Ghosh et al. Journal of Ecology and Environment (2020) 44:4
https://doi.org/10.1186/s41610-020-0149-9

Journal of Ecology and Environment

RESEARCH Open Access

Foraging behaviour and preference of pollen sources by honey bee (*Apis mellifera*) relative to protein contents

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Abstract

Background: Pollen is an important source of protein and lipids for many animals including honey bees. In order to understand the foraging behaviour of honey bee colonies and preference among the available floral resources, pollen collections from three experimental healthy colonies of honey bees were analysed in the month of June.

Results: The amount of pollen collections were related to the colony's need which was indicated by the number of larval and adult bees present in the hive. Interesting was the sequence of pollen collection from different floral sources. All honey bee colonies collected pollens from *Trifolium repens* first, then *Erigeron annus* and the third choice was *Coreopsis drummondii* and *Oenothera biennis* flowers. Total protein content of *Trifolium* pollen was the highest (20.0 g/100 g DM), and the others were in the range of 8.9–11.4 g/100 g DM.

Conclusion: The results indicated that the first criteria for honey bee foraging preference of pollens would be the nutritional contents of protein and the resource availability of the lesser nutritious floral sources. This information can help pollinator protection programmes of habitat manipulation using flowering plants for nectar and pollen sources.

Keyword: *Trifolium*, *Erigeron*, *Oenothera*, *Coreopsis*, Amino acid, Nutrition

Background

Animals including insects forage food in order to obtain appropriate nutrients for their sustenance which includes growth, development and reproduction. In the case of the social insects, it is not just to satisfy the requirements of individuals but the foragers collect the nutritional resources also for the other members living in the hive (Hölldobler and Wilson, 2009). Bees forage nectar and pollen as their nutritional source. Nectar is a sugary fluid secreted by plant especially within the flower undergoes modification inside the bee's honey stomach with the help of enzymes like invertase, diastase and glucose oxidase and is resulting in honey (Winston, 1987). On the other hand, pollen is the primary source for protein, lipid and micronutrients like minerals and minerals necessary for bee's health (Ghosh and Jung 2017). Thus, nutrient quality and quantity, of course, can be counted as one of the key parameters followed by other influential factors like the appearance of flower including colour, shape, morphology, display area and odour (Willmer, 2011; Brunet et al., 2015) to understand foraging decision-making of insect foragers. This, in turn, is also essential to figure out the formulation of feed and prepare supplementary in order to rear the species.

All insect foragers do not appear the same in regard to their food collection. The differences exist between the foraging pattern of honey bees and bumblebees. It has been shown that bumblebees collect pollens from at least twice as many plant species than honey bees generally do (Leonhardt and Blüthgen 2012). Honey bees show a highly flower constant foraging behaviour whereas bumblebee collects even up to six different species during one foraging trips (Kratochwil and Kohl, 1988). Studies indicated that the honey bees do not forage preferentially on pollen with higher protein

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concentrations (van der Moezel et al., 1987; Roulston et al., 2000; Pernal and Curie, 2001). In contrast, bumblebees show a preference for a higher protein containing pollen (Kitaoke and Nieh, 2009; Leonhardt and Blüthgen 2012) and in fact, a higher quality of protein (Robertson et al., 1999; Hanley et al., 2008). The present pilot study has been carried out in order to understand the foraging behaviour of honey bee colonies and preference among the available floral resources.

Results

Honey bee foragers collected different pollen during the study period. Figure 1 represents the SEM photographs of the bee collected pollens, which were then identified by the morphometric characteristics. Figure 2 represents the amount of pollen per day foraged by three colonies. The third honey bee colony collected the highest amount of pollen (3845.7 mg) followed by 2nd (3159.3 mg) and 1st (1613 mg) during these three days although that there is no significant differences among the amounts of pollen collections of three experimental honey bee colonies (ANOVA, F = 1.078; Sig. = 0.398). Figure 3 represents the relationship between foraging efficiency and temperature. It shows that the temperature around 20–28 °C was found favoured and resulted in higher return of pollen and lower temperature of 15 °C or higher temperature of 30 °C was not favoured. Figure 4 represents the respective amount of collected pollen from each floral origin by three different honey bee colonies. It has been found that all three honey bee colonies collected the majority of pollen from *Trifolium repens* followed by *Erigeron annus*. *Oenothera* and *Coreopsis* pollen were collected mostly by 3rd colony and pollens obtained from other floral resources were significantly less. The amino acid composition of the studied pollens has been represented by Table 1. Total amino acids was found the highest in *Trifolium repens* (19.97 g/100 g pollen) followed by *Coreopsis drummondii* (11.44 g/100 g pollen), *Erigeron annus* (9.67 g/100 g pollen) and *Oenothera biennis* (8.89 g/100 g pollen). Altogether, seventeen amino acids were detected and estimated. Tryptophan was not recovered because of the entire degradation of it by acid hydrolysis. Among the essential amino acids, leucine and lysine was found predominating and among the non-essential amino acids glutamic acid was found the most abundant.

Discussion

The requirement of food depends on several factors in the bee colony. Besides many other factors like genotype, seasonal availability of resources, foragers primarily depend on the number of larvae present in the hive and amount of stored food in order to make the decision about the amount of pollen foraging (Camazine, 1993, Bilisik et al., 2008). As one mechanism, Pankiw et al.'s (1998) study demonstrated that brood pheromone stimulates pollen foraging behaviour of forager honey bees. Jung and Burgett (2011) and Jung et al. (2011) tested if the synthetic brood pheromone effects on colony level of honey bee showing higher foraging activity and pollen collection. Also, the number of very active foragers in a hive is an important factor. In a very recent study, researchers showed that only 19% of the total forager performed 50% of the colony's total foraging trip (Klein et al., 2019). Thus, these factors could be plausible reasons for different foraging pollen amounts of the honey bee colonies.

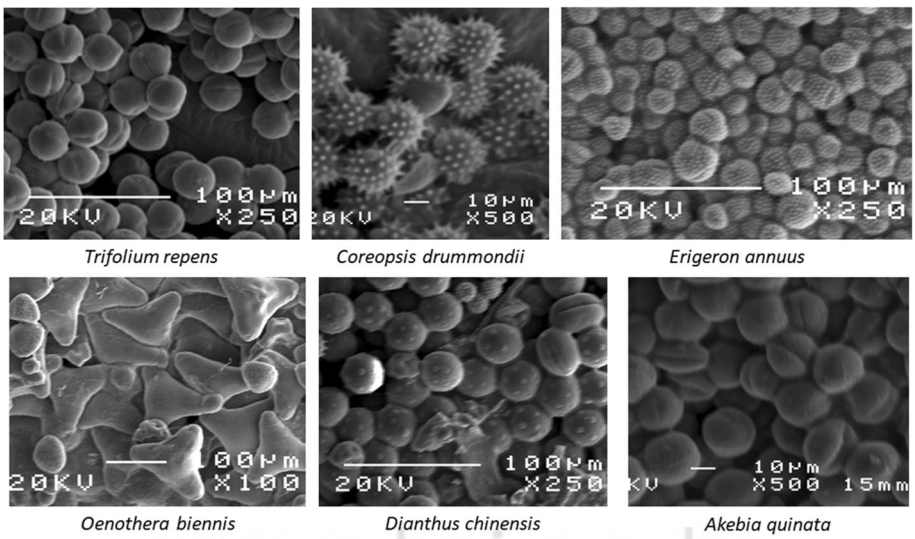

Fig. 1 SEM photographs of pollens collected by honey bee during the study

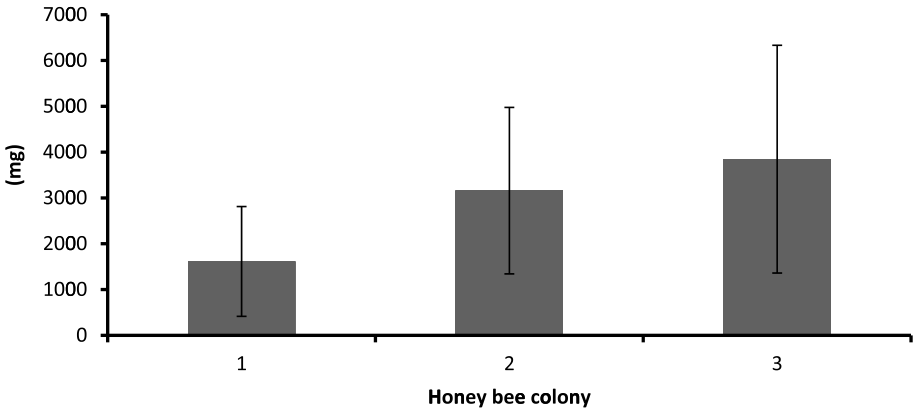

Fig. 2 Total amount (mg/day) of pollen collected by foraging workers by the three experimental honey bee colonies during the study in June 2017

In almost all cases, the foraging activity was found the highest at 1 pm because it had the highest amount of pollen foraged during this period of the day. Assuming the amount of pollen collected is proportionate to the activity of foragers, the study demonstrated a clear trend that the honey bees were more active in the afternoon than in the morning. Pernal and Curie (2001) reported a higher foraging rate in the afternoon that in the morning. In another study, researchers found that foragers showed higher visitation and foraging of onion flower during 11 to 12 in a day (Yucel and Duman, 2005). In a simple legitimate way, this highest activity of foragers during a day depends on the time at which foragers can have the highest reward from their visit to a flower, so in turn, it depends on the flowering timing of plant species. The next important factor is of course temperature. The most favoured temperature was found around 20–28 °C (Fig. 3) and resulted in higher return of pollen. Lower temperature of 15 °C or higher temperature of 30 °C was not favoured. Honey bees maintain the inside colony temperature fairly constant over the season (Seeley, 1985). Temperature of the brood area is narrowly controlled to be 35 °C for optimum larval development while the temperature inside the bee ball cluster during winter is around 21–24 °C (Farhenholz et al., 1989; Yi and Jung, 2010; Jeong et al., 2016). However, foraging is largely limited by the outside temperature because of the small sized-body with high energy demand for flight muscle movement (Seeley, 1985). Honey bees rarely work below 13 °C and above 38 °C (Abou-Shaara, 2014). Foragers carry relatively little fluid during the hottest period and pollen foraging decreases at high ambient temperature (Cooper et al. 1985).

The next objective is to figure out the preference among the available resources. In order to understand the possible reason behind the choice of pollens (Fig. 4), we carried out the amino acid analysis of four mostly

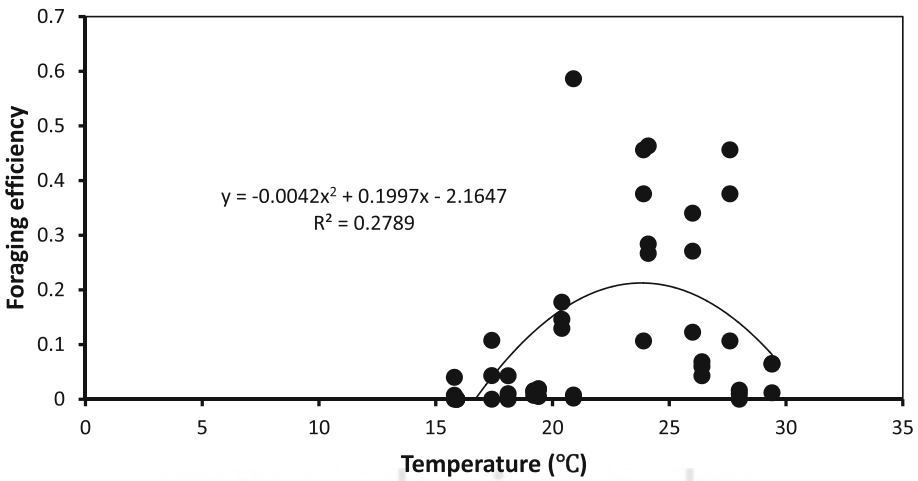

Fig. 3 Relationship between temperature and pollen foraging efficiency

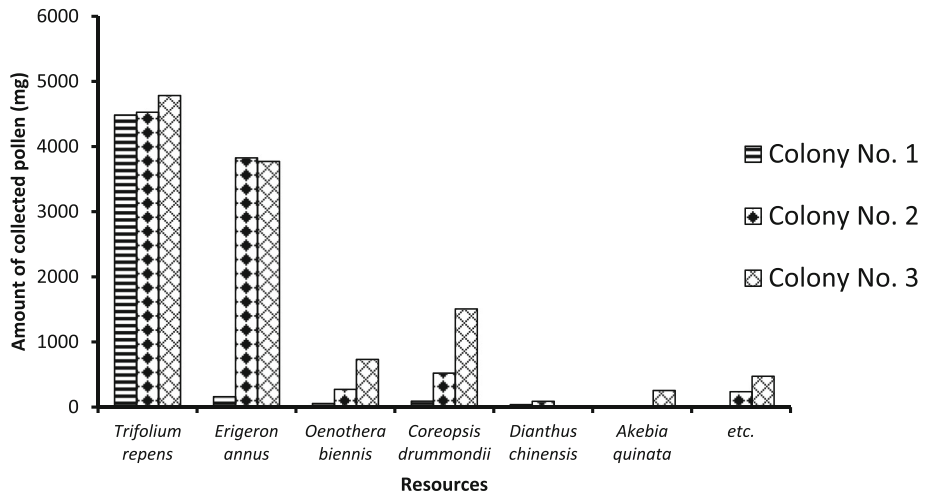

Fig. 4 Amount of pollen collected from each floral resource during the study in June 2017

bee collected pollen (Table 1). As it is a well-established fact that pollen serves as a major protein source for honey bees, we focussed on the amino acid composition at first. Protein and amino acid concentration in pollen play crucial roles in bees including modulation of immunocompetence, reproduction, etc. (Alaux et al., 2010; Vanderplanck et al. 2014; Moerman et al. 2016). The preference often depends on interaction of macronutrients, in terms of ratio, rather than a single nutrient. To cite some examples, protein to carbohydrate ratio is commonly found to be associated with nutrient regulation in herbivores (Lee et al., 2006; Behmer, 2009); however, in bees, protein to lipid ratio has also been found effective to figure out the preference (Vaudo et al.,

Table 1 Amino acid compositions of pollens of *Trifolium repens*, *Coreopsis drummondii*, *Erigeron annus* and *Oenothera biennis* (g/100 g dry matter)

Amino acid	Resources			
	Trifolium repens	*Coreopsis drummondii*	*Erigeron annus*	*Oenothera biennis*
Leucine[a]	1.64	0.96	0.66	0.58
Valine[a]	1.18	0.59	0.47	0.42
Isoleucine[a]	0.96	0.54	0.42	0.38
Methionine[a]	0.24	0.14	0.07	0.15
Lysine[a]	1.58	1.08	1.17	1.12
Threonine[a]	0.65	0.40	0.52	0.36
Histidine[a]	0.88	0.77	0.49	0.42
Phenylalanine[a]	0.56	0.34	0.24	0.23
Arginine[a]	1.20	0.55	0.48	0.37
Tyrosine	0.73	0.36	0.32	0.29
Aspartic acid	1.61	0.80	0.66	0.60
Glutamic acid	2.65	1.29	1.14	1.06
Serine	1.64	0.89	0.81	0.82
Proline	1.61	0.93	0.67	0.69
Glycine	1.14	0.73	0.67	0.59
Alanine	1.43	0.84	0.70	0.61
Cysteine	0.27	0.23	0.18	0.20
Total	19.97	11.44	9.67	8.89

[a]Essential amino acid for honey bee

2016; Donkersley et al. 2017). Vaudo et al. (2016) showed that in the case of *Bombus impatiens* the most preferred pollen exhibited protein to lipid ration is 4.6:1. However, it would not be overstated that protein content (and amino acid) of pollen plays the most significant roles and thus it is considered as central to the discussion for several reasons. The bees collect nectar which is a source of carbohydrate. The lipid content of pollens is generally within the range of 5 to 12% (Ghosh and Jung, 2017; unpublished data) and except a few of the fatty acids can be synthesised by bees. On the other hand, about half of the proteinergic amino acids, i.e. 10 amino acids, are essential to bees (de Groot, 1953). Bees must obtain these amino acids from their diet for growth, somatic maintenance and reproduction. Nursing adult bees consume the protein rich pollen to biosynthesize the brood food, royal jelly which is the main source of protein for developing larvae and the queen (Winston, 1987, Paoli et al., 2014).

Considering the total amino acids as protein *Trifolium repens* contained the highest amount of protein followed by *Coreopsis drummondii*, *Erigeron annus* and *Oenothera biennis*. Altogether seventeen amino acids were detected and estimated. Tryptophan was not recovered because of the entire degradation of it by acid hydrolysis. Except for tryptophan, all the essential amino acids demonstrated by de Groot (1953) for honey bees were present in the studied pollen. Among the essential amino acids, leucine was found predominating in the case of *Trifolium repens* which was in agreement with previous reports for oak and hardy kiwi pollen (Ghosh and Jung, 2017, Yang et al., 2013; Human and Nicolson, 2006). The reverse was found true for the other three studied pollen in which lysine was found higher followed by leucine. Lysine plays a very important role in honey bee physiology. Fortification of pollen substitute with DL lysine and methionine significantly raised the size of the fat body (Rogala and Szymaś, 2004). Further, the highest counts of haemocytes in haemolymph were recorded for bees which received pollen substitute supplemented with L isomers of methionine, lysine, histidine and threonine (Rogala and Szymaś, 2004). The next amino acid predominated was arginine. Honey bee larvae fed with L-arginine- and abscisic acid-supplemented diet was found to enhance the fitness via nitric oxide and granulocyte immune response (Negri et al., 2017). On the other hand, non-essential amino acids are also worthy. Among the non-essential amino acid, glutamic acid and proline was found predominating which is in agreement with other reports (McCaughey et al., 1980). Proline is used as fuel for flight in insects including honey bees. Also, proline helps in increasing cold hardiness (Mollaei et al., 2013). Although the amino acid content of the studied pollens varied the distribution was almost similar.

Conclusion

The amount of pollen collections were found to exhibit the relation to the colony's need which was indicated by the number of larval and adult bees present in the hive. Further, the results revealed that honey bees foraged the pollens with higher protein content. However, protein and amino acid is not the only component responsible for the well-being of the honey bee and thus exploring the interaction with other nutrients especially macronutrients is also important in the context.

Materials and methods

Pollen collection by honey bee colony

We have recruited three different healthy honey bee (*Apis mellifera*) colonies from the experimental apiary of Andong National University. The no. 1 colony contained 2 brood frames of developing larvae and the no. 2 colony consisted with 3 frames with brood, while no. 3 contained 4 brood frames. Queens were sourced from the same breeding line and requeened in autumn in the previous year. Honey bee colonies were placed in a landscape with floral resources like *Trifolium repens*, *Erigeron annus*, *Oenothera biennis*, *Coreopsis drummondii*, *Diaanthus chinensis*, *Akebia quinata*, etc. in the month of June 2017. We observed the amount of pollen collection in five different times, i.e. 7 am, 10 am, 1 pm, 3 pm and 5 pm on the consecutive three dates 6th, 7th and 8th of the month. Further, we sorted the bee collected pollen according to their floral origin. In order to understand the environmental conditions favouring honey bee foraging activities we recorded the temperature and precipitation during the foraging time of each day.

Scanning electron microscopy study

The pollens were identified based on their SEM study. We mounted pollen samples on the SEM stubs and sputter coated with gold, using Cressington Sputter coater 108 Auto. Specimens were examined and photographed obtained using Hitachi S-2500C SEM instrument in the public laboratory of Andong National University.

Amino acid analysis of the bee collected pollen

Following the standard procedure of AOAC (1990) we estimated the amino acid composition of the bee collected pollen. The samples were analysed on an as-is basis. Firstly, the pollen samples were undergone hydrolysis process in 6 N hydrochloric acid (HCl) at 110 °C for 24 h followed by the reconstitution of the samples with physiological buffer (0.12 N, pH 2.2) and analysed by the amino acid analyser (Sykam, Germany) associated with LCA K07/Li (PEEK column 4.6 × 150 mm) column.

Statistical analysis

Analysis of variance (ANOVA) procedure was used to compare the mean amount of pollen collected by different size of honey bee colonies. The relationship between temperature and pollen collection efficiency was analysed by non-linear regression. Each amino acid content was compared with the minimum requirement value proposed by de Groot (1953) for honey bee development. The statistical analysis was carried out by using SPSS ver. 16.0.

Abbreviations

SEM: Scanning electron microscope; ANOVA: Analysis of variance; DL: Dextro Levo; SPSS: Statistical Package for Social Sciences

Acknowledgements

This research was funded by Climate Change and Insect monitoring Research Project, RDA, grant number PJ013463042018, and by the BSRP through the National Research Foundation of Korea (NRF), Ministry of Education, grant number NRF-2018R1A6A1A03024862.

Author's contributions

All authors have participated in the research and manuscript writing. SG analysed chemically and wrote the manuscript. HJ collected data from the field. CJ designed the study and supervised the research and manuscript. All authors read and approved the final manuscript.

Funding

This research was funded by Climate Change and Insect monitoring Research Project, RDA, grant number PJ013463042018, and by the BSRP through the National Research Foundation of Korea (NRF), Ministry of Education, grant number NRF-2018R1A6A1A03024862.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Received: 21 November 2019 Accepted: 7 January 2020
Published online: 21 January 2020

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