First posted online on 9 January 2018 as 10.1242/jeb.171470
Access the most recent version at http://jeb.biologists.org/lookup/doi/10.1242/jeb.171470

Stress decreases pollen foraging performance in honeybees

Célia Bordier[1], Simon Klein[2,3], Yves Le Conte[1], Andrew B. Barron[3,*] & Cédric Alaux[1,*]

[1] INRA, UR 406 Abeilles et Environnement, 84914 Avignon, France

[2] Research Center on Animal Cognition, Center for Integrative Biology, National Center for Scientific Research (CNRS), University Paul Sabatier (UPS), Toulouse, France

[3] Department of Biological Sciences, Macquarie University, North Ryde, NSW 2109, Australia

[*] joint senior authors

Correspondance:
Cédric Alaux
INRA, UR 406 Abeilles et Environnement,
Domaine Saint-Paul, CS 40509
84914 Avignon
France
E-mail: cedric.alaux@inra.fr
Tel.: +33 (0)4 32 72 26 19

Keywords: immune challenge, pollen foraging, biogenic amine, RFID

Summary statement

By tracking honeybees with a Radio-Frequency Identification device (RFID) and a camera at the colony entrance, we provided experimental evidence for a decrease in pollen-foraging performance in stressed bees.

Abstract

For honeybees, foraging is energetically demanding. Here we examined whether stressors, which increase metabolic demands, can impair foraging performance. A controlled non-pathogenic stressor (immune challenge) resulted in a change in foraging preferences of bees. It reduced pollen foraging, and increased the duration of trips in pollen foragers. Stress also reduced the amount of octopamine in the brain of pollen foragers (a biogenic amine involved in the regulation of foraging and flight behaviour in insects). According to the literature, flight metabolic rate is higher during pollen foraging than nectar foraging, and nectar gives a higher energetic return relative to the foraging effort when compared to pollen. We thus propose that stress might be particularly detrimental to the performance of pollen foragers, and stressed bees prefer the energy-rich resource of nectar. In conclusion, stress, even at low levels, could have consequences on both bee foraging behaviour and thereby the nutritional balance of the colony.

Introduction

For honeybees, which are central-place foragers relying on pollen and nectar from flowers, foraging behaviour places demands on both cognitive capacities (Klein et al., 2017), and metabolic capacity: indeed, insect flight is known to be among the most intense and energy-demanding physiological process in the animal kingdom (Dudley, 2000). The metabolic rates of flying insects, mainly fuelled by carbohydrates, can be up to 170 times higher than resting individuals (Bartholomew and Casey, 1978). As a consequence, it is expected that environmental stressors (e.g. parasites and temperature changes), which often impose increased metabolic demands (Bordier et al., 2017a; Johnson and White, 2009), could compromise foraging performance. Deciphering how stress impacts honeybee foraging performance might therefore help us better understand the mechanisms underlying colony decline and failure, which continues to be an issue of widespread concern (Goulson et al., 2015; Potts et al., 2010).

Stressors may directly limit bees' energetic reserves and thus reduce foraging performance. Indeed, there are several reports of a reduction of global flight activity in parasitized bees due to energy depletion (Kralj and Fuchs, 2010; Alaux et al., 2014; Naug, 2014; Wolf et al., 2014). Stressors may also affect forager decision-making processes as a consequence of the energetic challenges of the stressor, in which case bees may show a preference for carbohydrate rich resources to supply their own energy needs. The finding that the gene coding for the pheromone biosynthesis-activating neuropeptide, a neuropeptide known to be higher in nectar foragers than pollen foragers (Brockmann et al., 2009), is over-expressed in parasitized bees (McDonnell et al., 2013), provides some indirect support for this hypothesis. Stress can decrease sucrose responsiveness (Pankiw and Page, 2003), which is lower in nectar foragers than in pollen foragers (Pankiw and Page, 2000), suggesting that stress might cause a change in foraging preference. In addition, it has been shown that parasitized bees are less likely to forage for pollen (Lach et al., 2015). Together these findings suggest that stressed bees may favour nectar over pollen foraging. This could have consequences for the nutritional balance and development of the colony, since the majority of larva protein intake indirectly comes from pollen supply (Brodschneider and Crailsheim, 2010; Pernal and Currie, 2000). Moreover, pollen nutrition promotes immunocompetence and parasitism tolerance of adult bees (Alaux et al., 2010; Di Pasquale et al., 2013).

To test the hypothesis that stress can induce a change in foraging performance, without any potential effects of parasite manipulation of host metabolism (Adamo, 2012; Biron and Loxdale, 2013), we exposed bees to a non-pathogenic immune-challenge. Immune responses

are energetically costly, and even simple responses, like encapsulation, can raise metabolic rate by up to 28% in insects (Ardia et al., 2012; Freitak et al., 2003). We then tracked their foraging behavior throughout their life with a Radio-Frequency Identification device (RFID), and a camera at the colony entrance to identify if they carried pollen loads. Finally, we assessed the influence of stress on brain biogenic amine levels, which is known to be involved in the regulation of bee behaviour (Schulz and Robinson, 2001; Schulz et al., 2002).

Materials and methods

Experiments were performed from January to April 2016 with honeybees (*Apis mellifera*) obtained from the research apiary at Macquarie University (Sydney, Australia). We tested the influence of stress on foraging behaviour (Experiment 1) and brain biogenic amine signalling (Experiment 2). Frames containing late-stage pupae were collected from 3 donor colonies and placed into an incubator overnight at 34°C. Newly emerged bees were marked on the thorax with either a RFID tag for experiment 1, or a paint mark for the experiment 2, and released into host colonies. They were then re-captured when 7 days old and placed in plastic cages with *ad libitum* sugar solution (50% w/v). Half of the bees were given an immune challenge, which consisted of piercing the cuticle between the third and the fourth tergites of the abdomen using a 0.15-mm needle. If a haemolymph drop was released after the pin prick, the bee was discarded. Previous studies have already shown that the bee's immune system is activated by this wounding, without pathogen infection (Alaux et al., 2014; Evans et al., 2006; Siede et al., 2012). Control bees did not receive any pin prick. Handled bees (control and immune challenged) were given an additional paint mark on the abdomen to identify them by their treatment group before release back into their colony. This experiment was repeated 3 times.

Experiment 1: Impact of immune challenge on foraging performance

Following the stress treatment at 7 days, 380 control and 370 stressed bees in total (n = 3 trials) were released into a small nucleus hive equipped with a modified entrance. Bees had to use a specific path to exit the hive and another one to enter inside the hive. Each path was made of transparent 1cm of diameter plastic tubing (Bunnings, Gordon, Australia). To avoid bees using the wrong path, a plastic gate with plastic bristles, which bees could use in only one direction, was placed at the end of each path. The traffic of bees was also regulated using infrared activated gates placed at the beginning of each path Arduino technology (Arduino, Adafruit and little birds electronics, Hornsby, Australia). Each time a bee broke the infrared

beam, the linked gates were closed behind the bee for 10 seconds, which was the time needed for bees to cross the path and RFID system. Each path was equipped with a RFID reader (Invengo, Guangzhou, China; Perry et al., 2015; Søvik et al., 2015) to monitor each of the entering and exiting channels. Each RFID tag (diameter 4 mm; weight 1 mg) had a unique digital identifier read by the antennae at the entrance and exit. The entrance path was also equipped with a digital video camera (Logitech) and a white LED light enclosed in a plastic box. Motion detection video recording software (ZoneTriger, Omega Unfold Inc. Canada) was used to visually identify whether bees carried pollen or not.

Experiments continued until the last recording of the last bee, i.e. 55 days. RFID data, i.e. bee ID, direction (in or out of the hive) and time (day, hours, minutes and seconds) were recorded in .csv files. From this data, we were able to reconstruct trips outside the hive for each bee. RFID readings were time-matched with readings from the camera, and videos taken from 10 seconds before RFID detection were inspected to identify the resource of returning bees (pollen or not-pollen). Only data for bees with an RFID tag and paint marks on their abdomen were analysed. Trips shorter than 30 seconds were not considered as foraging flights and were excluded. As in Perry et al. (2015), trip longer than 8 h were also removed.

Of the 380 control and 370 immune challenged bees, a completed foraging flight was recorded at least once from 96 and 74 bees, respectively. This loss of bees could be due to the loss of tag prior to leaving the hive, ejection from the colony by nestmates or the death during its first flight. In total, 979 flights identified as pollen (n = 154) or non-pollen (which can be nectar, water or an empty crop; n = 825) foraging flights were recorded. The number of foraging flights appeared to be relatively low for a total of 170 bees, but it was likely explained by the experimental device composed of one entrance and one exit paths (one bee at a time could use the path), and the fact that many bees completed a very low number of flights (median, first and third quartiles: 4, 2, 8 foraging trips per bee, respectively). A maximum of 83 completed foraging trips per bee was recorded and 20 bees completed more than 20 trips.

Experiment 2: Impact of immune challenge on brain biogenic amines levels

After the stress treatment at day 7, 637 control and 695 immune challenged bees in total were introduced into a normal Langstroth colony (n = 3 trials). Bees returning to the colony when they were between 24 and 28 days old were sampled and immediately flash-frozen into liquid nitrogen. Whether they carried pollen or not was also noted. Frozen heads were freeze-dried for 60 min at a pressure below 300 mTorr (VirTis Benchtop^{TM}) and -35°C and then stored at

-80°C until brain dissection and biogenic amine analysis. Brain dissections (including optic lobes, antennal lobes, the central brain and gnathal ganglion) were performed on dry ice.

Brain biogenic amine (octopamine-OA, dopamine-DA, tyramine-TYR and serotonin-5-HT) levels were measured using High-Pressure Liquid Chromatography (HPLC) following the protocol described by Søvik et al. (2013) and also used later (Scheiner et al., 2014; Søvik et al., 2015). Briefly, the HPLC system was composed of a pump and an autosampler (Agilent 1200 Series; Agilent Technologies, Santa Clare, CA, USA), coupled to an electrochemical detector (ESA Coulochem III) connected to an analytical cell (ESA 3011A, Chelmsford, MA, USA). A 100 mm Hypersil BDS octadecylsilane column was used to separate samples (Thermo Fisher Scientific Waltham, MA, USA). Signals were integrated using the Chemstation software with reference to a standard curve obtained from perchloric acid solutions containing 10 pg/µl of dihydroxybenzylamine and varying amount of OA, DA, TYR, 5-HT, (Sigma-Aldric, St. Louis, MO, USA).

In total, we obtained information on brain levels of biogenic amines for 94 control bees (32 with pollen and 62 without pollen) and 50 immune challenged bees (12 with pollen and 38 without pollen). Tyramine was detected in only 14% of brains, and thus was not analysed.

Statistical analysis

All statistics were performed using the statistical software R version 3.2.1 (R Core Team, 2015). For the RFID experiment, the last day any individual bee was detected was assumed to mark the date of bee death. We then compared the probability of survival between stressed and control bees using the Kaplan-Meier test ("surfit" function of the survival package on R) (Therneau and Lumley, 2014).

Aspects of the foraging performance of bees were analysed using mixed models. The choice of best-fit model was based on the smaller sample size-corrected Akaike's Information Criterion (AICc) (Burnham and Anderson, 2004). Variation in total number of completed foraging flights per bee, the collected resource (pollen or not-pollen) and foraging trip duration were each analysed using different mixed models and fitted with a Poisson, binomial and Gaussian distribution, respectively (based on the distributions of our experimental data). To analyse the number of trips and the collected resource, the treatment (immune challenged or control) and trial were set as fixed and random explanatory variables, respectively. To analyse foraging trip duration, collected resource and honeybee identification were added as fixed and random explanatory variables, respectively.

The normality and the homoscedasticity of brain biogenic amines levels were such that parametric analyses were appropriate for these data. Biogenic amine amounts were analysed using a repeated measures ANOVA followed by Tukey's post-hoc comparison. Treatment and the resource collected (pollen or not-pollen) were analysed as fixed factors while the trial was analysed as random factors.

Results

Experiment 1: Impact of immune challenge on survival and foraging performance

Survival probability did not differ between the control and immune challenged groups (Kaplan-Meier test, $P = 0.42$; Fig. 1A).

The best-fit model explaining the variability in the number of trip per bee (lowest AICc) included a significant effect of treatment (Table 1). Immune challenged bees completed slightly more flights than control bees (mean predicted values with 95% confidence interval: 6.46 [6.12-6.80] versus 5.22 [4.95-5.49], respectively).

A significant switch in foraging preference was detected, with immune challenged bees performing 1.9 times fewer pollen foraging trips (9.14% [8.32-9.96]) than control bees (17.56% [16.20-18.91]; Fig. 1B and Table 1).

Considering foraging trip duration, the best-fit model included a significant interaction between treatment (immune challenged or control) and the collected resource (pollen or not-pollen) (Table 1). Pollen foraging trips were longer than non-pollen foraging trips (Fig. 1C), but trip duration for each collected resource also varied with treatment. Immune challenged bees performed slightly shorter non-pollen foraging trips than control bees (Fig. 1C), but when foraging for pollen immune challenged bees performed 30% longer trips than control bees (Table 1).

Experiment 2: Impact of immune challenge on brain biogenic amines levels

Brain dopamine and serotonin levels did not differ significantly between treatment groups (ANOVA: $P = 0.67$; $P = 0.14$, respectively) or the collected resource (ANOVA: $P = 0.75$; $P = 0.27$, respectively; Fig. 2A and B). However, we found a significant treatment by resource interaction on brain octopamine (OA) levels (ANOVA: $P = 0.02$; Fig. 2C). No difference in brain OA levels was found in non-pollen foraging bees (Tukey's post-hoc tests: $P = 1$), however when sampled returning to the hive carrying pollen, immune challenged bees had significantly less OA in the brain than control bees (around 27% less, Tukey's post-hoc tests: $P = 0.032$; Fig. 2C).

Discussion

In this study, we have provided experimental evidence for a stress-induced decrease in pollen-foraging performance in honeybees. The non-pathogenic immune challenge stress applied did not affect bee survival as has been found previously (Alaux et al., 2014), but did induce a shift in resource collection. An increase in non-pollen foragers (water foragers, nectar foragers and/or empty bees) was observed at the expense of pollen foragers. Since more than 90% of non-pollen foragers are nectar foragers and empty bees (Bordier et al., 2017b) and those bees have lower sucrose responsiveness than pollen foragers (Pankiw and Page, 2003), we could reasonably assume that stress decreased bee sucrose responsiveness. Stressed bees may prefer to forage for resources that are rich in carbohydrates to overcome the energetic cost of the stress, as has been observed with parasitism of honeybees (Lach et al., 2015). Indeed, compare to pollen, nectar gives a higher energetic return relative to the foraging effort (8:1 gain pollen vs 10:1 gain nectar; Winston, 1987). Similarly, bumblebees exposed to pesticides were found to exhibit lower pollen foraging performances (Feltham et al., 2014; Gill and Raine, 2014).

Such changes in foraging decision-making could cause a nutritional imbalance with a pollen deficit at the colony level, and thereby affect colony development. Indeed, pollen shortage may have detrimental effects on brood care, resulting in undernourished larvae (Blaschon et al., 1999) and emerging adults with behavioural deficiencies (Scofield and Mattila, 2015). Moreover, pollen nutrition during adult stage is essential for stress tolerance (DeGrandi-Hoffman et al., 2010; Di Pasquale et al., 2013; Wahl and Ulm, 1983). Finally, under extreme pollen shortage, nurse bees may reduce the number of larvae to feed and cannibalize eggs and young larvae (Schmickl and Crailsheim, 2001).

Pollen foraging trips were also 30% longer for immune challenged bees, suggesting a significant effect of the stressor on foraging capacity. It has been found that the thorax temperature differs between different classes of foragers and ranks pollen > nectar > water foragers (Feuerbacher et al., 2003). Those differences were linked to flight metabolic rate, with pollen foragers exhibiting a 10% higher hovering metabolic rate than nectar foragers, regardless of their loads (Feuerbacher et al., 2003). The authors suggested that pollen foragers require more power output to generate the same vertical lift as nectar foragers. We therefore propose that immune challenged bees spend more time on pollen collecting trips, since it is the most energetically demanding resource to collect (Feuerbacher et al., 2003) and the

stressor likely decrease the energy budget of bees. The increase in foraging trips duration may simply reflect more time resting rather than any others changes in flight characteristics (e.g. distance, speed…) (Wolf et al., 2014). It is also possible that a lower energy budget induced by the stressor caused cognitive impairment in pollen foragers and thus affected their navigation capacities (Jaumann et al., 2013), lengthening their trip times.

Finally, we found that brain OA level was depressed in immune challenged pollen foragers. OA is known to increase sucrose responsiveness in bees (Scheiner et al., 2002) and stimulate flight activity (Fussnecker et al., 2006), and therefore the drop in OA level is in accordance with the behavioural changes observed in pollen foragers after stress exposure. A previous study reported a rapid decrease in OA and DA but not 5-HT levels in response to stress exposure (chilling anesthesia and vertical spin, Chen et al., 2008). We did not find variation in DA levels after our stress exposure. However, to conclude on the nature of the causal role of biogenic amines in honeybee stress responses, functional studies involving manipulations of OA, DA, and 5-HT signalling would be required.

In conclusion, our study suggests that the highly energy-demanding foraging activity of pollen foragers make them susceptible to stress, even at low levels, which could potentially affect the colony nutrient balance (pollen vs nectar). Therefore, future studies on whether stress might narrow the colony foraging flexibility to environmental changes might help to better understand colony decline.

Acknowledgements

We thank F. Kamhi and B.V. Entler for their help with laboratory work, A. Cabirol for her help with fieldwork, and H. Dechatre and A. Faure for their help in data analyses.

Funding

C. Bordier was supported by an ANR (Agence Nationale de la Recherche) project (ANR-13-ADAP-0002) and by a travelling fellowships grant of the Company of Biologists sponsored by The Journal of Experimental Biology (JEBTF-150809).

Author contributions

Conceived and designed the experiments: CB ABB CA; Performed the experiments: CB SK; Provided experimental material: ABB; Analyzed the data: CB SK; Contributed to the writing of the manuscript: CB CA; Reviewed the manuscript: SK YLC ABB

Conflicts of interests

The authors declare that they have no competing interests

References

Adamo, S. A. (2012). The strings of the puppet master: how parasites change host behavior. In *Parasitic Manipulation* (ed. D. Hughes and F. Thomas), pp. 36-51. Oxford: Oxford University Press.

Alaux, C., Ducloz, F., Crauser, D. and Le Conte, Y. (2010). Diet effects on honeybee immunocompetence. *Biol. Lett.* **6**, 562–565.

Alaux, C., Crauser, D., Pioz, M., Saulnier, C. and Le Conte, Y. (2014). Parasitic and immune-modulation of flight activity in honey bees tracked with optical counters. *J. Exp. Biol.* **217**, 3416–3424.

Ardia, D. R., Gantz, J. E., Schneider, B. C. and Strebel, S. (2012). Costs of immunity in insects: An induced immune response increases metabolic rate and decreases antimicrobial activity. *Funct. Ecol.* **26**, 732–739.

Bartholomew, G. A. and Casey, T. M. (1978). Oxygen consumption of moths during rest, pre-flight warm-up, and flight in relation to body size and wing morphology. *J. Exp. Biol.* **76**, 11–25.

Biron, D. G. and Loxdale, H. D. (2013). Host-parasite molecular cross-talk during the manipulative process of a host by its parasite. *J. Exp. Biol.* **216**, 148–60.

Blaschon, B., Guttenberger, H., Hrassnig, N. and Crailsheim, K. (1999). Impact of bad weather on the development of the brood nest and pollen stores in a honeybee colony (Hymenoptera: Apidae). *Entomol. Gen.* **24**, 49–60.

Bordier, C., Suchail, S., Pioz, M., Devaud, J.-M., Collet, C., Charreton, M., Le Conte, Y. and Alaux, C. (2017a). Stress response in honeybees is associated with changes in task-related physiology and energetic metabolism. *J. Insect Physiol.* **98**, 47-54.

Bordier, C., Dechatre, H., Suchail, S., Peruzzi, M., Soubeyrand, S., Pioz, M., Pélissier, M., Crauser, D., Conte, Y. Le and Alaux, C. (2017b). Colony adaptive response to simulated heat waves and consequences at the individual level in honeybees (*Apis mellifera*). *Sci. Rep.* **7**, 3760.

Brockmann, A., Annangudi, S. P., Richmond, T. A., Ament, S. A., Xie, F., Southey, B. R., Rodriguez-Zas, S. R., Robinson, G. E. and Sweedler, J. V (2009). Quantitative peptidomics reveal brain peptide signatures of behavior. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 2383–2388.

Brodschneider, R. and Crailsheim, K. (2010). Nutrition and health in honey bees. *Apidologie* **41**, 278–294.

Burnham, K. P. and Anderson, D. R. (2004). Multimodel Inference: Understanding AIC and BIC in Model Selection. *Sociol. Methods Res.* **33**, 261–304.

Chen, Y. L., Hung, Y. S. and Yang, E. C. (2008). Biogenic amine levels change in the brains of stressed honeybees. *Arch. Insect Biochem. Physiol.* **68**, 241–250.

DeGrandi-Hoffman, G., Chen, Y., Huang, E. and Huang, M. H. (2010). The effect of diet on protein concentration, hypopharyngeal gland development and virus load in worker honey bees (*Apis mellifera* L.). *J. Insect Physiol.* **56**, 1184–1191.

Di Pasquale, G., Salignon, M., Le Conte, Y., Belzunces, L. P., Decourtye, A., Kretzschmar, A., Suchail, S., Brunet, J. L. and Alaux, C. (2013). Influence of Pollen Nutrition on Honey Bee Health: Do Pollen Quality and Diversity Matter? *PLoS One* **8**, 1–13.

Dudley, R. (2000). The Biomechanics of Insect Flight: Form, Function, Evolution. Princeton University Press.

Evans, J. D., Aronstein, K., Chen, Y. P., Hetru, C., Imler, J. L., Jiang, H., Kanost, M., Thompson, G. J., Zou, Z. and Hultmark, D. (2006). Immune pathways and defence mechanisms in honey bees *Apis mellifera*. *Insect Mol Biol* **15**, 645–656.

Feltham, H., Park, K. and Goulson, D. (2014). Field realistic doses of pesticide imidacloprid reduce bumblebee pollen foraging efficiency. *Ecotoxicology* **23**, 317–323.
Feuerbacher, E., Fewell, J. H., Roberts, S. P., Smith, E. F. and Harrison, J. F. (2003). Effects of load type (pollen or nectar) and load mass on hovering metabolic rate and mechanical power output in the honey bee *Apis mellifera*. *J. Exp. Biol.* **206**, 1855–1865.
Freitak, D., Ots, I., Vanatoa, A., Hõrak, P. and Horak, P. (2003). Immune response is energetically costly in white cabbage butterfly pupae. *Proc. R. Soc. B-Biological Sci.* **270**, S220–S222.
Fussnecker, B. L., Smith, B. H. and Mustard, J. A. (2006). Octopamine and tyramine influence the behavioral profile of locomotor activity in the honey bee (*Apis mellifera*). *J. Insect Physiol.* **52**, 1083–1092.
Gill, R. J. and Raine, N. E. (2014). Chronic impairment of bumblebee natural foraging behaviour induced by sublethal pesticide exposure. *Funct. Ecol.* **28**, 1459–1471.
Goulson, D., Nicholls, E., Botías, C. and Rotheray, E. L. (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *SciencExpress* **2010**, 1–16.
Jaumann, S., Scudelari, R. and Naug, D. (2013). Energetic cost of learning and memory can cause cognitive impairment in honeybees. *Biol. Lett.* **9**, 20130149.
Johnson, E. C. and White, M. P. (2009). Stressed-out insects: Hormonal actions and behavioral modifications. *Horm. Brain Behav. Online* 1069–1097.
Klein, S., Cabirol, A., Devaud, J.-M., Barron, A. B. and Lihoreau, M. (2017). Why bees are so vulnerable to environmental stressors. *Trends Ecol. Evol.* **2201**, 1–11.
Kralj, J. and Fuchs, S. (2010). *Nosema* sp. influences flight behavior of infected honey bee (*Apis mellifera*) foragers. *Apidologie* **41**, 21–28.
Lach, L., Kratz, M. and Baer, B. (2015). Parasitized honey bees are less likely to forage and carry less pollen. *J. Invertebr. Pathol.* **130**, 64–71.
McDonnell, C. M., Alaux, C., Parrinello, H., Desvignes, J.-P., Crauser, D., Durbesson, E., Beslay, D. and Le Conte, Y. (2013). Ecto- and endoparasite induce similar chemical and brain neurogenomic responses in the honey bee (*Apis mellifera*). *BMC Ecol.* **13**, 25.
Naug, D. (2014). Infected honeybee foragers incur a higher loss in efficiency than in the rate of energetic gain. *Biol. Lett.* **10**, 20140731.
Pankiw, T. and Page, R. E. (2003). Effect of pheromones, hormones, and handling on sucrose response thresholds of honey bees (*Apis mellifera* L.). *J. Comp. Physiol. A Neuroethol. Sensory, Neural, Behav. Physiol.* **189**, 675–684.
Pankiw, T. and Page, R. E. (2000). Response thresholds to sucrose predict foraging division of labor in honeybees. *Behav. Ecol. Sociobiol.* **47**, 265–267.
Pernal, S. F. and Currie, R. W. (2000). Pollen quality of fresh and 1-year-old single pollen diets for worker honey bees (*Apis mellifera* L.). *Apidologie* **31**, 387–409.
Perry, C. J., Søvik, E., Myerscough, M. R. and Barron, A. B. (2015). Rapid behavioral maturation accelerates failure of stressed honey bee colonies. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 3427–32.
Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O. and Kunin, W. E. (2010). Global pollinator declines: Trends, impacts and drivers. *Trends Ecol. Evol.* **25**, 345–353.
R Development Core Team. (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
Scheiner, R., Plückhahn, S., Öney, B., Blenau, W. and Erber, J. (2002). Behavioural pharmacology of octopamine, tyramine and dopamine in honey bees. *Behav. Brain Res.* **136**, 545–553.

Scheiner, R., Toteva, A., Reim, T., Søvik, E. and Barron, A. B. (2014). Differences in the phototaxis of pollen and nectar foraging honey bees are related to their octopamine brain titers. *Front. Physiol.* **5**, 1–8.
Schmickl, T. and Crailsheim, K. (2001). Cannibalism and early capping: Strategy of honeybee colonies in times of experimental pollen shortages. *J. Comp. Physiol. - A Sensory, Neural, Behav. Physiol.* **187**, 541–547.
Schmidt, J., Thoenes, S. and Levin, M. (1987). Survival of honey bees, Apis mellifera (Hymenoptera: Apidae), fed various pollen sources. *J. Econ. Entomol.* **80**, 176–183.
Schulz, D. J. and Robinson, G. E. (2001). Octopamine influences division of labor in honey bee colonies. *J. Comp. Physiol. - A Sensory, Neural, Behav. Physiol.* **187**, 53–61.
Schulz, D. J., Barron, A. B. and Robinson, G. E. (2002). A role for octopamine in honey bee division of labor. *Brain. Behav. Evol.* **60**, 350–359.
Scofield, H. N. and Mattila, H. R. (2015). Honey bee workers that are pollen stressed as larvae become poor foragers and waggle dancers as adults. *PLoS One* **10**, 1–19.
Siede, R., Meixner, M. D. and Büchler, R. (2012). Comparison of transcriptional changes of immune genes to experimental challenge in the honey bee (*Apis mellifera*). *J. Apic. Res.* **51**, 320–328.
Søvik, E., Cornish, J. L. and Barron, A. B. (2013). Cocaine Tolerance in Honey Bees. *PLoS One* **8**, 1–10.
Søvik, E., Perry, C. J., Lamora, A., Barron, A. B., Ben-Shahar, Y., Sovik, E., Perry, C. J., Lamora, A., Barron, A. B. and Ben-Shahar, Y. (2015). Negative impact of manganese on honeybee foraging. *Biol. Lett.* **11**, 20140989–20140989.
Therneau, T. M. and Lumley, T. (2014). Package "survival". Survival Analysis. R package version 2.37-7.
Wahl, O. and Ulm, K. (1983). Influence of pollen feeding and physiological condition on pesticide sensitivity of the honey bee *Apis mellifera carnica*. *Oecologia* **59**, 106–128.
Winston, M. L. (1987). The biology of the honey bee. Harvard University Press.
Wolf, S., McMahon, D. P., Lim, K. S., Pull, C. D., Clark, S. J., Paxton, R. J. and Osborne, J. L. (2014). So near and yet so far: Harmonic radar reveals reduced homing ability of nosema infected honeybees. *PLoS One* **9**, e103989.

Figures

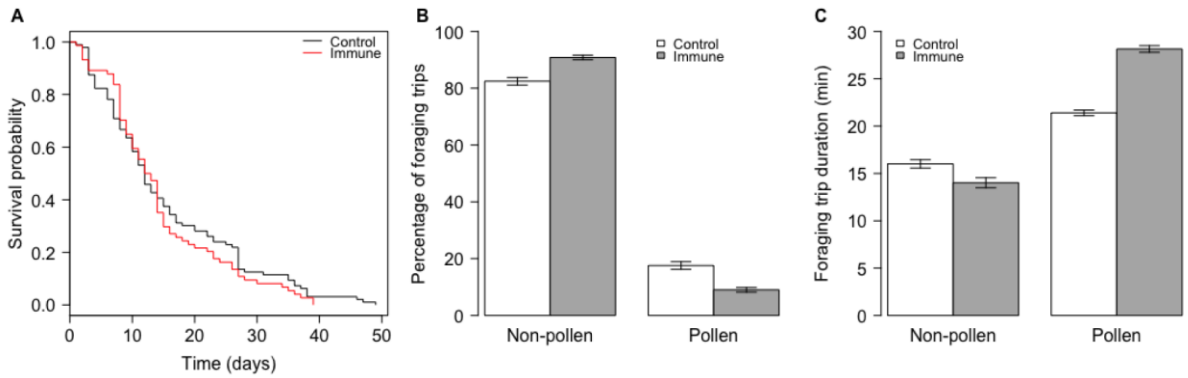

Figure 1. Survival probability and foraging trip characteristics according to the treatment. Data shown the survival over 49 days for control bee (black line) and immune challenged bees (red line) (A). Day 0 was the day of stress exposure. Bees from the two treatment groups did not differ in survival probability (Kaplan-Meier test, $P = 0.42$). For the percentage of pollen and non-pollen foraging trip (B) and the foraging trip duration (C), mean and 95% confidence intervals predicted by the model (Table 1) are shown according to the collected resource and the treatment: control (white bars; n = 100 pollen and 401 non-pollen foraging trips) and immune challenge (grey bars; n = 54 pollen and 424 non-pollen foraging trips). Immune-challenged bees performed less but longer pollen foraging trips than control bees.

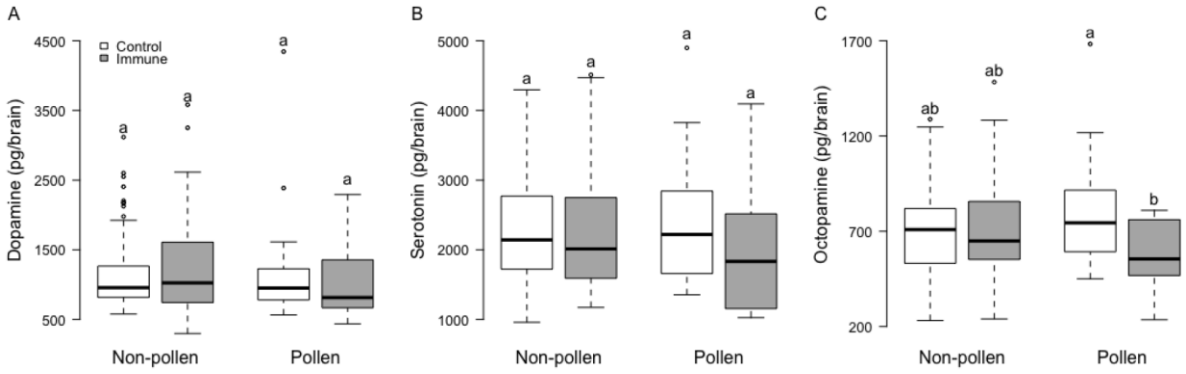

Figure 2. Brain biogenic amines levels in response to immune challenge in different forager groups. The amount of (A) dopamine-DA, (B) serotonin-5-HT and (C) octopamine-OA, is shown for control (white boxes; n = 32 pollen and 62 non-pollen foragers) and immune-challenged bees (grey boxes; n = 12 pollen and 38 non-pollen foragers), and according to the collected resource. Boxes show 1st and 3rd interquartile range with line denoting median. Whiskers encompass 90% of the individuals, beyond which each outlier is represented by a point. Plots that do not share a common superscript are statistically different ($P < 0.05$, ANOVA followed by Tukey's post hoc comparisons).

Table 1. Summary of best-fit mixed models to analyse the impact of immune challenge on foraging behaviour. Three models were fitted to analyse the number of foraging trips, foraging trip duration and foraging preference (pollen or not-pollen). Only summaries of the best-fit models are shown. For each model, fixed and random explanatory variables, number of statistical units, degree of freedom (df) and corrected Akaike's information criterion (AICc) are detailed. For each dependent variable, the selected model, i.e., with the lowest AICc, is indicated in bold.

Dependent variables	Fixed explanatory variables	Random explanatory variables	Number of statistical units	df	AICc
Number of foraging	**Treatment**	Trial	170 bees	**3**	**1594.7**
	Null		belonging 3 trials	2	1606.1
Foraging trip duration	**Treatment * Resource**	Trial/Bee	979 observations	**6**	**9120.3**
	Treatment + Resource		of 170 bees	5	9129.0
	Treatment		belonging 3 trials	4	9148.3
	Resource			4	9130.9
	Null			3	9150.2
Foraging preference	**Treatment**	Trial	170 bees	**3**	**379.2**
	Null		belonging 3 trials	2	391.3