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Variability and Evolution of the venom of two biological control agents from the genus Psyttalia.

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Parasitoid wasps are widely used in biological control programs since they are naturally efficient to control the population of their host because of their antagonistic interactions. These antagonistic interactions lead to strong co-evolution between parasitoid virulence and host resistance. For instance, insect immune response to the intrusion of a foreign object, such as a parasitoid egg, is the encapsulation process, which requires both cellular and humoral components (Carton et al. 2008). To evade or counteract the host immune reaction, endoparasitoids have evolved different strategies (Poirié et al. 2009). The most often described is the injection of venom and/or ovarian factors that can include proteins, vesicular, or virus-like, components (also described as Virus-Like Particles, VLPs) or symbiotic viruses (such as polydnaviruses, PDVs) (Beckage and Drezen, 2012). As a consequence, the success of parasitoids used in biological control is likely to depend on the content of their venom and its efficiency to counteract the host immune reaction.

Previous studies focusing on venom protein content and diversity at inter-specific level have shown that venom is very diversified even between closely-related species (Crawford et al. 2008; Dominique et al. 2012). The intra-specific and intra-population variability can be studied thanks to protein electrophoresis coupled with new methods of image analysis that enable to quantify the variability of individual venom profiles. These methods have recently lead to results showing that the variability is surprisingly high even between individuals (Colinet et al. 2012). This high variability suggests that the evolutionary potential of venom is high and that venom components thus likely vary among populations of candidate biological control agents. This means that the screening of venom might be relevant to monitor biological control agents. Indeed, a high evolutionary potential is expected to affect chances of success of biological control as it is often assumed that establishment relies on adaptation to new environments. However, the high evolutionary potential may also select laboratory-fit individuals. For instance, biological control agents may become more adapted to their alternative host used in an industrial settings than to their natural (and target) host. The screening of such venomic diversity might thus permit following adaptation in response to the laboratory or to the release.

However, such screening would prove useful only if variation of venomic content is correlated with variation of parasitism success against target pests or substitution host used for laboratory rearing. Many venom proteins have already been shown to be involved in parasitism success (Asgari et al. 2003, Colinet et al. 2009). At the intra-specific level, several studies have shown an correlation between variability in host preference and variability in virulence (Kraaijeveld et al. 1995). Moreover, a correlation could be found between

quantitative variation of one venom protein essential for parasitism success and variability in virulence (Colinet et al. 2010, Dubuffet et al. 2009).

In this work, we have characterized and compared the venom content of various populations of the biological control agent *Psyttalia lounsburyi* (Silvestri) (Hymenoptera: Braconidae), used against the olive fruit fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae). Then, we have studied the evolution of the venom and of the parasitic success of various populations in controlled conditions.

The venom content was characterized by a combined transcriptomic and proteomic approach in order to identify the putative function of venom's proteins and explain the observed variation in venom contents. The venomic variability between individuals was studied at the individual level by 1D SDS-PAGE (Colinet et al. 2012) notably, to compare intra-population and inter population variability.

For this study, we used two *P. lounsburyi* strains from South Africa and Kenya. Both are reared in the laboratory on the substitution host *Ceratitis capitata* (Weideman) (Diptera: Tephritidae) for more than 6 years (i.e. more than 100 generations). Our results reveal quantitative differences for some bands on venom profiles between Kenyan and South African laboratory strains for which original populations are strongly genetically differentiated.

To study the effect of the host shift between the natural and substitution hosts, we have recently obtained new samples from the field in South Africa and Kenya. This allowed us to follow the evolutionary response to the host shift from *B. oleae* to *C. capitata* in several replicates and to compare laboratory strains to wild strains. Preliminary results show a higher inter-individual variability in field populations than in laboratory strains (Fig 1).

Overall, this work provides encouraging results about the relevance of rearing quality-control methods based on venom analysis. Indeed, venom protein electrophoresis is cheap and simple to carry out once venom apparatus are obtained, and they reveal venomic inter-individual variability, which complements the usual quality-control procedures based on phenotypic trait.

Figure 1. (Reprinted from Colinet et al. 2012)

Electrophoretic comparison of venom proteins from Psyttalia lounsburyi individuals.

Venom glands were dissected in insect Ringer solution supplemented with a protease inhibitors cocktail (PI; Roche) and residual tissues were removed by centrifugation. The individual protein contents of 12 individual venom glands, was analyzed under reducing conditions on a 12.5% SDS– PAGE and visualized by silver staining. 6 individuals originate from a population sampled in Sirimon Forest (Kenya), the other 6 being issued from a strain collected in Kenya but reared in laboratory conditions for more than 8 years. Arrows point to examples of inter-individual variation with the presence/absence of bands in the natural

population or quantitative variation of specific bands in the laboratory strain. Molecular weight standards are in kDa.

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