

Diet effects on honeybee immunocompetence

Cédric Alaux, François Ducloz, Didier Crauser and Yves Le Conte

Biol. Lett. published online 20 January 2010
doi: 10.1098/rsbl.2009.0986

Supplementary data

["Data Supplement"](#)

<http://rsbl.royalsocietypublishing.org/content/suppl/2010/01/18/rsbl.2009.0986.DC1.html>

References

[This article cites 19 articles, 1 of which can be accessed free](#)

<http://rsbl.royalsocietypublishing.org/content/early/2010/01/18/rsbl.2009.0986.full.html#ref-list-1>

P<P

Published online 20 January 2010 in advance of the print journal.

Subject collections

Articles on similar topics can be found in the following collections

[ecology](#) (1221 articles)

[health and disease and epidemiology](#) (190 articles)

Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click [here](#)

Advance online articles have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Advance online articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Advance online articles must include the digital object identifier (DOIs) and date of initial publication.

To subscribe to *Biol. Lett.* go to: <http://rsbl.royalsocietypublishing.org/subscriptions>

Diet effects on honeybee immunocompetence

Cédric Alaux*, François Ducloz,
Didier Crauser and Yves Le Conte

INRA, UMR 406 Abeilles et Environnement, Laboratoire Biologie et Protection de l'abeille, Domaine Saint-Paul, 84914 Avignon, France

*Author for correspondence (cedric.alaux@avignon.inra.fr).

The maintenance of the immune system can be costly, and a lack of dietary protein can increase the susceptibility of organisms to disease. However, few studies have investigated the relationship between protein nutrition and immunity in insects. Here, we tested in honeybees (*Apis mellifera*) whether dietary protein quantity (monofloral pollen) and diet diversity (polyfloral pollen) can shape baseline immunocompetence (IC) by measuring parameters of individual immunity (haemocyte concentration, fat body content and phenoloxidase activity) and glucose oxidase (GOX) activity, which enables bees to sterilize colony and brood food, as a parameter of social immunity. Protein feeding modified both individual and social IC but increases in dietary protein quantity did not enhance IC. However, diet diversity increased IC levels. In particular, polyfloral diets induced higher GOX activity compared with monofloral diets, including protein-rich diets. These results suggest a link between protein nutrition and immunity in honeybees and underscore the critical role of resource availability on pollinator health.

Keywords: immunocompetence; diet; pollen; honeybee; social immunity

1. INTRODUCTION

The maintenance of the immune system is one of the most costly physiological systems in animals (Lochmiller & Deerenberg 2000; Schmid-Hempel 2005) and deficient nutrition can impair immune function and increase the susceptibility of individuals to disease. In humans, dietary protein deficiency reduces the concentrations of most amino acids in plasma and compromises the immune system (Li *et al.* 2007). Therefore, an adequate provision of proteins is required to sustain normal immunocompetence (IC), defined as the capacity of an organism to mount an immune response (Wilson-Rich *et al.* 2008). However, besides a few studies showing that the dietary protein quantity and quality can enhance immune functions (Lee *et al.* 2006, 2008), this relationship has been poorly investigated in insects. Also, to our knowledge, it is unknown whether diet diversity also affects IC in insects.

To fill this gap, we tested the effect of both dietary protein quantity and diet diversity on honeybee

(*Apis mellifera*) IC by feeding bees with mono- and polyfloral pollen diets. The honeybee is a valuable model for such studies, because they usually pollinate a variety of plants but sometimes are forced to feed on single crops (monocultures). In addition, pollen, which is the main source of dietary protein and contains essential amino acids for their development (de Groot 1953), can influence longevity, the development of hypopharyngeal glands (HPG) and ovaries (Pernal & Currie 2000) and the susceptibility to pathogens (Rinderer *et al.* 1974; Rinderer & Elliott 1977); but to what extent pollen can affect IC is not known. Finally, honeybee populations have been declining over the last years and a current idea suggests that honeybee colonies may suffer from a compromised immune system (van Engelsdorp *et al.* 2008), which could be related to poor nutrition commonly associated with colony losses (van Engelsdorp *et al.* 2008). Such a study will provide a framework to better understand how the abundance and diversity of environmental resources can shape an organism's immunity.

The effects of pollen diet on the capacity for disease resistance were empirically tested by measuring haemocyte concentration and fat body content as indirect assessments of cellular and humoral IC, respectively, and phenoloxidase (PO) activity, which is involved in both. Haemocytes are involved in the phagocytosis and encapsulation of parasites, the latter process requiring PO activity, and fat body is the main site of antimicrobial peptide synthesis. As social organisms, honeybees depend not only on individual immunity, but also on the overall functioning of the hive. So, we also analysed glucose oxidase (GOX) activity as a parameter of social immunity. Mainly expressed in the HPGs (Ohashi *et al.* 1999), GOX catalyses the oxidation of β -D-glucose to gluconic acid and hydrogen peroxide, the latter having antiseptic properties. The antiseptic products are secreted into larval food and honey, which contributes to colony-food sterilization (White *et al.* 1963), and hence, to the prevention of disease contamination at the group level.

2. MATERIAL AND METHODS

Experiments were performed in Avignon (France) with local hybrid colonies (*A. m. ligustica/A. m. mellifera*). For controlling the pollen intake, 1-day-old bees were reared in cages in the dark at 32°C and 70 per cent RH. They were obtained from honeycombs containing late-stage pupae removed from source colonies. Bees were fed ad libitum with candy (30% honey from the source colonies, 70% powdered sugar), water and one of the pollen diets prepared with 1/10 water. Pollen diets were replaced every day for 10 days and bees were collected at days 5 and 10 for IC sampling. To simulate as much as possible the colony rearing conditions, cages were exposed to a Beeboost (Pherotech), releasing one queen-equivalent of queen mandibular pheromone per day.

(a) Pollen diets

Seven blends of fresh pollen commonly found in France and with a respective predominance of *Acer*, *Castanea*, *Cistus*, *Erica*, *Quercus*, *Salix* and *Taraxacum* pollen were obtained from Pollenergie (France) and frozen at -20°C. To produce monofloral pollen diets, we sorted, by colour, pellets of the predominant pollen from each of the commercial blend. Nitrogen and then protein contents of the different pollens were determined by microkjeldahl analysis using a Vapodest 45 (Gerhardt) and according to the procedures described in ISO 5983 (1997). In order to test the effect of protein quantity and pollen diversity, groups of 80 bees were, respectively, fed with monofloral diets that differed in the quantity of protein or

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rsbl.2009.0986> or via <http://rsbl.royalsocietypublishing.org>.

Table 1. Mono- and polyfloral diets of pollen. The percentage of the different pollens used for each polyfloral blend is given. The protein content of *Acer*, *Erica* and *Salix* pollen was 25.9, 17.1 and 25.8%, respectively.

diet	pollen type	% protein	blend composition
monofloral	<i>Cistus</i>	15.5	—
	<i>Taraxacum</i>	19.8	—
	<i>Castanea</i>	23.6	—
	<i>Quercus</i>	29.6	—
polyfloral	first pollen blend	19.8	32% <i>Erica</i> , 28% <i>Cistus</i> , 16% <i>Castanea</i> , 12% <i>Salix</i> , 12% <i>Acer</i>
	second pollen blend	23.6	24% <i>Quercus</i> , 20% <i>Salix</i> , 20% <i>Taraxacum</i> , 18% <i>Acer</i> , 18% <i>Cistus</i>

polyfloral diets that had the same amount of protein as monofloral diets (table 1). Control groups had no pollen. The experiment was repeated on five different colonies.

(b) Immunocompetence

IC was assessed indirectly in the absence of an actual pathogen challenge. To determine the haemocyte concentration, haemolymph was extracted with micro capillaries (10 μ l) from the second abdominal tergite and diluted 1:5 in ice-cold phosphate-buffered saline (pH 7.4). The number of haemocytes per microlitre of haemolymph was counted using a phase contrast microscope (200 \times) with haemocytometer. Fat body mass was estimated using an ether extraction method according to Wilson-Rich *et al.* (2008), then the relative mass of fat body was given as the per cent change in abdominal weight after the ether wash. There are no data yet showing how the fat body size correlates with immune response, but this tissue is the main site of immunoproteins synthesis, energy and protein storage used for brood food and vitellogenin synthesis involved in longevity (Amdam & Omholt 2002). PO and GOX activity were, respectively, measured on whole abdomen and head (see electronic supplementary material). Diet and age effects on IC were determined using two-way ANOVA followed by Newman-Keuls post hoc tests.

3. RESULTS

The amount of pollen consumed per bee and per day did not differ between the diets (one-way ANOVA: $F_{5,204} = 0.863$, $p = 0.51$, 5.6 ± 2.1 mg per bee per day) but we found a significant effect of pollen diet on the different immune parameters. Control bees had a higher haemocyte concentration compared with bees fed with pollen ($F_{6,489} = 5.27$, $p < 0.001$; figure 1a). Regarding PO activity, a slight pollen-diet effect was observed ($F_{6,428} = 2.54$, $p = 0.02$), which was due to a higher PO activity in bees fed with the protein-richest polyfloral blend compared with control bees (figure 1b). The fat body contents increased with pollen diets ($F_{6,489} = 6.74$, $p < 0.001$). In addition, the first polyfloral blend induced a higher fat body content compared with its monofloral counterpart (same protein content) and the protein-lowest monofloral diets (figure 1c). Pollen consumption also greatly increased GOX activity, and furthermore, the type of diet had a pronounced effect on its activity ($F_{6,446} = 19.9$, $p < 0.001$). Both, polyfloral diets induced a higher GOX activity compared with the same protein-level monofloral diets and the protein-richest diet (figure 1d). Finally, for each immune parameter,

no difference was observed between the different monofloral diets (figure 1a–d).

A significant age effect was also observed on the different immune parameters. Haemocyte concentration and fat body contents decreased with age ($F_{1,489} = 19.1$, $p < 0.001$ and $F_{1,489} = 25.2$, $p < 0.001$, respectively) contrary to PO and GOX activity, which increased with age ($F_{1,428} = 24.9$, $p < 0.001$ and $F_{1,446} = 123.6$, $p < 0.001$, respectively). In addition, the effect of diet on IC was consistent between 5 and 10-day-old bees, as no significant interaction between the diet and age factors was found ($p > 0.05$ for each immune parameter).

4. DISCUSSION

In this study, we provided experimental evidence for a link between pollen nutrition and baseline IC in honeybees. These results suggest that the abundance and diversity of environmental resources can have a direct impact on pollinator's health.

Haemocyte concentration was augmented in bees fed a diet with no protein. Higher haemocyte concentrations are expected to be associated with protein supply and higher resistance to disease. However, bees fed with protein might invest more resources in certain types of haemocytes (e.g. granulocyte or plasmotocyte) at the expense of others types. An investment in the type of haemocytes that are more costly to produce would ultimately lead to an overall decrease in haemocyte numbers. Or, since the metabolic activity of haemocytes increases with a pollen diet (Szymaś & Jedruszuk 2003), haemocyte concentration might increase in bees fed without protein to compensate for the lower metabolic activity. This age decrease in concentration was also found by Schmid *et al.* (2007). However, Wilson-Rich *et al.* (2008) observed a lower haemocyte concentration in 1-day-old bees compared to foragers, perhaps because collected bees were very young and in the process of reaching the maximum haemocyte concentration. Pollen feeding did not clearly affect the PO activity. Similar results were found in bumble-bees, where limited nutrition did not affect the encapsulation response mediated by PO (Schmid-Hempel & Schmid-Hempel 1998). Perhaps, the costs induced by pathogens would reveal a critical role of protein diet on PO activity. On the contrary, GOX activity, which does not provide an immune protection to the bee itself but allows prevention of infectious disease in the colony, was greatly affected by the protein diets, suggesting that bees would invest more resources in social rather than individual immunity. Indeed, honeybees possess only one-third the numbers of immune response genes known for solitary insects (Evans *et al.* 2006), which indicates that other types of defence against pathogens might be important (e.g. GOX).

Regarding the diet quality, the different monofloral diets did not induce changes in the IC levels. This could be explained by a nutritive compensation of bees fed with protein-poor pollen; however, the amount of pollen consumed per bee did not differ between the diets. Interestingly, a comparable range of protein content induces differences in ovary and

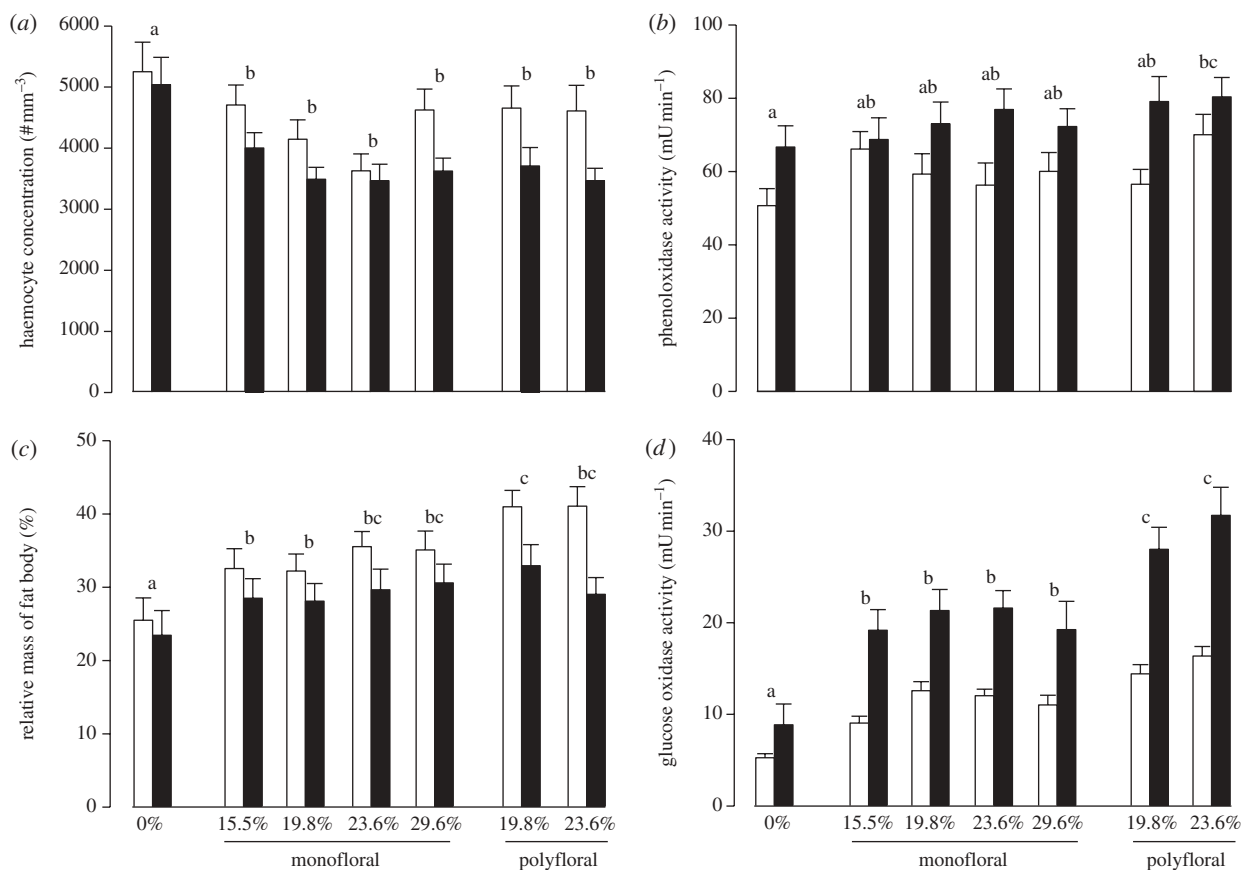


Figure 1. Effect of pollen diet on IC in 5 (open bars) and 10 days old bees (filled bars). (a) Haemocyte concentration, (b) phenoloxidase activity, (c) fat body mass and (d) GOX activity. Eight bees per cage for each experimental group were analysed for each immune parameter. The protein percentage of each pollen diet is indicated on the x-axis. Each letter indicates significant differences between diets ($p < 0.05$, Newman–Keuls post hoc tests). No significant interaction between the diet and age factors was found ($p > 0.05$ for each immune parameter). Data show mean \pm s.e.

HPG development (Pernal & Currie 2000), suggesting that IC in bees is not sensitive to the amount of protein. However, we cannot exclude the possibility that other pollen with poorer or richer protein content or challenges with pathogens would induce differences in IC.

Interestingly, polyfloral diets enhanced some immune functions compared with monofloral diets, in particular GOX activity, meaning that the diversity in floral resources confers bees with better in-hive anti-septic protection. This demonstrates that diet diversity is important and that a minimal nutrient diversity may not meet all honeybee needs. Because nitrogen content was equal between the mono- and polyfloral diets, additional properties might be present in the pollen mix. For example, essential amino acids from protein digested are required in specific proportions to complete their normal growth and development (de Groot 1953). Low pollen diversity might represent a major limiting factor for honeybee's development, but a polyfloral diet might increase the diversity and/or the proportion of specific proteins and amino acids required for tissue development (fat body and HPGs). This assumption is supported by the study of Tasei & Aupinel (2008) showing that bumble-bee larvae fed with a polyfloral blend were heavier than larvae fed with monofloral diets of higher protein content.

If nutrition is a critical factor in immune response, then malnutrition is probably one of the causes of immunodeficiency in honeybee colonies. This work also emphasizes the importance of diet diversity and underscores the need for further studies to test different blends of proteins and identify protein 'cocktails' essential for developing normal immune function.

We thank Pollenergie for pollen supply, J. L. Brunet, D. Feuillet, F. Mondet, M. R. Schmid and B. Vaissière for help and advice and A. Brockmann, C. M. McDonnell and S. F. Pernal and three anonymous referees for comments that improved the manuscript. Funding was provided by HFSP (RGP0042/2007). C.A. was supported by an INRA young researcher position (INRA SPE department).

- Amdam, G. V. & Omholt, S. W. 2002 The regulatory anatomy of honeybee lifespan. *J. Theor. Biol.* **216**, 209–228. (doi:10.1006/jtbi.2002.2545)
- de Groot, A. P. 1953 Protein and amino acid requirements of the honey bee (*Apis mellifica* L.). *Physiol. Comp. Oecol.* **3**, 197–285.
- Evans, J. D. *et al.* 2006 Immune pathways and defence mechanisms in honey bees *Apis mellifera*. *Insect. Mol. Biol.* **15**, 645–656. (doi:10.1111/j.1365-2583.2006.00682.x)
- ISO 5983 1997 *Animal feeding stuffs. Determination of nitrogen content and calculation of crude protein content—Kjeldahl*

- method.* Geneva, Switzerland: International Organization for Standardization.
- Lee, K. P., Cory, J. S., Wilson, K., Raubenheimer, D. & Simpson, S. J. 2006 Flexible diet choice offsets protein costs of pathogen resistance in a caterpillar. *Proc. R. Soc. B* **273**, 823–829. (doi:10.1098/rspb.2005.3385)
- Lee, K. P., Simpson, S. J. & Wilson, K. 2008 Dietary protein-quality influences melanization and immune function in an insect. *Funct. Ecol.* **22**, 1052–1061. (doi:10.1111/j.1365-2435.2008.01459.x)
- Li, P., Yin, Y. L., Li, D., Kim, S. W. & Wu, G. 2007 Amino acids and immune function. *Br. J. Nutr.* **98**, 237–252. (doi:10.1017/S000711450769936X)
- Lochmiller, R. L. & Deerenberg, C. 2000 Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* **88**, 87–98. (doi:10.1034/j.1600-0706.2000.880110.x)
- Ohashi, K., Natori, S. & Kubo, T. 1999 Expression of amylase and glucose oxidase in the hypopharyngeal gland with an age-dependent role change of the worker honeybee (*Apis mellifera* L.). *Eur. J. Biochem.* **265**, 127–133. (doi:10.1046/j.1432-1327.1999.00696.x)
- Pernal, S. F. & 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. (doi:10.1051/apido:2000130)
- Rinderer, T. E. & Elliott, K. D. 1977 Worker honey bee response to infection with *Nosema apis*. *J. Econ. Entomol.* **70**, 431–433.
- Rinderer, T. E., Rothenbuhler, W. C. & Gochnauer, T. A. 1974 The influence of pollen on the susceptibility of honey-bee larvae to *Bacillus* larvae. *J. Invertebr. Pathol.* **23**, 347–350. (doi:10.1016/0022-2011(74)90100-1)
- Schmid, M. R., Brockmann, A., Perk, C. W., Stanley, D. W. & Tautz, J. 2007 Adult honeybees (*Apis mellifera* L.) abandon hemocytic, but not phenoloxidase-based immunity. *J. Insect. Physiol.* **54**, 215–221.
- Schmid-Hempel, P. 2005 Evolutionary ecology of insect immune defenses. *Annu. Rev. Entomol.* **50**, 529–551. (doi:10.1146/annurev.ento.50.071803.130420)
- Schmid-Hempel, R. & Schmid-Hempel, P. 1998 Colony performance and IC of a social insect, *Bombus terrestris*, in poor and variable environments. *Funct. Ecol.* **12**, 22–30. (doi:10.1046/j.1365-2435.1998.00153.x)
- Szymaś, B. & Jędruszek, A. 2003 The influence of different diets on haemocytes of adult worker honey bees, *Apis mellifera*. *Apidologie* **34**, 97–102. (doi:10.1051/apido:2003012)
- Tasei, J. N. & Aupinel, P. 2008 Nutritive value of 15 single pollens and pollen mixes tested on larvae produced by bumblebee workers (*Bombus terrestris*, Hymenoptera: Apidae). *Apidologie* **39**, 397–409. (doi:10.1051/apido:2008017)
- van Engelsdorp, D., Hayes Jr, J., Underwood, R. M. & Pettis, J. 2008 A survey of honey bee colony losses in the U.S., fall 2007 to spring 2008. *PLoS ONE* **3**, e4071. (doi:10.1371/journal.pone.0004071)
- White, J. W. J., Subers, M. H. & Schepartz, A. I. 1963 The identification of inhibine, antibacterial factor in honey, as hydrogen peroxide, and its origin in a honey glucose oxidase system. *Biochem. Biophys. Acta* **73**, 57–70. (doi:10.1016/0006-3002(63)90359-7)
- Wilson-Rich, N., Dres, S. T. & Starks, P. T. 2008 The ontogeny of immunity: development of innate immune strength in the honey bee (*Apis mellifera*). *J. Insect Physiol.* **54**, 1392–1399. (doi:10.1016/j.jinsphys.2008.07.016)