Honeybees' physiological and behavioural immunity deficit induced by DWV

Baracchi D[1,3], Di Prisco G[2], Gentili V[3], Pennacchio F[2], Turillazzi S[3]

[1]*Queen Mary University of London,* [2]*Università degli Studi di Napoli,* [3] *Università degli Studi di Firenze*

INTRODUCTION

As a result of co-evolutionary dynamics between parasites and hosts, honeybees have evolved several adaptations to face the increased risk of epidemic diseases linked to social habits. One of the most important defences possessed by honeybees is represented by antimicrobial peptides present either in the hemolymph and the venom [1]. In particular, the venom application on the cuticle and on the nest wax to protect themselves against pathogens has been proposed [2-3] as a form of behavioural immunity [4].

AIM

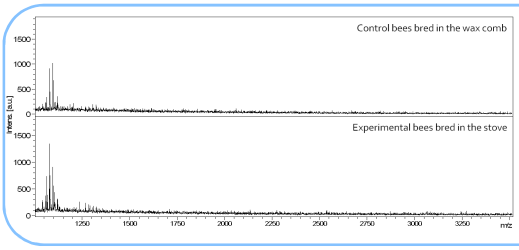
It is not yet clear whether honeybees actively add venom to their cuticle by means of auto-grooming (as prophylaxis) and whether they modulate this behaviour (and the production of venom peptides) to counteract an ongoing infection and protect the colony from an epidemic.

METHODS

1: Thirty prepupae were collected from the comb and bred in a stove. An equal number of prepupae were bred in their comb kept in a stove as controls. Emerged bees were sacrificed and the presence of the venom on their cuticle was ascertained by means of MS techniques. 2: healthy newly emerged bees were kept isolated and without the possibility to perform self-grooming (n =20 +20 controls). The presence of the venom on the cuticle of each 5-days-old bee was ascertained. 3: venom of healthy (n =30) and DWV infected bees (n =30) was analysed and compared. 4: newly emerged bees were orally infected with DWV and the grooming behaviour was quantified (n=30+30 controls). Finally DWV load (qRT-PCR), grooming activity and the presence of venom on the cuticle (MS MALDI-TOF) were correlated one another.

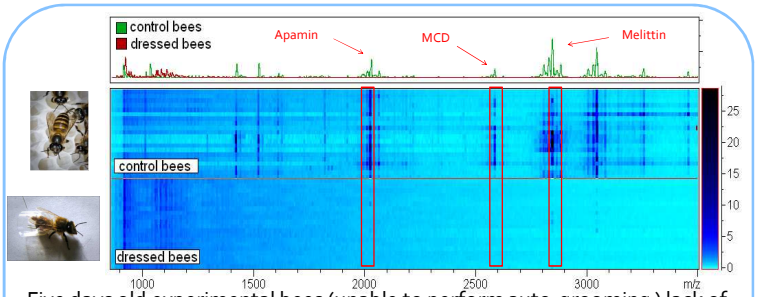
RESULTS

1



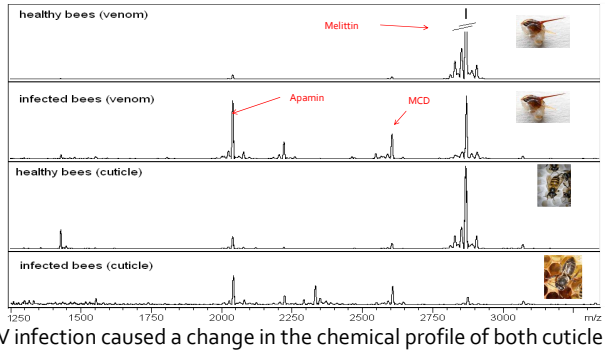
No peptides were found in any cuticular extract of both groups of bees. Cuticular peptides do not come from the wax in which pupae are developed

2

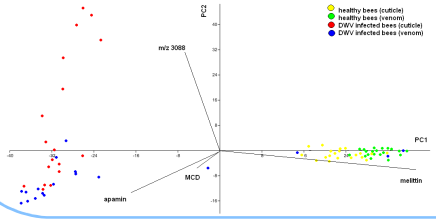


Five days old experimental bees (unable to perform auto-grooming) lack of venom peptides on the cuticle. By contrast, control bees showed several venom peptides on their cuticle.

3

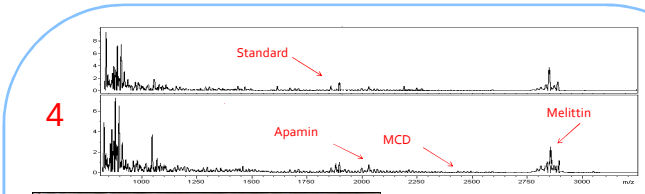


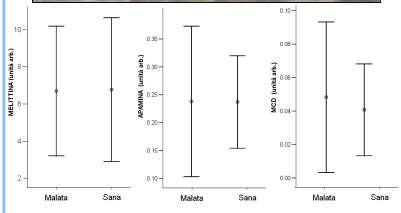
DWV infection caused a change in the chemical profile of both cuticle and venom, probably inducing an additional immunological deficit in sick bees



Infected bees showed less melittin and more variable cuticular chemical profiles than healthy bees

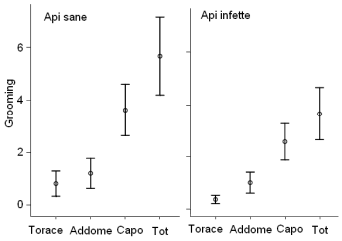
4





Orally DWV infected bees (N=30) and control bees (N =30) showed comparable amounts of venom on the cuticle (melittin, P=0.97; apamin, P=0.87; MCD,P=0.77). However, at the end of the experiment both groups showed very high parasite loads (DWV copies).

The different parts of the body received different amounts of grooming (thorax-abdomen t=-2.99, P=0.004; thorax-head t=-8.91, P=0.001; abdomen-head t=-6.51, P=0.001).



Controls groom themselves more than DWV bees (N=60, t=2.52, P=0.015). Grooming activity differs between healthy and infected bees at the level of the head (t=2.08, P=0.042) and the thorax (t=2.4, P= 0.002), but not of the abdomen (t=1.04, P=0.3).

DISCUSSION

Behavioural experiments showed how grooming behaviour is responsible for the presence of the venom layer on the cuticle of healthy bees suggesting that bees are able to mount a prophylactic defence against pathogens. We found that in nature, when the DWV infection occurred at the larval stage, it induced changes in the venom causing a significant reduction of antimicrobial peptides such as melittin. Unfortunately it has been not possible to clarify whether honeybees are able to mount a therapeutic defence against pathogens by increasing auto-grooming activity during ongoing infections.

Thanks to Dr. Ginevra Cusseau e Dr. Antonio Fadda for helping in collecting data

[1] Zasloff M (2002) Nature 415 389-395; [2] Baracchi D, Turillazzi S (2010). J. Insect phys. 56 366-375; [3] Baracchi D, Francese S, Turillazzi S (2011). Toxicon 58 550-557 [4] de Roode JC, Lefèvre T (2012) Insects 3 789-820

Dr. D. Baracchi's research activity is supported by a Marie Curie Intra European Fellowship