Honeybees and bumblebees: two ways of living lead to differences in immune responses and fitness costs

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Introduction

Immune responses of invertebrates imply more than developing a merely unspecific response to an infection. Great interest has been raised to unveil whether this investment into immunity also involves fitness costs associated to the individual or the group. Focusing on data from our laboratory regarding immune responses of honeybees and, using the well-studied insect bumblebee for comparison, we studied whether the foraging activities of honeybees and bumblebees are affected upon immune stimulation by assessing the flight performance. Additionally, the acceptance behavior of nestmates toward immune-challenged honeybees was determined by introducing bees in observation colonies.

Materials and Methods

Honeybees and bumblebees were immune-stimulated by injection of heat-killed *Paenibacillus larvae (Pl)*-bacteria and 24 h later their flight performance was quantitatively evaluated on a roundabout by measuring overall flight duration, covered distance, maximum speed reached and flight average speed. All bees used in this experiment were 15 to 20 days old foragers . Bees were randomly assigned to one of the following groups; naïve, ringer-injected (2 µL ringer buffer) or heat-killed *Pl*-injected (2 µL 10^8 CFU/ml), kept in separate cages for 24 h and then used to assess their flight performance. After a first emptying flight, bees were fed with 10 µL of a 2 M glucose solution and attached to the rotator arm of the roundabout to start the flight. All data generated during the flight were automatically recorded.

For the acceptance experiment, honeybees were assigned to one of the following groups: naïve, ringer-injected or LPS-injected (0.5 mg/mL of solution), marked individually and introduced in two observation colonies (colony 1: naïve=348, ringer-injected=265, LPS-injected=277; colony 2: naïve=343, ringer-injected=342, LPS-injected= 331). During the next 25 days, daily screenings of marked bees were carried out in order to obtain survival data for the three experimental groups. Screenings were done in the morning (8:30 a.m.) and in the afternoon (4 p.m.) during one and a half hour every time and for both colonies at the same time.

Results

From all parameters tested (flight duration, covered distance, maximum speed and averaged speed), flight duration was statistically significantly affected by insect treatment (F (2.66) = 4.89, p = .010). Within bumblebees, the flight duration was significantly longer in the naïve group (adjusted mean = 36.62 minutes) than in the ringer-treated group (adjusted mean= 26.74) or *Pl*-treated group (adjusted mean = 22.28) (fig. 1). Whereas wounding of individuals (ringer group) is not enough to produce a significant difference to the naïve group (p = .066, SD = 2.44), wounding and injection of heat-killed bacteria (*Pl* group) produces a significant difference to the naïve group (p = .009, SD = 2.32). No significant differences were observed between the ringer and the *Pl* group (p = 1.000, SD = 2.37). Covered distances by insect are shown in fig. 2. No significant differences were found for honeybees in any of the flight parameters. Interestingly, with the same amount of sugar solution, the flight duration of bumblebees was longer than in honeybees (adjusted mean = 36.62 min and 25.91 min, respectively, p = .033, SD = 5.05).

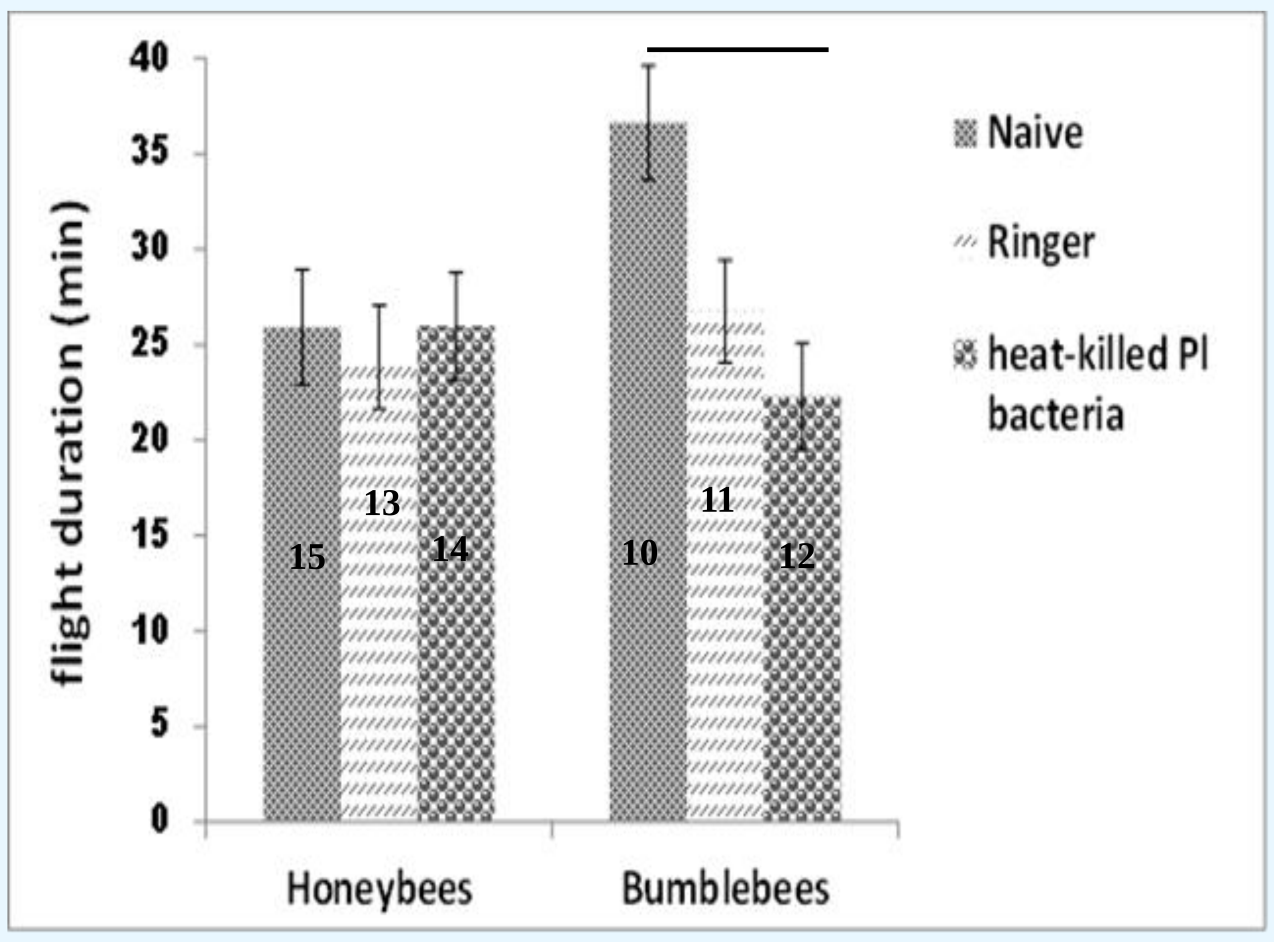

fig.1. Flight duration in minutes of honeybees and bumblebees.

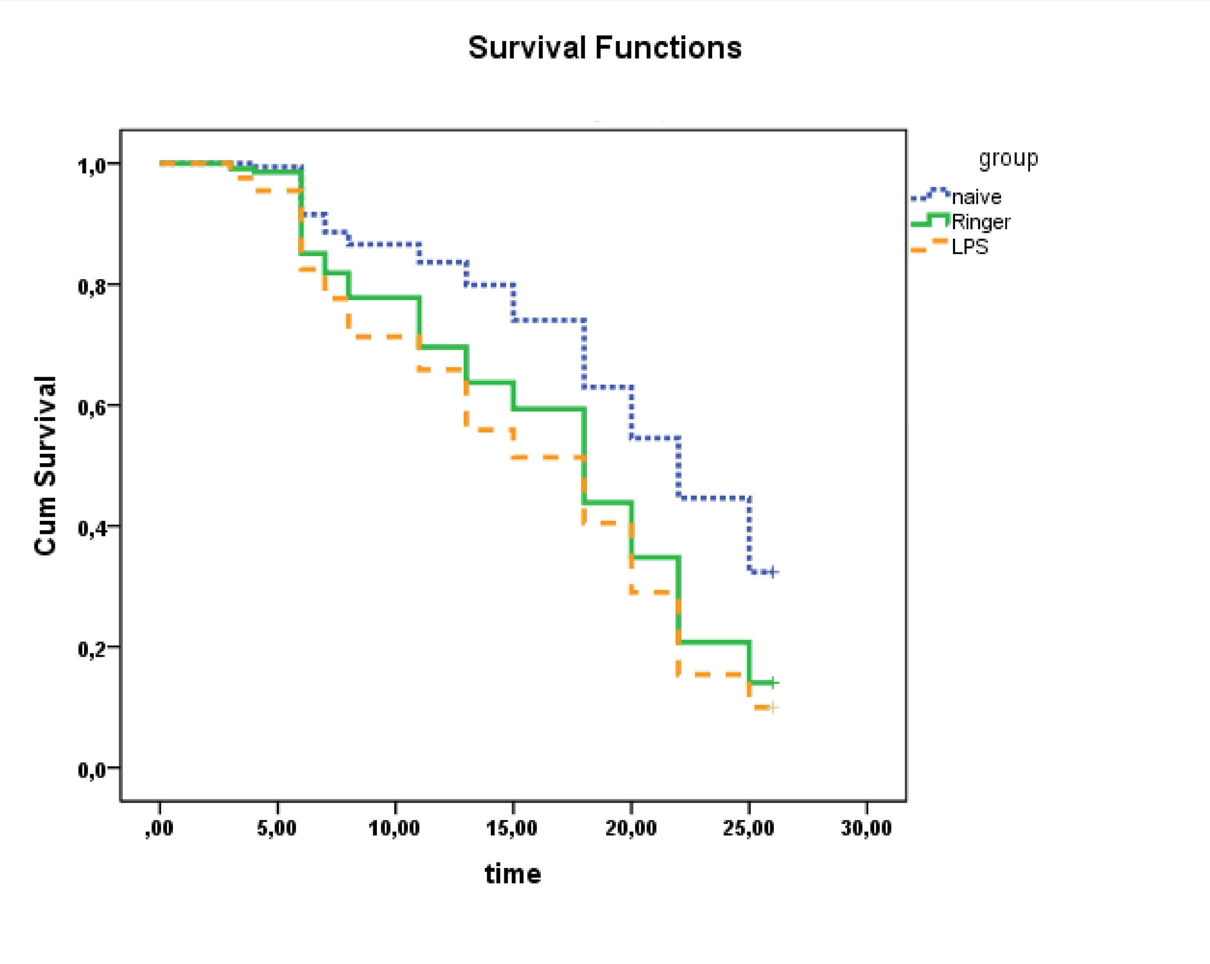

fig.3. Cumulative survival of naïve, ringer-injected and LPS-injected bees introduced in observational colonies.

Regarding survival data obtained over the 25 days observation period in colonies, a Cox regression analysis revealed statistically significant differences between naïve bees and ringer- or LPS-injected bees (treatment; c^2= 85.443, d.f.=2, p-value=0.000) (fig. 3). Both ringer and LPS injections cause a reduction in the survival profile of these bees as compared to naïve bees (fig.3).

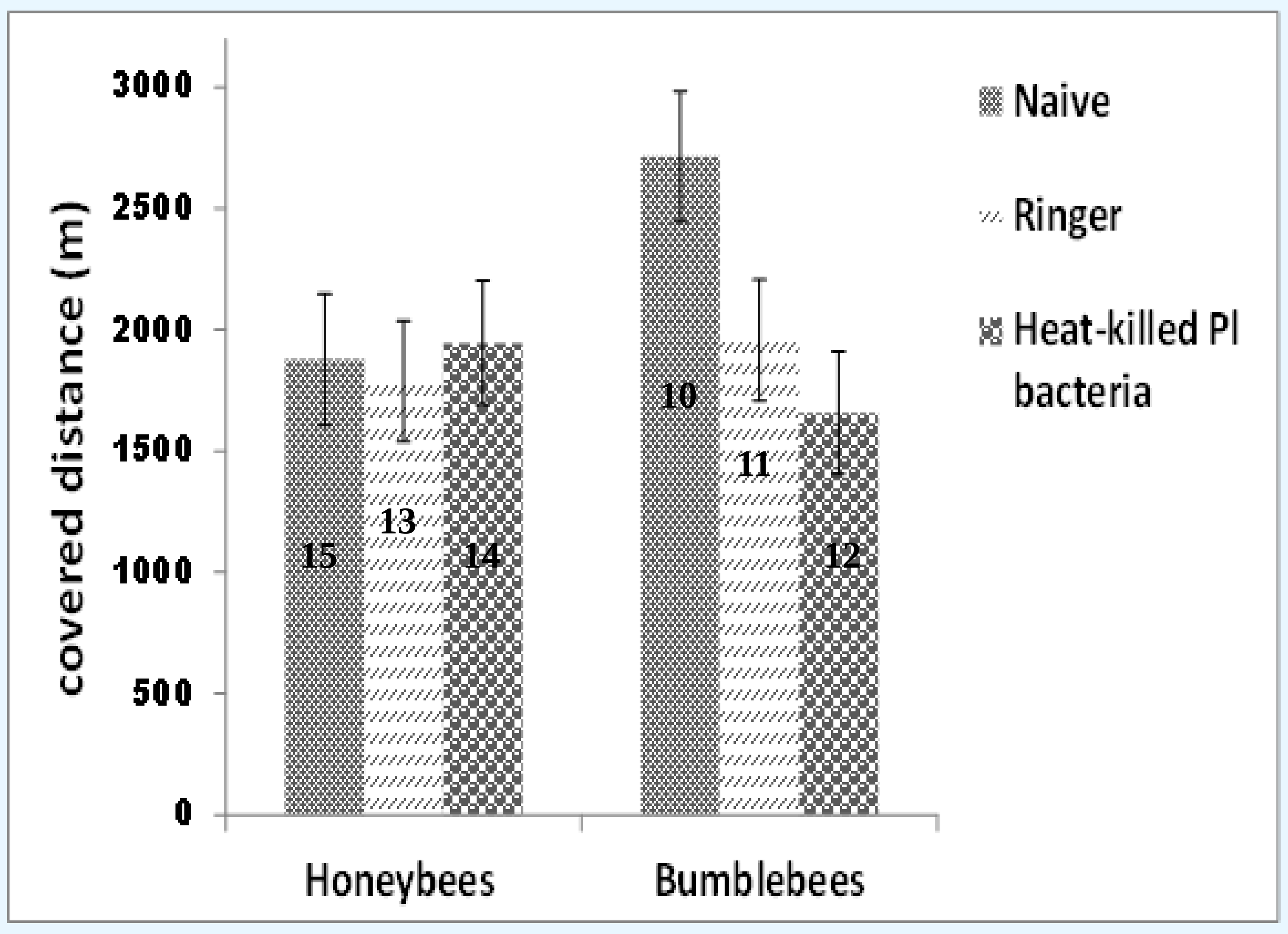

fig.2. Covered distance in meters of honeybees and bumblebees.

Discussion

Results show that despite stimulating the immune system of honeybees, they were not affected by an immune-challenge in their flight performance whereas bumblebees showed significant flight impairment. Several authors reported learning deficits and loss of orientation associated to infections in honeybees (1, 2). Although the observed malfunction of the nervous systems does not necessarily need to be connected with our work on flight parameters, we found no fitness costs associated to immune activation in flight performance in honeybees, unlike in bumblebees.

Immune-challenged honeybees showed lower survival rates than naïve individuals when introduced into a regular colony.
The proportion of chemicals expressed in the cuticle of honeybees increases during infection which causes rejection of infected individuals by other nestmates. Changes in the cuticle were significant both for ringer- and for LPS-injected bees as compared to naïve bees (3). This might explain the different survival profile of ringer- and LPS-injected honeybee workers found in our experiments carried out in observation colonies. Injected honeybees, independently of the effect of wounding or injection of LPS, showed lower survival rates than naïve bees. Besides rejection, other explanations might account for the observed decrease of treated bees in both colonies. For instance, immune-stimulated workers (ringer and LPS) may begin foraging sooner than naïve workers, which will shorten their life expectancy. Alternatively, treated bees may also have abandoned their social function and remove themselves from the colony or may have suffered of secondary infections (4).

Literature

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Acknowledgments

Funding for J. Hernadez-Lopez was provided by an FWF Einzelprojekt (P20510).

6th EUROPEAN CONFERENCE OF APIDOLOGY. Murcia, Spain 2014.