Wolbachia alters gene expression related to immunity and energy metabolism in *Chorthippus parallelus* (Orthoptera: Acrididae)

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Wolbachia pipientis is a mainly maternally transmitted obligate endosymbiotic bacterium, widely distributed in insects, with which it establishes complex symbiotic relationships. The continuity of the symbioses may rely on the physiological advantages that *Wolbachia* may confer to their host. Two subspecies of the grasshopper *Chorthippus parallelus* (Orthoptera: Acrididae), the Iberian endemism *C. p. erythropus* and *C. p. parallelus,* which is widely distributed throughout the rest of Europe, differ in morphological, behavioural, mitochondrial, nuclear and chromosomal characters, but also in the strains of the maternally transmitted bacterial endosymbiont *Wolbachia* infecting them. The distribution of both subspecies overlaps in the Pyrenees where they form a stable hybrid zone (HZ), so representing an appropriate system to identify 'key genes' that actually maintain genetic boundaries between emerging species. In fact, *Wolbachia* contributes to the reproductive barrier between both subspecies inducing in them uni- and bidirectional cytoplasmic incompatibilities.

In this work, we *de novo* characterised relevant genes in *C. parallelus*, as potential molecular biomarkers that show the physiological responses in individuals infected by *Wolbachia*, with particular attention to energy metabolism and immunity. *Wolbachia* induces the expression of carbohydrates and lipids metabolic genes as well as some others related to the immune system. This research explores the expression of reporter genes in the gonads of infected and uninfected adults of both sexes performed by means of quantitative real-time PCR. Reproductive organs were chosen since they are the main target of *Wolbachia* infection. Significant *Wolbachia* -and sex- dependent transcriptional effects were observed for most of the analysed biomarkers in infected and non-infected adults. Our data show how *Wolbachia* interferes with essential systems of *C. parallelus*, providing more information about its symbiotic relationship. Our initial, promising results show new sensitive biomarkers suitable for the study of the reproductive barrier that *Wolbachia* induces in the hybrid zone.

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