

Evolutionary and ecological interactions among selected taxa of beetles and endosymbiotic bacteria

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Summary of doctoral thesis

Endosymbionts of insects play a crucial role in biology of their hosts. However many aspects of their interactions with hosts are still a mystery. Among endosymbiotic bacteria the specific group are so called 'male-killers' that by various manipulations of their host's reproduction are changing sex ratio in the population. The best known and so far best studied endosymbiont is *Wolbachia*. This Gram-negative α -proteobacteria infects arthropods and nematodes (estimated 66% of insects are infected by it). With development of molecular techniques scientists discovered other bacteria with similar influence on their hosts such as: *Rickettsia*, *Spiroplasma* or *Cardinium*, however knowledge about their prevalence and diversity is still unsatisfactory. Along with increasing knowledge about the role of 'male-killers' in the hosts biology (including protection against viruses and providing of nutrients) their classification changed from parasites to endosymbionts. Endosymbionts influence on population differentiation have led to develop a thesis about coevolution between endosymbiont and its host. On the other hand more evidence has risen on behalf of horizontal transmission of *Wolbachia* between infected and uninfected species. The problem with full understanding of interactions between such bacteria and their hosts was due to studies analyzing either single species or focusing on selected hosts traits. Coleopterans are perfect organisms for studying relationship between endosymbionts and their hosts. Beetles are highly diversified group in terms of biodiversity (they represent 40% of all insect species), phylogenetic relations and ecological dependencies (this group consist representatives of many alternative evolutionary strategies including most of known trophic guilds).

There are presented the results of doctoral studies published already in six scientific articles (Kolasa et al. 2017 Eur J Entomol 114:446–454; Kotásková et al. 2018 Entomol Sci 21:385-395; Kolasa et al. 2018 Bull Insect 71:193-300; Kolasa et al. 2018 Folia Biol-Krakow 66:165-178; Kajtoch et al.

2019 Sci Rep 9:847; Kolasa et al. 2019 Microb Eco doi:10.1007/s00248-019-01358-y). These studies were focused on following aims explaining:

i) prevalence and diversity of endosymbiotic bacteria from the "male-killers" group among beetles; ii) co-evolution between *Wolbachia* and its hosts; iii) correlation between the hosts ecological and biological traits and *Wolbachia* infection; iv) possible ways of horizontal transmission of *Wolbachia* among beetles from various environments and trophic guilds; v) influence of *Wolbachia* on inducing parthenogenesis in *Strophosoma* weevil; and vi) determining whether the composition of the microbiota in beetles is shaped by the phylogenetic relationships of hosts, hosts trophic affinity or endosymbionts abundance.

The research was carried out on a very diverse group of beetle species (in total 297), each time selected for a specific purpose, using a comprehensive knowledge of their systematics, biology and ecology, and using molecular analyzes (bacterial genotyping and microbiota metabarcoding).

As a result of the conducted research *Wolbachia* has been determined as the most frequently occurring endosymbiont from the group of "male killers" in beetles. Among studied beetles 27% were hosts for *Wolbachia*, 7.7% were infected by *Rickettsia*, 3% by *Spiroplasma* and 2% by *Cardinium*. In addition, the results indicate that *Wolbachia* may be associated with hosts in a short evolutionary perspective (within genera), but we cannot talk about co-evolution with the host. On the other hand, the data obtained indicate that some categories of traits (such as reproduction method and trophic affinity, to a lesser extent: microhabitat preferences, distribution, mobility and body size) may be associated with *Wolbachia* infection status, while some others (e.g. range, thermal preferences) are not related to infection. Nevertheless, it is not possible to talk about a clear pattern of links between a specific set of traits and an infection.

Molecular analyzes provided evidences that the host plant (*Asparagus*) could be a vector for horizontal transmission of *Wolbachia* in the *Crioceris* leaf-beetles (Chrysomelidae). However, there is lack of evidence for a similar mechanism in the case of predatory beetles (in presented research riverine ground beetles Carabidae and rove beetles Staphylinidae). The results also indicate that hybridization not *Wolbachia* infection was responsible for the induction of parthenogenesis in one of the species of *Strophosoma* weevils (Curculionidae).

Research on the composition of bacterial microbiota of 24 beetle species from three trophic guilds (carnivorous, herbivorous and detritivorous) and five families (Carabidae, Staphylinidae, Curculionidae, Chrysomelidae, Scarabaeidae) showed that trophic affinity and phylogenetic relationships are the main factors shaping bacterial communities in beetles.

Although endosymbionts have been shown to be a factor that shapes microbiota composition of their host, their effect is negligible.

Presented studies are coherent and together significantly extends knowledge not only about the prevalence of “male killer” bacteria among beetles, but also provide new insight into the relationship between endosymbionts and their hosts. The conclusions presented above open the perspectives for further research, the most interesting of which may be those requiring experimental manipulation of infection in hosts with different phylogenetic relationships, breeding systems and ecological dependencies.