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Modulation of host immunity and reproduction by horizontally acquired *Wolbachia*

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ABSTRACT

The *Wolbachia* are symbiotic bacteria vertically transmitted from one host generation to another. However, a growing amount of data shows that horizontal transfers of *Wolbachia* also frequently occur within and between host species. The consequences of the arrival of new symbionts on host physiology can be studied by their experimental introduction in asymbiotic hosts. After experimental transfers of the eight major isopod *Wolbachia* strains in the isopod *Porcellio dilatatus* only two of them (*w*Con and *w*Dil) were found to (1) have no pathogenic effect on the host and (2) be able to pass vertically to the host offspring. In the present work, we studied the influence of these two strains, able to complete an horizontal transfer, on immunity and reproduction of *P. dilatatus* at two stages of the transfer: (1) in recipient hosts that encounter the symbionts: to test the influence of symbiont when acquired during host life and (2) in vertically infected offspring: to test the influence of a symbiotic interaction occurring all lifelong. The impact of *Wolbachia* varied depending on the stage: there were clearer effects in vertically infected individuals than in those that acquired the symbionts during their lives. Moreover, the two *Wolbachia* strains showed contrasted effects: the strain *w*Con tended to reduce the reproductive investment but to maintain or increase immune parameters whilst *w*Dil had positive effects on reproductive investment but decreased the investment in some immune parameters. These results suggest that horizontally acquisition of *Wolbachia* can influence the balance between host immune and reproductive traits.

1. Introduction

Wolbachia, considered the most widespread symbionts in arthropods, are intracellular bacteria vertically transmitted from mother to offspring that are known to manipulate their host reproduction in order to increase such a path of transmission (Werren et al., 2008). However, it is increasingly recognised that the widespread distribution of *Wolbachia* in arthropods is also linked to horizontal transfers that constitute an important way to colonize new hosts at both intra and interspecific levels (Baldo et al., 2008; Huigens et al., 2004; Le Clec'h et al., 2013; Narita et al., 2006; Shoemaker et al., 2002). Once *Wolbachia* are in the recipient host, both the genomes of the host and the symbiont jointly produce an extended phenotype that often differs from the initial one. For instance, the immune phenotype of the host has been shown to be modified by *Wolbachia* in many ways leading to either an increase or a decrease in the level of immune parameters (Braquart-Varnier et al., 2008; Hughes et al., 2011; Kambris et al., 2009, 2010; Moreira et al., 2009; Sicard et al., 2010; Thomas et al., 2011). To date, *Wolbachia* are among the most studied symbionts regarding influence of symbiotic interaction on host resistance towards other invaders (Eleftherianos et al., 2013), especially because it has been demonstrated that their presence modify immune competence of arthropod vectors against several viruses, bacteria and also filarial and malaria parasites (Hedges et al., 2008, 2011; Kambris et al., 2009, 2010; Moreira et al., 2009; Osborne et al., 2009; Teixeira et al., 2008; Ye et al., 2013; Zélé et al., 2012). However, artificially introduced *Wolbachia* have been recognised to induce stronger modifications in immune phenotype than the naturally associated ones (Hughes et al., 2011;

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Kambris et al., 2009, 2010; Moreira et al., 2009; Thomas et al., 2011). This is true in the trans-infected individuals (i.e., in the animals receiving injection of *Wolbachia*; Kambris et al., 2010) but also in their offspring (i.e., F1, F2, etc, Hughes et al., 2011; Kambris et al., 2009; Moreira et al., 2009). A possible explanation is that in the case of artificial association, the host faces a new symbiont with which it did not co-evolved and this unusual interaction can drive an over investment in immunity. As immune functions are costly to implement for the host (Moret and Schmid-Hempel, 2000), the immune variations induced by *Wolbachia* can also indirectly influence reproduction *via* a trade-off (Chen et al., 2000).

The *Wolbachia*-isopod symbiotic systems set good examples to study the impact of symbiosis on immunity. For instance, previous studies have demonstrated that the *w*VulC feminizing strain of *Wolbachia* modified immune parameters such as Phenoloxydase (PO) activity and Total Haemocyte Count (THC) in its native host *Armadillidium vulgare* (Braquart-Varnier et al., 2008; Sicard et al., 2010). *Porcellio dilatatus* is another terrestrial isopod for which some populations exhibit almost only infected individuals, harbouring a strain responsible for cytoplasmic incompatibility (CI) called *w*Dil, whilst some other populations comprise only uninfected individuals (Sicard et al., 2014). A recent study showed that *P. dilatatus* offers interesting opportunities to address the impact of different *Wolbachia* strains on the same host. Indeed, Le Clec'h et al. (2013) used the isopod strains of *Wolbachia* that were previously characterised on their host phenotype manipulation (i.e., mainly feminization or cytoplasmic incompatibility) and injected them to asymbiotic *P. dilatatus* as recipient hosts. Whilst all the *Wolbachia* were capable of colonizing all the tested organs of this recipient host, their consequences on host life history traits (i.e., growth, mobility, survival) were quite contrasted: (1) the feminizing *w*VulC, *w*VulM and *w*PruIII resulted in the death of most of the individuals (Le Clec'h et al., 2012, 2013), (2) *w*Ase, *w*Bre, *w*Pet were found unable to pass to the next generation (Le Clec'h et al., 2013) and (3) *w*Dil and *w*Con were the only ones capable of performing a complete horizontal transfer by finding their vertical way to the offspring (Le Clec'h et al., 2013). By using the two latter host-symbiont combinations (i.e., one intraspecific horizontal transfer with *w*Dil and one interspecific horizontal transfer with *w*Con), we addressed the question of the influence of horizontally acquired *Wolbachia* on immune and reproductive investments of the host *P. dilatatus*. Concerning immunity, we have measured three primordial cellular and humoral parameters: the haemocyte density, the phagocytosis activity and the total PO activity. To assess reproductive investment, we counted the number of oocytes per ovary. This way, we highlighted that the investment in both immune and reproductive life history traits of the terrestrial isopod *P. dilatatus* change due to (i) the acquisition mode (i.e., during life by injection or vertical acquisition) and (ii) the genotype of the *Wolbachia*.

2. Materials and methods

2.1. Biological materials

The biological model of this study is the terrestrial isopod *Porcellio dilatatus dilatatus*. In this species, reproduction only occurs once a year. Before reproduction, females undergo a preparturial moult leading to sexual receptivity. Then, males and females copulate and the fecundated oocytes are laid in the female marsupium. After an incubation period in the marsupium, the offspring is released in the environment. Then, young animals (i.e., pulli) are grown at 20 °C in plastic breeding boxes, in natural photoperiod, on moistened potting mix derived from peat from sphagnum moss (pH = 6.4 and conductivity = 50 mS/m) with dead lime-tree leaves as a food source. From animals collected in the field, two lines of *P. dilatatus* are maintained in the laboratory. The first one called *dilatatus* A which is asymbiotic (i.e., without any *Wolbachia*) was initially sampled in the village of Rom (France) whilst the second one, called *dilatatus* S which is naturally infected with *Wolbachia* (*w*Dil) was initially sampled in Sainte Marguerite Island (France). In a previous study, the native *Wolbachia* strain (*w*Dil) responsible for CI in *P. dilatatus* (Sicard et al., 2014) and another CI *Wolbachia* strain from *Cylisticus convexus* (*w*Con, Moret et al., 2001) have been artificially introduced by injection in the *dilatatus* A genetic background (Le Clec'h et al., 2013). Both *Wolbachia* were found to be able to vertically transmit from injected mothers to the individuals of the F1 generation which have been used in the present experiments (Le Clec'h et al., 2013). These F1 animals were used in order to study the impact of vertically transmitted *Wolbachia* strains (*w*Dil & *w*Con) on the life history traits of *P. dilatatus*. They are hereinafter referred to as 'F1 *dilatatus* A-*w*Dil' and 'F1 *dilatatus* A-*w*Con'. Experiments were conducted in parallel with animals which are naturally infected with *w*Dil but exhibit a different host genetic background than '*dilatatus* A' (Sicard et al., 2014). They are herein after refereed as '*dilatatus* S'.

To study the impact of *w*Dil and *w*Con on *P. dilatatus* when horizontally acquired during the life of the host (i.e., trans-infection), the *Wolbachia* strains were injected to 6-months-old virgin females and males. To do so, filtered suspensions of crushed ovaries of the donors (*dilatatus* S females for *w*Dil and *C. convexus* females for *w*Con) were injected to recipient *dilatatus* A animals. For each batch of injection, ovary suspensions were prepared with the ovaries of (i) five *dilatatus* S females, (ii) five *C. convexus* females or (iii) five *dilatatus* A females (i.e., control). The ovaries were collected and crushed into 1 ml of Ringer solution. The resulting suspension was filtered through a 1.2 μm pore membrane, and 1 μL of each filtrate was injected in a small hole pierced in each individual cuticle, using a thin glass needle, into the general cavity, at the posterior part of animals. These injected animals are hereinafter referred to as '*dilatatus* A injected *w*Dil', '*dilatatus* A injected *w*Con' or '*dilatatus* A injected control'. Measurements of life history traits were conducted six months after the injection, when the animals were aged of one-year-old. This led us to compare these trans-infected animals with vertically infected ones of the same age. Moreover, this delay of 6 months has been previously demonstrated as being enough time to obtain an infection as intense in trans-infected animals as the one measured in individuals that were vertically infected (Le Clec'h et al., 2013). Control asymbiotic animals received an equivalent injection of crushed ovaries coming from individuals that did not host *Wolbachia*.

Importantly, all animals (infected with *w*Dil, *w*Con or control) selected for experiments were of a similar weight. Females analysed were virgin and in their intermoulting period (i.e., no calcium plates) as described in Beauché and Richard (2013).

2.2. Measurement of the different life history traits

On animals from the different treatments: injection *versus* vertical acquisition of *w*Dil or *w*Con and their respective controls, we monitored a maximum of five parameters: (1) Total Haemocyte Count (THC), (2) PhenolOxidase Activity (PO), (3) Phagocytosis Activity, (4) Reproductive investment: number of oocytes per ovary, (5) *Wolbachia* titer: *Wolbachia* quantification in gonads. The same animals were used to measure *Wolbachia* titers, THC and either PO activity or phagocytosis activity because the last immune parameters required too much haemolymph to perform both of them on the same animal. For all females, the number of oocytes per ovary was counted. The sample size for the

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