Surviving in the city: a metabolomic-based approach to investigate urban stressors and adaptation strategies implemented by bees

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Abstract: Pollinator insects play a fundamental role in sustaining ecosystem functions and human wellbeing by supporting the reproduction of wild plants and crops. Despite their importance, in recent decades we have witnessed a decline in pollinators' richness and abundance, due to different anthropic stressors, among which urbanization and land use intensification are some of the main causes. While several species of wild pollinators are threatened by urbanization, others manage to thrive in this challenging environment, however it remains unclear at what cost and whether they are developing adaptations to this human-altered environment.

In this thesis project, we aim to shed light on the effects of the urban environment on pollinators through untargeted metabolomic. This approach has the advantage of being universal, since several metabolites and the essential pathways are conserved among organisms, and it can be representative of phenotypic changes, allowing the comparison between different species and environments. Specifically, we aim at comparing samples from various degrees of urbanization to identify reliable stress biomarkers and potential signs of adaptations involving metabolic processes. To do this we collected about 300 samples belonging to three wild bee species, namely *Bombus terrestris* (Linnaeus, 1758), *Bombus pascuorum* (Scopoli, 1763) and *Osmia cornuta* (Latreille, 1805) in four metropolitan cities, namely Rome, Milan, Florence and Turin. Sampling sites were characterized by different degrees of urbanization and were settled both in natural and urbanized environments. Other environmental variables such as the availability of green area in the surrounding of sampling sites, the green area fragmentation and the mean temperature during the sampling season were measured and taken into account for their influence on individuals metabolome. Furthermore, to minimize the variability among samples, only female bees were considered in the analysis. Moreover, the age of individuals was estimated by analyzing wing wear and taken into account as it could influence metabolism.

The final results of this thesis will enhance our understanding of the biological mechanisms underlying pollinators' adaptation strategies in urban environments and identify specific metabolites associated with urban stressors. These findings will provide effective markers to evaluate pollinator health across diverse contexts and assess the impact of nature-based solutions and green space management policies on supporting urban pollinator populations.