



Presence of cryptic species in host insects forms a hierarchical *Wolbachia* infection pattern

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With 1 figure and 1 table

Abstract: Although *Wolbachia* spp. are described as one of the most prevalent groups of endosymbiotic intracellular bacteria in invertebrates, studies often found large variations in *Wolbachia* infection rate at the species level. The underlying mechanisms of these variations however remain unclear. Given some phenotypic effects of *Wolbachia* such as cytoplasmic incompatibility and difficulties to be transmitted among host populations with deep genetic divergence, we hypothesized that cryptic species could play an important role in the spreading success of *Wolbachia*. Here we used *Wiebesia pumilae*, the morphospecies specifically pollinating *Ficus pumila* to test our hypothesis. We collected individuals from 40 locations covering most of its distribution range in China. Using a mtDNA gene, we suggested a total of three cryptic species in *W. pumilae* with a parapatric distribution pattern. At the morphospecies level, we detected an overall *Wolbachia* infection rate of 52.8%. In contrast, at the cryptic species level, we found that all infected individuals belonged to only one cryptic species. These results support our hypothesis and indicate that cryptic species boundaries can create a hierarchical *Wolbachia* infection pattern and the potential role of *Wolbachia* infection in restricting gene flow among cryptic species.

Keywords: cryptic species, *Wolbachia*, infection pattern, morphospecies, pollinating fig wasps, *Wiebesia*

1 Introduction

Wolbachia (Rickettsiales, Rickettsiaceae) spp. are a group of endosymbiotic intracellular bacteria that prevalently infect invertebrates and can profoundly affect the population dynamics and even the evolutionary trajectories of their hosts (Sanaei et al. 2021; Werren et al. 2008). This is because *Wolbachia* strains can manipulate hosts' reproduction by the phenotypic effects of cytoplasmic incompatibility (CI, causing the death of offspring between individuals of incompatible infection status) (Poinsot et al. 2003), feminization of genetic males (Hiroki et al. 2002), thelytokous parthenogenesis (Weeks & Breeuwer 2001), and male killing (Duplouy et al. 2010). Moreover, other phenotypic effects of *Wolbachia* strains like resistance against viral pathogens and influences on egg production of hosts can be both beneficial and parasitic, and therefore the tradeoff between costs and

benefits will impact host fitness (Werren et al. 2008). If disadvantages in selection occur in infected individuals, spread of *Wolbachia* strains may be restricted, impeding the fixation of *Wolbachia* infection. Besides vertical transmission from ancestor hosts, *Wolbachia* can also infect new hosts sharing the same environments with their original hosts through horizontal transfer (Turelli et al. 2018).

Wolbachia spp. are expected to evolve to adapt to their hosts and spread across host populations. Although there are many phenotypic effects such as CI can facilitate their spreading and fixation of *Wolbachia* is expected (Jansen et al. 2008; Tong et al. 2021a; Zug et al. 2012), in nature, such an all-infected pattern has been seldom observed. Instead, a large variation in the infection rate at species level (from near 0% to 76%) is often observed, forming a “most-to-few” infection pattern (Werren & Windsor 2000; Kikuchi & Fukatsu 2003; Nirgianaki et al. 2003; Hilgenboecker