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Original Article

INDIVIDUAL PRECOCITY, TEMPORAL PERSISTENCE, AND TASK-SPECIALISATION OF HYGIENIC BEES FROM SELECTED COLONIES OF *APIS MELLIFERA*

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Abstract

Hygienic behaviour is a complex trait that gives *Apis mellifera* L. resistance against brood diseases. Variability in the expression of hygienic behaviour is evidenced at the colony-level and is explained by the proportion and propensity of individual worker bees that engage in hygienic activities. We investigated the temporal performance and the dynamics of task-specialisation of individual bees over time, both in selected hygienic (H) and non-hygienic (NH) colonies. Then we evaluated the impact of these behavioural aspects on the colony performance. Bees that perform hygienic behaviour (hygienic bees) in our H colonies were more persistent in the hygienic activities throughout the days of the investigation. Such bees were more efficient in the removal of pin-killed brood than hygienic bees in the NH colonies. Hygienic bees in the H colonies were also specialist in the sub-tasks involved in the detection of odour stimulus from dead brood and continued to perform these activities throughout the days of the investigation (temporal persistence). Age-distribution of hygienic bees in the H colonies was asymmetrical, with a larger proportion of these bees performing hygienic activities early in life. At a colony-level, H showed higher efficiency compared to the NH colonies. The present results highlight the fact that individual behaviour may influence the collective dynamics of the hygienic behaviour in honeybee colonies. The results also note that the selection for highly hygienic colonies would result in changes in individual bees that improve the performance of the behaviour at the colony level. The relevance of task-partitioning and age-specialisation of hygienic bees on social immunity is discussed.

Keywords: honeybees, disease tolerance, pin-killed brood removal, polyethism, social immunity, task-specialisation

INTRODUCTION

Hygienic behaviour in the honeybee *Apis mellifera* L. **(**Hymenoptera: Apidae) is a complex trait that confers social immunity against brood diseases (reviewed by Cremer, Armitage, & Schmidt-Hempel, 2007; Wilson-Rich et al., 2009). This behaviour involves the detection and removal of diseased brood by adult worker bees (Rothenbuhler, 1964a,b) and hence prevents or reduces the transmission of brood pathogens in the hive and maintains colony health. Honeybee colonies that express hygienic behaviour are therefore economically important to beekeepers

(Spivak & Reuter, 2001).

Honeybee colonies show variability in the expression of this behaviour (Perez-Sato et al., 2009; Bigio et al., 2014). These differences can be explained by how quickly bees within a colony detect the presence of diseased brood in the nest (Palacio et al., 2010). Although all honeybees in the colony are capable of detecting and removing diseased brood (reviewed by Cremer, Armitage, & Schmidt-Hempel, 2007; Wilson-Rich et al., 2009), only those colonies with bees that do so rapidly and hence efficiently, limit disease transmission (Gramacho & Spivak, 2003). The word "hygienic" means workers that indeed perform any of the hygienic activities. A colony has a hygienic phenotype when over 50% of bees are hygienic and an increased percentage of these bees in the colony resulted in a higher efficiency of behaviour performance (Arathi & Spivak 2001; Palacio et al., 2010). Moreover, hygienic bees can modulate the extent of the performance of the behaviour depending on the genetic composition of the colony (Arathi & Spivak, 2001; Borsuk, 2009). Individual patterns of task performance are affected by the individual bee genotype and by the genotype of the nestmates in the colony. Thus, the combined and interactive effects of individual bees with diverse genetic propensities to detect and remove diseased brood affect the colony-level expression of hygienic behaviour (Perez-Santo et al., 2009). This occurs with other colony-level behavioural phenotypes that arise primarily from the interactions of workers with each other and also with the environment (Page & Mitchell, 1998, reviewed by Page et al. 2006). In fact, certain patterns of interactions and the temporal dynamics of these interactions among individuals or group members (e.g. caste, age or task-groups) have been claimed to provide "organizational immunity" (Naug & Camazine, 2002; Naug, 2008). This immunity together with sanitary behaviour (e.g., hygienic behaviour), contribute to social immunity by limiting pathogen spread at the colony-level (reviewed by Cremer, Armitage, & Schmid-Hempel, 2007; Wilson-Rich et al., 2009; Stroeymeyt, Casillas Perez, & Cremer, 2014).

Hygienic behaviour is generally partitioned into sub-tasks or activities: inspecting and detecting cells containing diseased brood, uncapping the wax cap over the brood, and removing cell contents (Arathi & Spivak, 2001; Palacio et al., 2010), although many bees are involved in completing each of the sub-tasks. According to the "threshold models" hypothesis, that explain the division of labour within a social insect colony (Calderone & Page, 1988; Robinson, 1992; Theraulaz, Bonabeau, & Deneubourg, 1998), the workers of a colony differ in their response thresholds to stimuli associated with certain tasks (Beshers & Fewell, 2001). Subgroups of honeybees in a colony that start engaging in a certain task, are likely to have a similar response threshold for a certain stimulus associated with that task and will constitute a task group (Page et al., 2006). In particular, the genetic propensity of individual bees (or a task group) to perform hygienic behaviour is explained by their high responsiveness to odour cues that stimulate them to initiate the hygienic activities (Masterman, Smith, & Spivak, 2000; Masterman et al., 2001; Spivak et al. 2003; Swanson et al., 2009; Palacio et al., 2010; Chakroborty, Bienefeld, & Menzel, 2015). Previous studies detected that hygienic bees show increased olfactory sensitivity and responsiveness to the odours of diseased brood compared to non-hygienic bees (Masterman, Smith, & Spivak, 2000; Masterman et al., 2001; Spivak et al., 2003; Palacio et al., 2010). In addition, the odour discrimination abilities are higher in bees that inspect (Palacio et al., 2010) and uncap the cells (Masterman, Smith, & Spivak, 2000; Gramacho & Spivak, 2003) compared to bees that remove the contents. These findings indicate a specialisation in the activities among hygienic bees depending on their differential response threshold to the odour stimulus emanating from the abnormal brood (Arathi & Spivak, 2001).

The behaviours of individual bees towards dead and diseased brood were explored in hygienic (H) and non-hygienic (NH) colonies by Palacio et al. (2010). These authors found that bees in H colonies showed more rapid detection of brood

affected by *Ascosphaera apis*, lower persistence of bees in hygienic activities (frequency of activities per day per bee), and higher efficiency in the removal of diseased brood compared to bees in NH colonies. They found similar results using pin-killed brood cells to evaluate hygienic behaviour. However, detailed information about the temporal performance, the dynamics of task-specialisation, and the interactions of individual middle-age workers over time in H and NH colonies, is still needed. Based on previous results and in order to understand how individual behaviour influences colony-level efficiency, we studied here the hygienic activities of individual bees towards pin-killed brood cells in H and NH colonies. Then, we analysed the performance, task-partitioning, and temporal persistence of the cohorts of tagged-bees. We discuss the consequences of the selection for hygienic behaviour and its potential influence on organisational patterns inside the colony.

MATERIAL AND METHODS

Bee Colonies

The experimental honeybees colonies of *A. mellifera ligustica* used here, were obtained from the MeGA Programme in Argentina, as described in Palacio et al. (2010). The Programme started in 1992 (Palacio et al., 1996) and for the last 10 years the colonies have been maintained in a closed-population breeding system. Hence, the bees share a common genetic background. The criterion for the selection of these colonies was based on their hygienic behaviour using a pin-killed brood assay (Newton & Ostasiewski, 1986; Palacio et al. 2000; 2010). Two hygienic (H) and two non-hygienic colonies (NH) exhibiting different levels of pin-killed brood removal within 24 hours (H: 90%, NH: 40%), were chosen from the breeding programme to be the source of worker bees, to set up observation hives. The experiments were entirely performed during spring (October-November 2010) in INTA-FCA Balcarce, Argentina. The experimental design was based on previous studies (Arathi & Spivak, 2001; Palacio et al., 2010).

Observation Hives

Four observation hives were installed to study the activities performed by worker bees as concerns the cells containing pin-killed brood. Each observation hive was provided with a frame containing stored honey and pollen, larvae, pupae, and empty combs, and approximately 1900 unlabeled bees of various ages from each corresponding colony (H1, H2, NH1, NH2). The hives were queenless for at least 24 hours prior to the introduction of a laying-egg queen. Each queen was introduced into the observation hive inside a queen chamber. Sugar candy was put at the top of the cage. The bees outside the chamber chewed through the candy and released the queen 4-5 days later. The queens were allowed to lay eggs in the central area of the hive, but the brood combs were replaced with empty combs every 18 days to ensure the absence of new emerged bees. This means that all the bees in the observation hives derived from the parental H and NH colonies. The entrance of pollen from natural sources was normal throughout the experiments and it was not necessary to supplement the diet with this source. During the experiments, the observation hives were provided with sugar syrup (sugar:water 2:1) in external feeders.

Combs containing pupae within 1-2 days of emerging, were removed from each colony (H_1, H_2, NH_1, NH_2) and placed in individual cages in an incubator (36°C and 60% RH). The emerging bees were individually marked with a numbered tag on the thorax. A different coloured tag was used each day to record the age of the honeybees. Every day, for 5 consecutive days, cohorts of 200 bees were labelled with the same coloured tag and added to each observation hive. A total number of 1000 labelled bees were present in each observation hive when the observations began.

Behavioural observations

To observe honeybees performing hygienic behaviour, a 10 x 5 cm comb section (experimental comb) was introduced in the centre of the comb of each observation hive. In order to elicit hygienic behaviour, 20 capped brood cells

containing pink-eyed pupae were perforated in each experimental comb using an entomological pin (No. 1) to kill the brood. An additional 20 capped brood cells containing undisturbed pink-eyed pupae were considered as the control cells (see Newton & Ostasiewski (1986) and Palacio et al. (1996; 2010) for methodological details). Behavioural observations began immediately after inserting the experimental comb in each observation hive.

Video recordings of the area of the experimental comb in the observation hives were made using a Panasonic AG-DP200E camera. Anti-reflection glass was used to enhance the filming images. Recordings began 11 days after the last 200 labelled bees were added to the observation hives; when the oldest tagged bees were 15 days old. A new experimental comb was introduced into each observation hive daily at 8:00 am and was removed from the colonies at 17:00 pm. The number of cells that were uncapped and removed after 9 h of filming was registered from the experimental combs of each colony (H_1, H_2, NH_1, NH_2). The recordings were made on 4 consecutive days for both H and NH colonies, resulting in 36 h of recording per colony (observation period).

Instantaneous scans of all the number-tagged bees present on each experimental comb section were made and their behaviours were registered from the videos. The activities performed by bees on the cells containing pin-killed brood and undisturbed brood were recorded as inspection (IN), uncapping (UN) or removal (RE). The number, colour and frequency of visits of individual tagged bees performing each activity on the cells were registered for each colony throughout the filming period.

Statistical analysis

To confirm the differential efficiency of hygienic behaviour between the H and NH colonies, the number of pin-killed brood removed daily (after nine hours of filming) by each colony, was calculated from the experimental combs. The differences were analysed by Fischer's Exact Test.

The rates of performance of each activity (IN, UN, RE) in terms of the total number of honeybees performing the activity, were calculated in H and NH colonies. Comparison was done with the Chi-square test of homogeneity, and presented as percentages for comparison between colonies and activities.

To evaluate the specificity of the honeybees in performing the activities of hygienic behaviour in each colony group (H and NH), the number of bees that performed the one, two or three activities of hygienic behaviour was registered and compared between colonies using Chi-square tests of homogeneity. Percentages were calculated from these data.

To explore the effects of bee age on the performance rates of the activities, a generalised linear model (GLM) was applied. The response variable was the percentage of honeybees performing hygienic behaviour. The analysis was conducted separately for each activity (IN, UN, RE) and each colony group (H, NH). Biological replicate (1, 2) and colour/cohort (1, 2, 3, 4, 5) as random factors, and age (12, 13, 14, 15, 16, 17) as a fixed factor, were all taken into consideration. The Shapiro-Wilks and Levene tests and the residue normality were analysed. The variance was modeled using Akaike criterion. To evaluate differences in performance rates among ages, the LSD Fisher test was used. The level of significance was set at 5%. The variance of random factors was also analysed. Kolmogorov-Smirnov tests were performed to analyse the bee age distributions for each activity performance in both, H and NH colonies. Data from bees which were 11 and 18 days old (only one cohort) were excluded from the statistical analyses and were analysed at a descriptive level.

The distributions of the number of visits per bee per day were compared between colonies using the Kolmogorov-Smirnov test. The number of bees that performed 1, 2, and 3 or more visits were taken into account. The persistence in each hygienic activity was calculated as the number of bees that performed more than 3 visits per day (spatial persistence) according to Arathi & Spivak (2001). In addition, we registered the number of bees that performed 3 or more visits to pin-killed cells during the days of the

experiment (temporal persistence). These variables were compared between colonies with Chi-square tests of homogeneity and presented as percentages. Statistical analyses were carried out with Statistica V 6.0 (Statsoft, 2001) and with InfoStat 2014 (Di Rienzo et al., 2014).

RESULTS

Our H colonies removed significantly more pin-killed brood cells (86.6%) compared to the NH colonies (52.5%) after the 9 hour period (Fisher's Exact test, $p<0.001$). No differences were observed among the four filming days or between replicates of each colony group. The control cells containing undamaged brood were not removed by tagged-workers in the H or NH colonies and no hygiene activity was observed on the videos. Fewer tagged bees were observed inspecting and uncapping cells containing pin-killed brood through the observation period in the H colonies as compared to the NH colonies (Chi-square = 9.82, $p = 0.002$ for IN; Chi-square = 5.48; $p = 0.019$ for UN; Table 1). There was no significant difference in the total number of bees performing RE activity between the H and NH colonies (Table 1). In the NH colonies, no differences were found in the proportion of bees performing each activity. In contrast, the percentage of inspectors and uncappers was lower than the percentage of removers for the H colonies (Chi-square = 1.91, $p = 0.385$ for NH; Chi-square = 8.25, $p = 0.016$ for H; Table 1).

The percentage of bees performing 1, 2, or 3 general sub-tasks of hygienic behaviour was similar between H and NH ($p = 0.94$). Between 56 and 58% of hygienic bees performed only one activity of hygienic behaviour (sub-task specialists) throughout the entire observation period for H and NH, with no significant differences between colony groups (Chi-square = 0.12, $p = 0.94$). A small proportion (13%) of the hygienic bees performed the three hygienic behaviour activities in both of the H and NH colonies.

Table 1

Values for the percentages of bees performing each activity (inspecting, uncapping and removing the pin-killed brood from the cells) through the entire observation period for both replicated-colonies. Values correspond to the mean percentages (± SE) on the replicates, as the replicates of each colony group did not differ significantly. Values with different Latin letters show significant differences between the colonies (hygienic, H vs non-hygienic, NH). Values with different Greek letters show significant differences among activities (inspection, uncapping and removing) in the same colony.

Colony	Inspection	Uncapping	Removing
H	10.1 ± 1.5 a, a	9.9 ± 1.2 a, a	13.5 ± 2.1 b, b
NH	14.8 ±1.3 b, a	13.3 ± 1.6 b, a	12.6 ± 1.9 b, a

The age distribution of tagged-bees performing each activity across the entire observation period between H and NH was dissimilar (Fig. 1). In particular, age distributions for the number of bees inspecting the pin-killed brood were significantly different between colonies (Kolmogorov-Smirnov Test, $D_{max} = 0.259$; $p = 0.001$; H: mean 14.15±1.29, median: 14; NH: mean 14.85±1.47, median: 15). In the H colonies, younger bees were more frequently involved in IN activity than older bees. In fact, the percentage of bees that were 13-14 days old and inspected the cells was significant higher than the percentage of bees that were 17 days old which performed this activity (results from GLM analysis; LSD Fisher, $p<0.05$; Fig. 1A). For the NH colonies, the percentage of inspectors increased as the bees aged (Fig. 1B). Since the errors were higher for this colony group we could not detect sig-

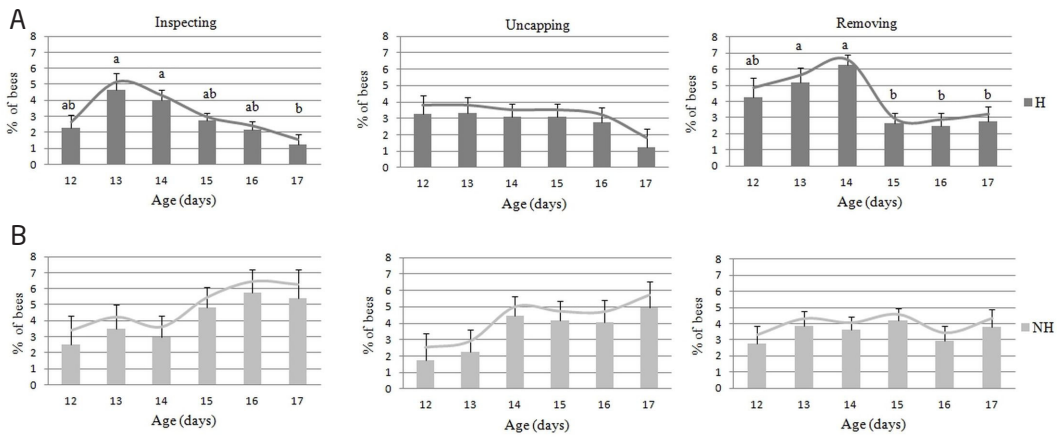

Fig. 1. Distribution of mean percentages of bees performing each activity (inspecting, uncapping, and removing the pin-killed brood from the cells) as a function of bee age for each colony group. A: hygienic colonies (H), B: non-hygienic colonies (NH). Bars with different letters show significant differences between the bees' ages for the variable measured (results from GLM analysis).

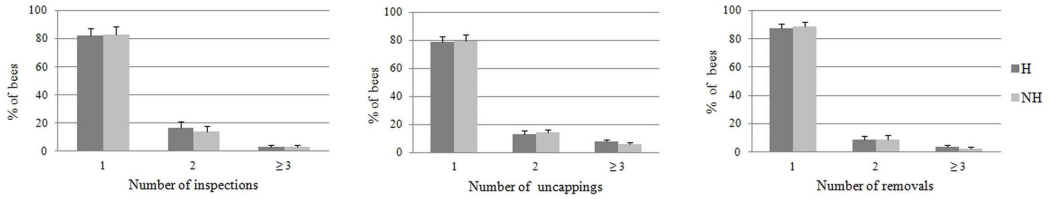

Fig. 2. Percentages of hygienic bees performing each activity as a function of the number of performances (inspections, uncappings, and removals) per day per bee in each colony group (H: hygienic and NH: non-hygienic). Values correspond to the mean percentages (± SE) on the replicates, as the replicates of each colony group did not differed significantly.

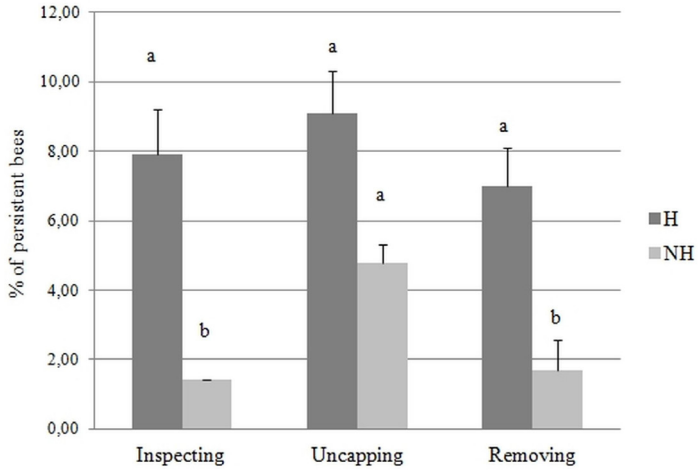

Fig. 3. Percentages of persistently hygienic bees throughout the days (with performances on > 3 consecutive days) for each activity (inspecting, uncapping, and removing the pin-killed brood from the cells). Values correspond to the mean percentages (± SE) on the replicates, as the replicates of each colony group did not differed significantly. Bars with different letters show significant differences between the colonies (H: hygienic and NH: non-hygienic) for the variable measured.

nificant differences among bee ages in the GLM analysis. While age distributions for the percentages of bees performing UN as concerns pin-killed brood, did not differ between colonies, significant differences were detected for RE between H and NH (Kolmogorov-Smirov Test, D_{max} = 0.162, p>0.1 for UN; D_{max} = 0.181, p = 0.024, H: mean 14.13±1.38, median: 14; NH: mean 14.50±1.46, median: 15 for RE). In particular, a higher percentage of bees that were 13-14 days old were detected removing the cells, compared to the percentage of bees that were 15-17 days old detected removing the cells in the H colonies (results from GLM analysis; LSD Fisher, p< 0.05). No differences were found in the percentages of bees performing this activity at any age, in the NH colonies (Fig. 1B). In addition, the descriptive analyses of the data that included only one cohort (the ones that were 11 and 18 days old) supported the same pattern. While the percentage of 11 days old bees that were involved in the hygienic activities was higher in the H compared to the NH colonies (IN: 1.5% NH vs 3.5% H; UN: 2.0% NH vs 4.0% H; RE: 2.5% NH vs 4.5% H), the percentage of 18 days oldbees was lower in the H than in the NH colonies (IN: 5.0% NH vs 0.5% H; UN: 5.5% NH vs 1.5% H; RE: 5.0% NH vs 2.5% H).

The distributions of the number of bees that performed 1, 2, or more 3 visits per day to the pin-killed cells were similar between the H and NH colonies for the three activities (results from Kolmogorov-Smirnov test; p>0.1; Fig. 2). For all activities, approximately 80-90% of the hygienic bees from both colonies performed only one visit to the cells per day (Fig. 2). There were no differences in the percentage of bees that performed more than 3 visits per day (bees with spatial persistence) between the H and NH colonies for the three activities (IN: Chi-square = 1.12, p = 0.29; UN: Chi-square = 0.21, p = 0.64; RE: Chi-square = 0.27, p = 0.60; Fig. 2).

The analysis of temporal persistence showed that the percentage of bees that persisted in each activity during the days of the experiment, was higher in the H than in the NH (Fig. 3). Particularly, significant differences were found in the percentage of persistent bees for IN and RE activities between colonies (Chi-square = 6.11, p = 0.013 for IN; Chi-square = 6.41, p = 0.011 for RE; Fig. 3). The proportion of persistent uncappers appeared to be higher in the H than in the NH colonies, but this difference did not reach the level of significance (Chi-square = 0.99, p = 0.319).

DISCUSSION

We presented here an in-hive temporal dynamics and task-partitioning of hygienic behaviour in order to dissect the basis of efficiency differences in this trait between hygienic and non-hygienic colonies. The results showed that hygienic behaviour in our colonies is exhibited by a small percentage (10-14%) of middle-aged bees, as was observed in previous studies (Arathi et al., 2000; Palacio et al., 2010; Panasiuk et al., 2010). Our results are also consistent with the observation that hygienic colonies are comprised of bees with varied genetic propensities to perform hygienic behaviour (Perez-Santo et al., 2009).

Our H colonies showed a high colony-level efficiency because 87% of pin-killed brood had been removed from the experimental combs. In contrast, hygienic bees in the NH colonies were not able to complete the hygienic activities as evidenced by 48% of the pin-killed cells still remaining capped at the end of each 9 hour period assay. As the H colonies that were chosen for the present study come from a 22 year breeding programme for hygienic behaviour (Palacio et al., 1996), this result reinforces the idea that selected colonies for high hygienic behaviour do in fact detect and remove pin-killed brood more rapidly compared to unselected (non-hygienic) colonies. Moreover, selected colonies from the same programme with over 80% of hygienic behaviour (based on the pin-killed brood assay), have shown a lower incidence of brood diseases and higher survival without chemical controls, especially for American foulbrood, when compared to non-hygienic colonies in previous studies (Palacio et al., 2000, 2010). The increased efficiency of our H colonies in removing pin-killed brood could not be explained by the higher total number of bees performing the three activities of hygienic behaviour. In

fact, the H colonies had fewer hygienic bees performing inspecting and uncapping activities than the NH colonies. In addition, the H colonies showed a dissimilar age-task distribution compared to the NH colonies. Particularly in the H colonies, younger hygienic bees were more frequently involved in hygienic activities than older bees. That is, a higher proportion of hygienic bees in these colonies could respond to the dead-brood stimulus by initiating the hygienic behaviour early in ontogeny. The opposite was found for the NH colonies, with the percentages of inspectors and uncappers increasing as they aged. Although Palacio et al. (2010) found no differences between H and NH colonies in the median age of bees performing the three activities (both colonies with a median age of 15 days), the NH colonies from their study presented a larger dispersion in bee ages. Greater flexibility in age performance was also evidenced by Arathi & Spivak (2001) in colonies with a low proportion of hygienic bees that continued performing hygienic activities even when they reached the normal foraging age. Thus, according to these authors, a more structured age distribution among hygienic bees within our H colonies, with more bees performing hygienic activities early in life, would partially explain the higher efficiency of the H colonies compared to the NH colonies. As selection for other colony-level traits that resulted in changes in behaviour at the individual level (Page et al., 2006), the selection for highly hygienic colonies would result in workers being more likely to perform odour-stimulus detection activities and also initiating hygienic activities earlier in life. In fact, Calderone & Page (1988) showed that workers from colonies selected for storing more pollen not only forage more successfully and are more likely to collect larger pollen load, but also forage about 1 day earlier in life. The precocity in initiating hygienic activities observed in the individual hygienic bees of our H colonies would have implications about the sanitary status of nuclei that have a high proportion of young bees potentially involved in hygienic activities.

Task partitioning among hygienic bees was evidenced in our colonies (both H and NH), with nearly 60% of hygienic bees performing only one of the hygienic activities (sub-task specialists) throughout the entire observation period. In addition, fewer total hygienic bees were involved in inspection and uncapping activities compared to the number of bees that performed the removing activity in the H colonies. Previous research on the partitioning of hygienic behaviour into subtasks within hygienic colonies, suggested that this specialisation could be determined by variability in response thresholds for odour stimulus detection of individual hygienic bees (Arathi & Spivak, 2001; Oxley, Spivak, & Oldroyd, 2010). In particular, bees involved in inspecting and uncapping cells are the most efficient bees in activities that involve odour discrimination because they rapidly identify the odour of abnormal brood (Masterman, Smith, & Spivak, 2000; Gramacho & Spivak, 2003; Palacio et al., 2010). Our results are in line with these studies and showed that these bees (inspectors and uncappers) constitute a subset of hygienic bees that are "specialists" in detecting the dead brood in H colonies. Moreover, these specialists responded to pin-killed brood (present study) in a way similar to that of hygienic bees exposed to freeze-killed brood (Arathi & Spivak, 2001), suggesting a common mechanism underlies the detection by hygienic bees of the stimulus emanating from abnormal brood. Thus, it appears that hygienic behaviour is a generalised response to the presence of abnormal brood. Besides, the "specialists" of our H colonies showed greater temporal persistence compared to those of the NH colonies. The greater temporal persistence of hygienic bees in our H colonies, particularly for inspection activity, provides further evidence that hygienic bees in these colonies have the lowest threshold for stimulus detection, which leads to specialisation in this activity and its performance during the days of the study. The specialisation within the hygienic bee group would reflect the adaptation capacity of the whole hive to improve the division of labour in

favor of a more efficient behaviour. Limited but efficient resources (in term of bees) invested for hygienic activities would leave middle-age workers available for other in-hive activities required for the colony. In fact, the presence of dead brood, and thus an increased need to uncap and remove the cell contents, results in a decreased performance frequency for the other behaviours of hygienic bees (e.g., walking, autogrooming, inspecting cells) (Arathi et al., 2000), that could be performed by other same-age workers. In addition, it is likely that learning could lead to better detection of the stimulus with continued exposure (Masterman, Smith, & Spivak, 2000). Learning may be a component of specialisation that influences the efficiency on this task performance; as occurred with other out-hive-tasks (Dukas & Visscher, 1994). In fact, a recent study showed that honeybees selected for increased hygienic behaviour against *V. destructor* performed better in learning and discriminating between the volatile odors of Varroa-infected and non-infected pupae than honeybees from the control colonies (Chakroborty, Bienefeld, & Menzel, 2015). If the learning capacity of bees has a genetic basis, then the temporal persistence to identify abnormal odours from the brood observed in our H colony could be associated with a higher learning capacity in these bees.

Disease transmission in insect societies is believed to depend greatly on the structure and dynamics of their social interaction networks (Charbonneau, Blonder, & Dornhaus, 2013; Stroeymeyt, Casillas Perez, & Cremer, 2014). Previous studies evidenced that the structure of social organisation of honeybee colonies that implies any behaviourally segregating structuring as age or task-group compartmentalisation, contributes to social immunity (Naug & Camazine, 2002; Cremer, Armitage, & Schmid-Hempel, 2007; Baracchi & Cini, 2014). In fact, honeybee workers might modulate social contacts depending on the age of interacting partners. Social segregation between age groups is thus reinforced, providing organisational immunity (Stroeymeyt, Casillas Perez, & Cremer, 2014). The results obtained in our H colonies are consistent with previous studies performed by Arathi et al. (2000), and Sun & Zhou (2013). They found that workers performing disease-risk tasks are usually highly specialised and have few interactions with other workers. This leads to their social isolation and hence, a limiting of the disease exposure risk. However, whether a colony-level pathogen-spread could be hindered depending on the number, identity, and degree of specialisation of the hygienic workers in the hygienic colonies from our breeding program, should be investigated in future studies. In addition, new efforts are necessary to increase the number of colonies from each group (H and NH) in order to generalise our results to the H and NH populations.

Overall, the greater efficiency evidenced by the H colonies could be explained at an individual level by their precocity (initiating the behavior early in life) and their greater persistence in the tasks during the days of the experiment. Greater efficiency at the colony level could be explained by a structured age-task distribution and an optimised use of bee resources that would reinforce social segregation between age sub-groups of hygienic bees, providing organisational immunity (Cremer, Armitage, & Schmid-Hempel, 2007). The present results highlight that individual behaviour may influence the collective dynamics of a complex behaviour in honeybee colonies, and showed that the selection for highly hygienic colonies can result in changes in individual bees that improve the performance of the colony behaviour as a whole.

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