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Honeybee biomarkers as promising tools to monitor environmental quality

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ABSTRACT

The aim of this study was to distinguish the impacts of two different anthropogenic conditions using the honeybee *Apis mellifera* as a bioindicator associated with a battery of biomarkers previously validated in the laboratory. Both the urban (RAV, Ravine des Cabris) and semi-natural (CIL, Cilaos) sites in La Reunion Island were compared in order to assess the impacts of two types of local pollution using the discriminating potential of biomarkers. Hives were placed at the CIL and RAV sites and honeybees were collected from each hive every three months over one year. Honeybee responses were evaluated with respect to several biochemical biomarkers: glutathione-S-transferase (GST), acetylcholinesterase (AChE), alkaline phosphatase (ALP) and metallothioneins (MT). The results showed a significant difference between the localities in terms of GST, AChE and ALP activities, as regarding midgut MT tissue levels. Compared to the CIL site, ALP and MT tissue levels were higher at the RAV site, although AChE activity was lower. GST displayed more contrasted effects. These results strongly suggest that the honeybees based in the more anthropized area were subjected to sublethal stress involving both oxidative stress and detoxification processes with the occurrence of neurotoxic pollutants, amongst which metals were good candidates. A classification tree enabled defining a decision procedure to distinguish the sampling locations and enabled excellent classification accuracy (89%) for the data set. This field study constitutes a strong support in favour of the in situ assessment of environmental quality using honeybee biomarkers and validates the possibility of performing further ecotoxicological studies using honeybee biomarkers.

1. Introduction

La Réunion Island benefits from considerable plant diversity and part of the island was granted the status of a UNESCO World Heritage Site in 2010. The island displays a high degree of endemism and rare terrestrial biodiversity. In view of the important and often irreversible impacts of human activity on this ecosystem, there is an increasing need to develop tools to monitor the impacts of pollution. Bioindicators represent good witnesses of environmental health and their presence, or the structure of their populations, could be considered as highly informative. However, characterization of the physiological integrity and functionality of individuals requires tools to act as biomarkers of exposure to environmental stressors. Biomarkers can be defined as observable or measurable modifications at the molecular, cellular, physiological or behavioural levels which reveal the exposure of an organism to xenobiotics (Lagadic et al., 1997). Biomonitoring programmes are usually based on studying a set of biomarkers in sentinel species of interest (Aguilera et al., 2012; Damiens et al., 2004; Lionetto et al., 2003; Stanic et al., 2006). In the terrestrial environment, the honeybee is a particularly pertinent model for the development of biomarkers in order to assess environmental contamination (Leita et al., 2004; Saifutdinova and Shangaraeva, 1997). Honeybees can constitute reliable indicators of environmental quality because their intense foraging activity brings them into contact with a large number of pollutants within a radius that generally ranges from 1.5 to 3 km around the hive, depending on food abundance (Chauzat et al., 2009). A decline in honeybee populations is currently being seen in many parts of the world, resulting in an active strategy for the monitoring and diagnosis of population health (Nguyen et al., 2009). The honeybee is therefore a species of particular interest in terrestrial ecotoxicology because its physiology, behaviour and ecology have been the subject of extensive study (Alaux et al., 2010; Decourtye et al., 2004; Henry et al., 2012).

The responses of some biochemical parameters, such as alkaline phosphatase, acetylcholinesterase and glutathione-S-transferase, have

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Table 1
General characteristics of the selected sites.

	CILAOS (CIL)	RAVINE DES CABRIS (RAV)
GPS coordinates	E55°27′19.9″ S21°15′45″	E55°28′36.3″ S21°17′12.7″
Anthropogenic pressure	Semi-natural	Dense urban
Altitude (m)	238	250
Sampling period	May 2009 to May 2010	May 2009 to May 2010

been characterized in laboratory studies after the exposure of honeybees to various chemicals (Bounias et al., 1985, 1996; Stefanidou et al., 1996). Their response profiles to chemicals mean that honeybees can be considered as promising tools for use in environmental biomonitoring programmes. However, no in situ validations have been performed to date. During previous studies, we validated the responses to a battery of metabolic and neural biomarkers of *Apis mellifera* honeybees after their exposure to chemicals under laboratory conditions (Badiou-Bénéteau et al., 2012). The purpose of the present study was therefore to validate honeybee biomarkers under field conditions. Urban (RAV, Ravine des Cabris) and semi-natural (CIL, Cilaos) locations in La Reunion Island were compared in order to evaluate the impacts of two types of local pollution using the discriminating potential of biomarkers. The biomarkers chosen for this study included non-specific and specific biomarkers of pollutant toxicity. We focused this study on metabolic biomarkers such as alkaline phosphatase (ALP) and glutathione-S-transferase (GST), a neural biomarker, acetylcholinesterase (AChE), and metal biomarkers such as metallothioneins (MT). Metals and pesticides were also quantified in the honeybees in order to determine the pollutants to which they had been exposed.

2. Materials and methods

2.1. Experimental design

The study sites were located in the south-western part of La Réunion Island and displayed contrasting degrees of anthropisation: a weakly anthropised rural site, CIL (Cilaos, E55°-27′-19.9″; S21°-15′-45″) and a strongly anthropised urban site, RAV (Ravine des Cabris, E55°-28′-36.3″; S21°-17′-12.7″) (Table 1 and Fig. 1). The rural site (CIL) was relatively landlocked between the feet of the Cilaos mountain (the hives being located close to the mountain) and the Cilaos ravine, inducing foraging activity where no industrial contamination could be detected. This site was chosen as the relative reference. The urban site (RAV) was located in the suburbs of Saint Pierre, separated from the CIL site by the cirque de Cilaos. To reduce any variations due to geographical factors (microclimates prevailing on La Réunion Island), the sampling sites were situated within the same ecoregion, separated by a distance of 3.7 km. It was assumed the foraging zones of the bees were relatively independent and restricted to their respective sites because (i) food resources were deemed to be sufficient in the area surrounding the hives, based on the amount of honey produced, and (ii) a broad and deep ravine separates the sites, dissuading the bees from crossing it. Six *A. mellifera* honeybee colonies were placed at the CIL and RAV locations (three colonies per site) and samples were collected every three months over a 1-year period. Foraging *A. mellifera* honeybees were captured at the hive entrance. Sampling for analysis was carried out simultaneously in the colonies of both sites, with approximately 2000 honeybees being collected each time (around 200 g of honeybees) from each hive.

2.2. Determination of honeybee races

In the subtropical island of La Réunion, the dominant race of honeybee is *A. mellifera unicolor* (Ruttner, 1975, 1988; Schneider, 1989), although several European races of *A. mellifera (carnica, ligustica, mellifera)* are known to have been introduced in the past (Schneider, 1989). It was therefore necessary to verify the races of the honeybee populations used during this study, and the homogeneity of our samples. Two workers per colony were taken from the samples collected for the biomarker study, and preserved in alcohol before extraction of their DNA. The mtDNA region including the tRNAleu gene, the COI-COII intergenic region and the 5′ end of the COII subunit gene were PCR-amplified according to a protocol detailed by Garnery et al. (1993). A fraction of the PCR product was run on 1% agarose gel for total size determination and the remaining product was restricted

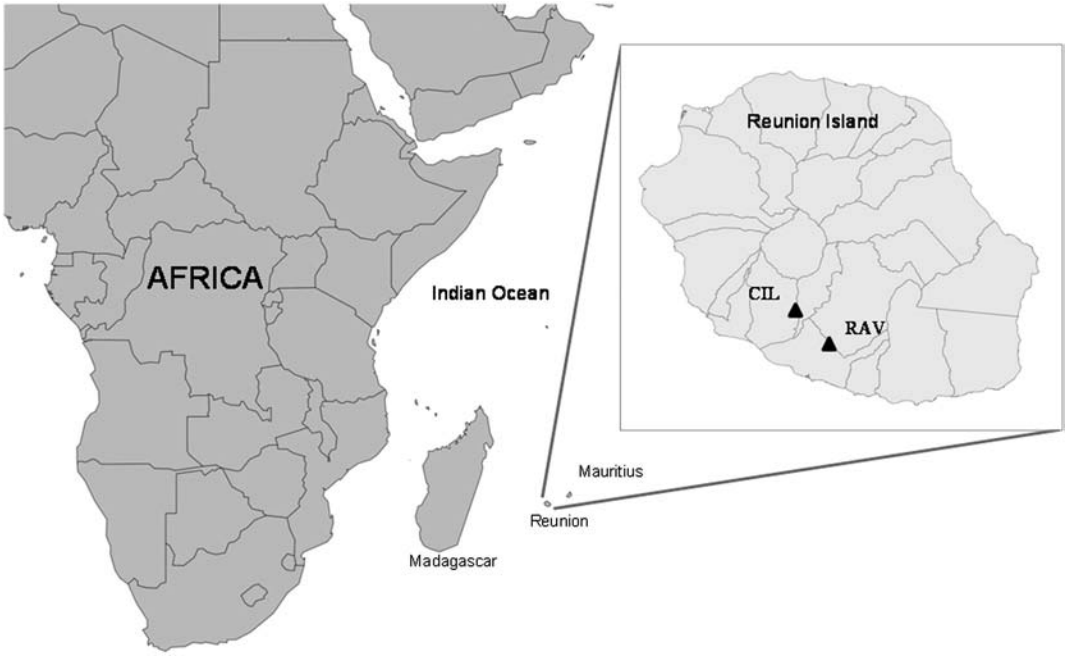

Fig. 1. Location of honeybee colonies at the CIL (Cilaos) and RAV (Ravine des Cabris) sites in Reunion Island (three colonies per site). These sites of interest were located in the south-western part of La Réunion Island and displayed contrasting degrees of anthropization: a slightly anthropized site (CIL) and a strongly anthropized site (RAV). CIL was chosen as the relative reference.