reversible evolution and the persistence of selfish elements: the evolutionary dynamics of cytoplasmic incompatibility. *Proc. R. Soc. Lond. B* 263: 97–104.
- Juchault, P. and J. P. Mocquard. 1993. Transfer of a parasitic sex factor to the nuclear genome of the host: A hypothesis on the evolution of sex-determining mechanisms in the terrestrial Isopod *Armadillidium vulgare* Latr. *J. evol. biol.* 6: 511–528.
- Merçot, H., B. Llorente, M. Jacques, A. Atlan and C. Montchamp-Moreau. 1995. Variability within the Seychelles cytoplasmic incompatibility system in *Drosophila simulans*. *Genetics* 141: 1015–1023.
- O'Neill, S. L., R. Giordano, A. M. E. Colbert, T. L. Karr and H. M. Robertson. 1992. 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. *Proc. Natl. Acad. Sci. USA* 89: 2699–2702.
- Rigaud, T., C. Souty-Grosset, R. Raimond, J. P. Mocquard and P. Juchault. 1991. Feminizing endocytobiosis in the terrestrial Crustacean *Armadillidium vulgare* Latr. (Isopoda): Recent acquisitions. *Endocyt. Cell Res.* 7: 259–273.
- Rousset, F. and E. De Stordeur. 1994. Properties of *Drosophila simulans* experimentally infected by different clones of the bacterium *Wolbachia*. *Heredity* 72: 325–331.
- Sinkins, S. P., H. R. Braig and S. L. O'Neill. 1995. *Wolbachia pipiensis*: bacterial density and unidirectional cytoplasmic incompatibility between infected populations of *Aedes albopictus*. *Exper. Parasitol.* 81: 284–291.
- Solignac, M., D. Vautrin and F. Rousset. 1994. Widespread occurrence of the proteobacteria *Wolbachia* and partial cytoplasmic incompatibility in *Drosophila melanogaster*. *C. R. Acad. Sci. Paris, ser. III* 317: 461–470.

- Stouthamer, R. 1990. Effectiveness of several antibiotics in reverting thelytoky to arrhenotoky in *Trichogramma* spp., pp 119–122. In INRA (ed.), *Trichogramma* and other egg parasitoids, San Antonio (Tx, USA).
- Turelli, M. 1994. Evolution of incompatibility inducing microbes and their hosts. *Evolution* 48: 1500–1513.
- Turelli, M. and A. A. Hoffmann. 1995. Cytoplasmic incompatibility in *Drosophila simulans*: dynamics and parameter estimates from natural populations. *Genetics* 140: 1319–1338.
- Turelli, M., A. A. Hoffmann and S. W. McKechnie. 1992. Dynamics of cytoplasmic incompatibility and mtDNA variation in natural *Drosophila simulans* populations. *Genetics* 132: 713–723.
- Wade, M. J. and L. Stevens. 1994. The effect of population subdivision on the rate of spread parasite-mediated cytoplasmic incompatibility. *J. Theor. Biol.* 167: 81–87.
- Werren, J. H., D. Windsor and L. Guo. 1995. Distribution of *Wolbachia* among neotropical arthropods. *Proc. R. Soc. Lond. B* 262: 197–204.
- Yen, J. H. and A. R. Barr. 1973. The etiological agent of cytoplasmic incompatibility in *Culex pipiens*. *J. Inv. Pathol.* 22: 242–250.