Review Article

DIETARY DYNAMICS IN HONEY BEE PHYSIOLOGY AND ECOLOGY

ABSTRACT

Honey bees collect and store floral nectar and pollen as honey and bee bread in their colonies. The social division of labour within the colony facilitates the gathering and consumption of these stored foods. Within-hive bees convert the stored pollen and honey into royal jelly, which, along with other glandular secretions, serves as the primary nourishment for growing larvae and the queen, and also fed to other colony members. Research indicates that bees regulate their macronutrient intake individually and collectively, with foragers playing a key role in maintaining these specific nutritional proportions.

***Keywords*:** honey bee, nectar, pollen, royal jelly, nutrition, bee bread

Abbreviations

(Gamma-AminoButyric Acid, Major Royal Jelly Proteins)

1. INTRODUCTION

Bees play an important role in world agriculture as pollinators, but their populations have come under threat in the past 30 years from parasites and pathogens, exposure to pesticides, and less abundant food. Nutritional stress due to habitat transformation and loss is thought to be among the major factors contributing to current declines in bee populations. Understanding bee nutrition is critical to overcoming population decline. Over their 90 million years of evolution alongside flowering plants, most bee species have adapted to a herbivorous diet consisting of floral nectar and pollen (Michener, 2000). Floral nectar serves as the primary energy source for bees, fueling their flight, thermoregulation, and wax production, while pollen provides essential proteins, fats, sterols, and micronutrients. The range of plant species that bees utilize as floral hosts varies significantly among different species. However, even those considered generalists exhibit selectivity in the pollen they gather (Minckley and Roulston, 2006).

2.NUTRIENT GATHERING

Most bee species are solitary. Female bees independently forage for nectar and pollen, which they collect and store in cells, providing a food supply for their eggs. In the case of primitively eusocial bumblebees, the colony is initially founded by a single queen. This queen takes on the sole

responsibility of foraging for pollen and nectar to feed the larvae until her first batch of daughters emerges. Once the first batchof daughters are mature, they take over the foraging duties for the colony. Meanwhile, drones (male bees) leave the colony and sustain themselves by feeding on floral nectar. (Goulson, 2003) (Fig. 1).

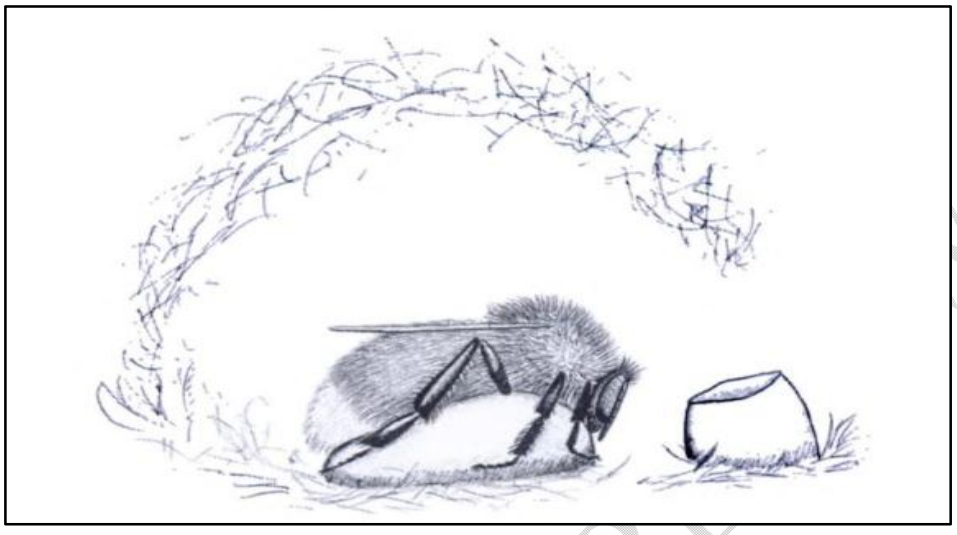


Fig.1 Queen of *Bombus lapidaries* (Linnaeus, 1758) *incubating the brood lump*

In honey bee colonies, the queen and the drones never visit flowers. Foraging is done exclusively by a specialized caste of sterile female workers. Honey bee workers progress through various behavioral tasks within the colony as they age, though their actions can also be shaped by genetic factors and environmental conditions (Robinson, 2002) (Fig. 2).Foragers collect nectar (and honeydew), pollen, water and tree resin (for propolis); the colony monitors foraging effort accordingly for each of these specific needs. The genetics of the queen and of worker patrilines influence the pollen-hoarding behavior of the colony and a forager's likelihood of collecting pollen or nectar (Robinson, 1989; Page, 2013). Honey bee foragers often specialize in collecting either pollen or nectar, but they may also collect both food sources simultaneously. Honey bees gather pollen and pack it onto the corbiculae, specialized structures on their hind legs.When collecting pollen, bees tend to show temporary specialization on one plant species' pollen (Gruter and Ratnieks, 2011). A study shows that peak pollen-collection times differ according to site. The number of pollen sources from trapped pollen pellets varied during the year, between sites, and between colonies in the same site, and ranged between 5 and 20 plant species per sampling date per site. The most abundant pollen source in each sample comprised between 22 and 94 per cent of the pollen pellets (Avni *et al*., 2009). Pollen collection is regulated according to a honey bee colony's need, but on average, colonies maintain about 1 kg of stored pollen (Brodschneider and Crailsheim, 2010).Nectar collection is regulated according to floral nectar availability, with increasing daily and seasonal fluctuations, with storage reaching tens of kilograms (Seeley, 1995). Water is collected mostly for evaporative cooling of the brood nest, and increases as a response to elevated temperature in the hive. Further, the study shows that the behavioural flexibility of a colony's water collectors enables them not only to satisfy

their colony's current water needs but also to buffer their colony against future extreme water stresses by storing water in their crops and combs (Ostwald *et al*., 2016). Propolis is plant-derived resin collected by bees to increase hive hygiene, as a form of social immunity. In social insects, behaviours that help reduce parasites in the colony are called "social immunity." For instance, honey bees gather resins from plants and use them in their nests. This practice can lower the need for individual bees to activate their immune responses, the study shows that colonies increase resin foraging rates after a challenge with a fungal parasite (*i.e., Ascophaeraapis*: chalkbrood).There is substantiated evidence indicating that colonies escalate the procurement of such resins in response to heightened levels of infection by the fungus responsible for the chalkbrood disease (Simone *et al*., 2012).

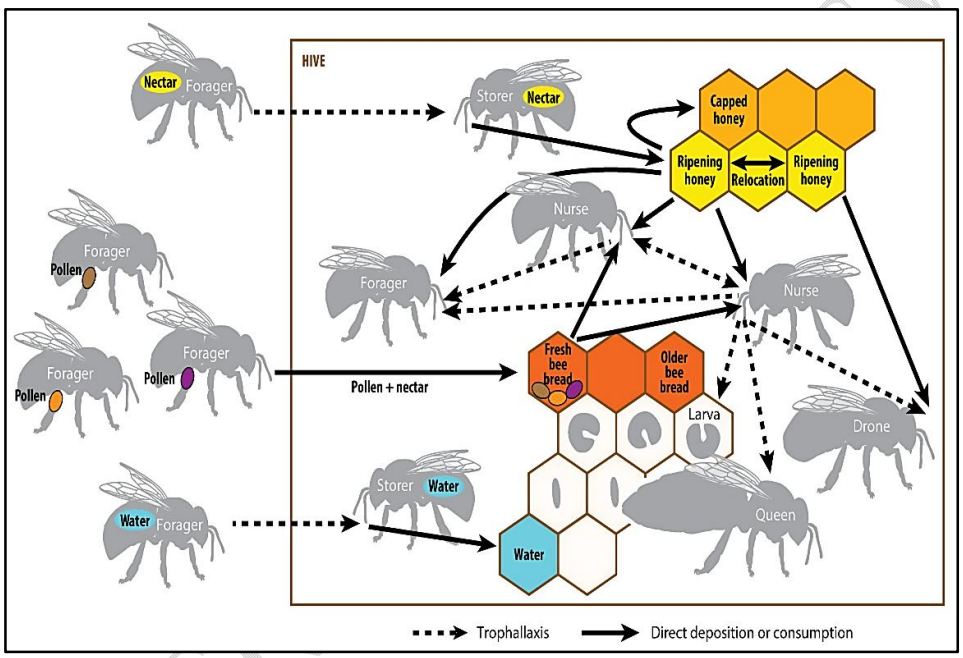


Fig.2 The flow of food and water in a honey bee colony

a) Nectar

Nectar properties tend to be similar for plants visited by the same kinds of pollinators, and much of the available information on nectar chemistry has been collected in the context of pollination syndromes. These are defined as broad associations between floral features and types of animal pollinators. The nutritional value of nectar is derived from three simple sugars- sucrose and its component monosaccharides, glucose and fructose. The proportions of these three sugars depend on the plant species from which nectar is collected, and the total sugar concentration in nectars ranges from about 10 to 70 per cent w/w (Nicolson *et al*., 2007). In general, honey bees collect sucrose in preference to fructose and fructose in preference to glucose, honey bees prefer nectar with a sugar concentration of 30–50 per cent (Waller, 1972), but in the field, they collect nectar of varying water content, depending on what is available in their local environment. The optimal concentration for

energy intake in honey bees is around 60 per cent; beyond this point, nectars become too viscous for efficient licking (Roubik and Buchmann, 1984).

Sucrose is preferred by foraging bees because it is more metabolically valuable per unit weight. Regardless of glucose concentration, worker bees need more sugar per meter flown and per gram of body weight and thorax weight compared to the significantly heavier drones and queens. Ultimately, the sugar composition of nectar, in terms of the proportion of sucrose, glucose and fructose, may not be physiologically important because bees digest sucrose rapidly and nectar sugars are efficiently assimilated (Gmeinbauer and Crailsheim, 1993).Many homopterans, such as aphids (Aphididae), coccids (Coccidae) and membracids (Membracidae) are involved in a mutualistic relationship with ants. These homopterans defecate 'honeydew', the sweet waste product of their sugar-rich but amino-acid-poor diet of plant sap.Honeydew sugar composition plays an important role in ant-homopteran interactions. Ants respond most intensively to honeydew containing high amounts of melezitose.This trisaccharide is synthesized in the aphid's gut from two units of glucose and one unit of fructose. Other sugar sources, such as the honeydew of phloem-feeding homopterans, are also collected by foraging bees; this sugar source is composed of sucrose, fructose and glucose but also contains the metabolizable trisaccharide melezitose in proportions that depend on plant-aphid-ant interactions (Fischer and Shingleton, 2001).

Nectar also contains amino acids in low concentrations (micromolar to millimolar) and non-essential amino acids tend to dominate the amino acid profile.The nutritional significance of these amino acids is unknown, but their presence may affect the taste of nectar and could also affect how bees learn floral traits (Simcock *et al*., 2014).

Free-flying honey bees prefer most essential amino acids above non-essential amino acids, with some exceptions (*e.g.*, methionine).Pollinator preferences have the most important effect, with phenylalanine being the most consequent discriminatory compound for the response of the nectar consumers in phrygana, predominantly for long-tongued bees, especially for Megachilidae. Gamma-aminobutyric acid (GABA) had a similar, even stronger influence on bees (long-tongued bees*viz.,*Anthophoridae andAndrenidae) and flies (Syrphidae and other Diptera), whereas asparagine behaves as a general repellent together with tryptophane. Phenylalanine is the most phago-stimulatory amino acid, and it is often the amino acid with the highest concentration in the nectar of plants visited by bees (Petanidou*et al*., 2006). The study shows that the bees collect water with a wide range of salt concentrations, but most collected water sources had relatively low salt concentrations, except seawater and swimming pools, which had >0.6% Na, whereas KCl being aversive (Lau and Nieh, 2016).Secondary metabolites in nectar such as alkaloids and phenolics are widespread in occurrence and varied in their actions which may be Deterrent (alkaloids, coumarins and saponins), Attractive (Terpenes), Toxic (Cardiac glycosides and Cyanogenic glycosides) (Detzel and Wink, 1993). Some metabolites also go undetected by bees but can influence bee behaviour (Wright *et al*.,2010), as when caffeine enhances honey bee memory of floral traits (Wright *et al*., 2013).

b) Pollen

Analyses are usually carried out on honey bees collected pollen obtained from pollen traps. The collection of powdery pollen by honey bees involves moistening the grains with nectar or honey and salivary secretions before packing it in the corbiculae. As a result, sugar levels in bee-collected pollen can be up to 50 per cent of dry mass, but usually, the proportion is not known (Nicolson, 2011). The protein component of pollen varies from 10 to 60 per cent dry mass in angiosperms. The study reveals that the crude protein levels in the analyzed pollen pellets varied from 9.2 per cent for *Hypochoerisradicata* to 37.4 per cent for *Echium plantagineum*, with an average of 25.9 per cent (Somerville and Nicol, 2006).

Apart from crude protein levels, the other common measure of pollen quality is its amino acid composition. The quantity of protein is less important than the amounts of essential amino acids relative to bee requirements (de, 1953). As in nectars, the proportion of nonessential amino acids, including proline, is high compared to essential amino acids, and some deficiencies in essential amino acids have been identified *e.g*., low concentration of histidine in maize pollen causes reduction in brood rearing and life span; pollen of maize is believed to be a minor food source for bees as it is thought to be lacking in proteins and essential amino acids (Hocherl*et al*., 2012).

Other macronutrients provided by pollen are carbohydrates and lipids. Carbohydrates include starch and starchy pollens contain less oil than starchless pollens, and the pollen-consuming insects will be deterred by starchy pollens because of an inability to digest starch or attraction to more oily pollens due to a greater caloric reward (Roulston and Buchmann, 2000) andfiber in the indigestible cell wall, in addition to nectar sugars added during collection. Lipids occur in pollen grains and their outer coating or pollenkitt and study shows that neutral lipids (energy storage and essential oil constituents) were most diverse in pollenkitt, while polar lipids (mostly membrane constituents) were found almost exclusively in the internal pollen fraction. Patterns in neutral lipid compositions suggest that pollenkitt may provide pollen with species-specific odours (Dobson, 1988), and total lipid content is 2–20 per cent dry mass (Roulston and Cane, 2000). The fatty acid composition of different pollens varies greatly, with the three most common fatty acids being palmitic, linoleic (omega-6), and alpha-linolenic (omega-3) acids, together comprising an average of 60–80 per cent of all fatty acids and many managed colonies are experiencing a shift in available forage toward a higher omega-6:3 ratio, which may be leading to colony declines (Arien *et al*., 2015). These three, in addition to oleic and stearic, are the main fatty acids inhoney bee bodies (Avni *et al*., 2014). Of greatest nutritional significance are the two essential fatty acids, linoleic and alpha-linolenic acid, with a deficiency in the latter impairing cognitive function (Arien *et al*., 2015). The concentration of linoleic acid is higher in bee bodies than in bee brains, whereas the opposite is true for alpha-linolenic acid. Pollens with high lipid concentrations and dominated by linoleic, linolenic, myristic and dodecanoic acids probably play a significant role in inhibiting the growth of the spore-forming bacteria, *Paenibacillus larvaelarvae* (American foulbrood), *Melissococcus pluton* (European foulbrood) and other microbes that inhabit the brood combs of beehives. Those pollens high in oleic and palmitic acids probably have a greater role in honey bee nutrition (Manning, 2001).

Pollen is the bee's main source of micronutrients and includes minerals, vitamins, and

essential sterols. Pollen sterols are diverse and include, but are not limited to, β-sitosterol, stigmasterol, avenasterol, and 24-methylene cholesterol (Villette *et al*., 2015).

Among the important minerals bees derive from pollen is iron, which accumulates at the periphery of the abdomen, partly as magnetite, with a suspected role in bee navigation (Wang *et al*., 2013) (Fig.3). However, high iron concentration in pollen-for example, from heavily fertilized crops-may induce lipid peroxidation and reduce bee longevity (Jumarie*et al*., 2017). Secondary metabolites (*i.e*., toxins, polyphenols, etc.) tend to be present at higher concentrations in pollen than in nectar and lowest in Honey (London *et al*., 2003). Polyphenols such as the flavonol quercetin are ubiquitous in pollen (Bonvehi and Jorda., 1997).

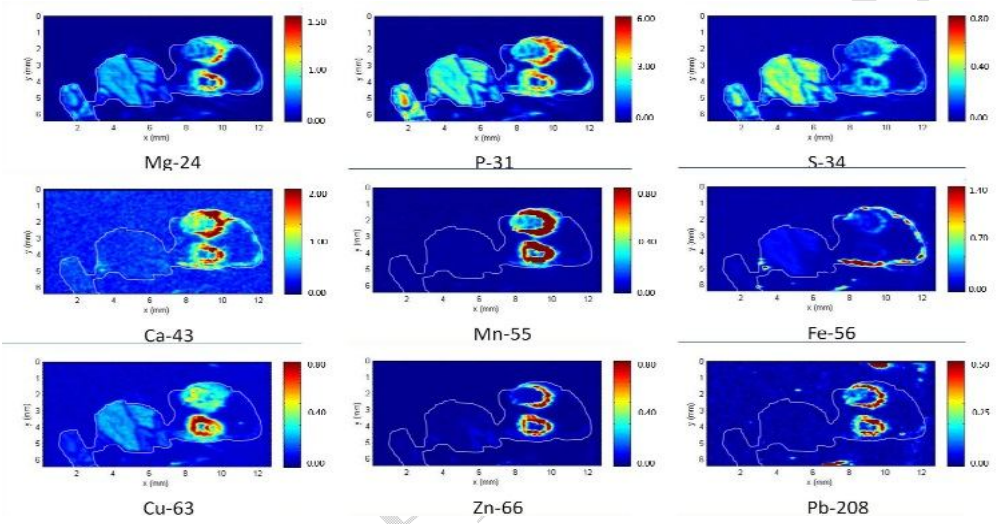


Fig.3. Spatial distributions of inorganic elements in a honeybee, measured using LA-ICP-MS. The unit shown in the figure is ng/mm2.

3) FOOD STORAGE

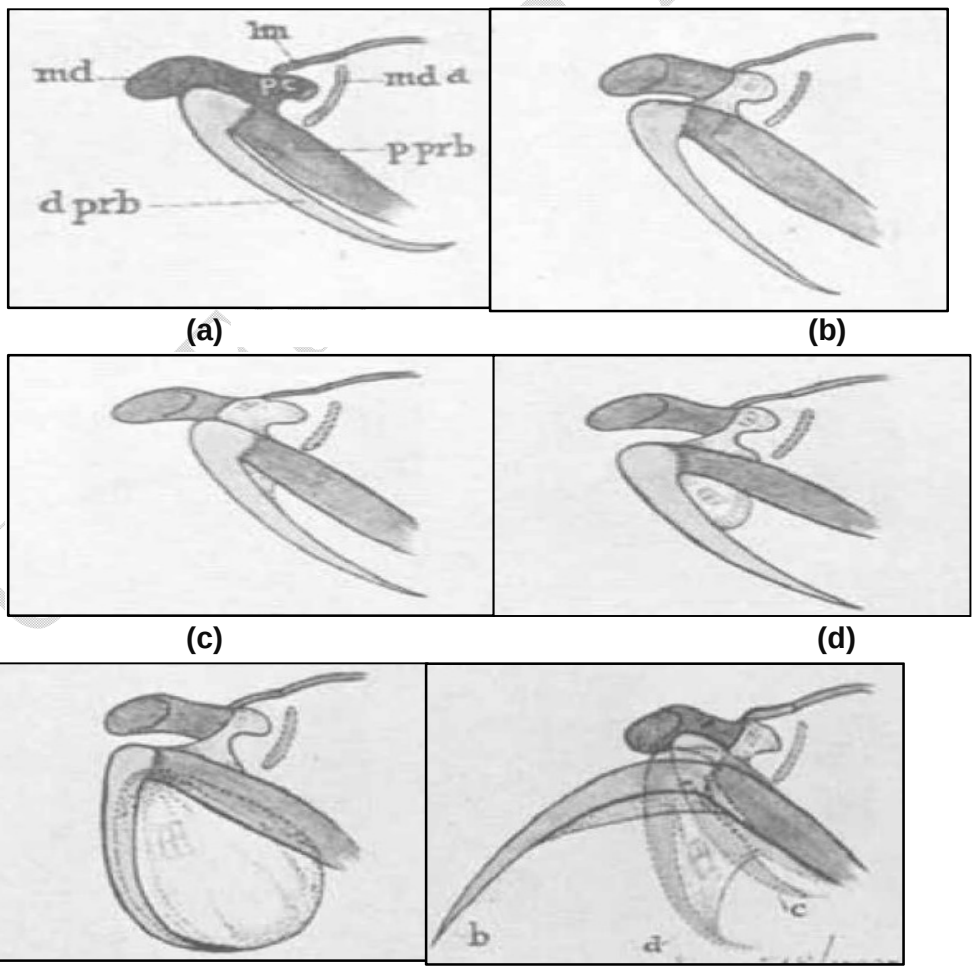
During food storage, honey bees process the food they gather, transforming it from its original form. They primarily store two types of food: honey, derived from nectar, and bee bread, made from pollen. This stored food is kept in a comb constructed from wax produced by the wax glands of adult worker bees.

a) Honey

A characteristic pattern of brood, pollen, and honey develops on the combs of a honey bee colony, consisting of three distinct concentric regions; a central brood area, a surrounding rim of pollen, and a large peripheral region of honey. Soon after entering the hive, honey bee nectar collectors pass their nectar loads to food-storer bees by trophallaxis; the food-storer bees then regurgitate the nectar into cells scattered throughout the comb (Camazine, 1991) (Fig. 4). Foragers

convert floral nectar into honey first by actively reducing the water content. Nectar is regurgitated and held between their mandibles to evaporate the water (Park, 1925) (Fig. 5). Nectar is passively evaporated within ripening cells within the colony; it has been shown that relocation between cells before final storage is essential to the honey ripening process and the honey is an inhomogeneous matrix (Eyer *et al*., 2016). Water elimination can also begin during transport to the hive (Nicolson and Human, 2008). The other part of the process is sucrose hydrolysis into glucose and fructose with invertase being added to nectar in the crop during collection (Oertel *et al*., 1951).

Fig.4a. Nectar being transferred from a loaded nectar-carrier to a house-bee **b. House bee ripening** **c. House-bee depositing honey**



(e) **(f)**

Fig. 5. Figures depicting the mouthparts of a bee engaged in honey ripening.

The resulting stored honey is characterized by a low water content of < 20 per cent and a high sugar concentration of > 80 per cent, dominated by the monosaccharide's glucose and fructose. Fructose is higher in concentration because the lower solubility of glucose means that it tends to crystallize. At hive temperature, glucose solubility increases with fructose concentration, resulting in a very concentrated sugar solution that inhibits microbial growth (Doner, 1977).

Free amino acids are also present in honey, including some added by bees so, proline is the most abundant. In addition, enzymes such as glucose oxidase, which converts a minor amount of glucose to gluconic acid and hydrogen peroxide, are added by bee salivary glands; others include diastase (α-amylase), which hydrolyzes starch, and invertases that convert sucrose to fructose and glucose. In addition to the high osmotic concentration, hydrogen peroxide and low pH enhance the antibiotic properties of honey (Kwakman*et al*., 2010). Many other minor compounds such as phenolics contribute to the color, aroma, and flavour of different kinds of honey and may be important for bee health (Mao *et al*., 2013). Honeydew honey can be distinguished from floral honey by its content of melezitose and other oligosaccharides (Bogdanov *et al*., 2004).

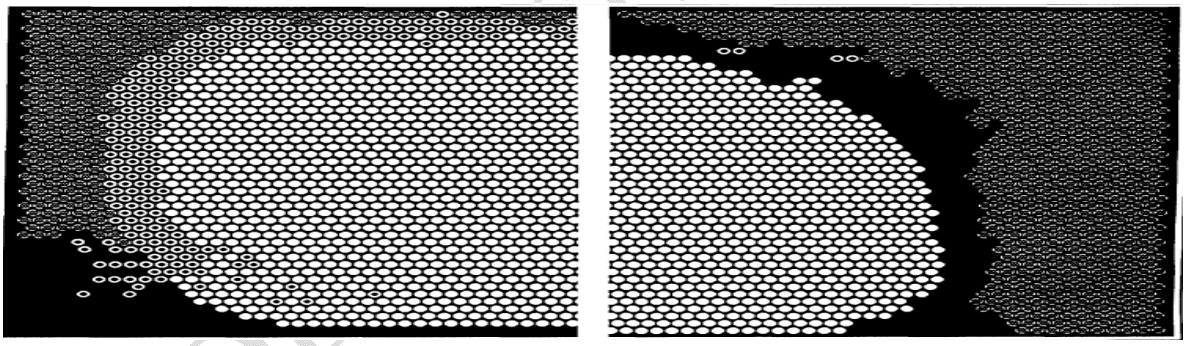


Fig.6a. A characteristic pattern of comb consisting of brood, pollen, and honey area.

b. Pattern of comb during early August

Most of the honey is consumed by the colony, with a preference for less concentrated honey, and only a small percentage is stored (Eyer *et al*., 2016). Honey storage comb area can still be large and reach many tens of kilograms in weight. The typical storage pattern that result is of brood at the center, surrounded by a small strip of pollen, and honey beyond (Camazine, 1991) (Fig. 6). A colony maintains only small reserves of water, mainly in the crops of water foragers and of in-hive reservoir bees to which the water collectors transfer their load; some water may also be stored in the combs (Ostwald *et al*., 2016).

b) Bee Bread

When they return, pollen-collecting foragers transfer their pollen loads directly into cells scattered throughout the comb (Camazine, 1991). Cells often already contain previous loads, potentially of different floral sources. Young hive bees further process the pollen, packing it tightly and adding regurgitated honey, whose antimicrobial properties preserve stored pollen (Anderson *et al*., 2014). Bees prefer to consume it fresh and preferentially from cells close to brood cells. Bees preferentially consume fresh pollen stored for less than three days. Newly collected pollen contains few bacteria, but these values decrease significantly when the pollen is stored for more than 96 hours. This results in relatively small reserves of stored pollen mostly remaining in a strip surrounding the brood cells (Camazine, 1991).

Pollen packed into cells for storage is called bee bread. The nutritional value of bee bread differs from floral pollen in that its protein content is in a smaller range of values from 10 to 30 per cent protein, lipids are also present in a narrower range of values than found in pollen; bee bread is 3 to 8 per cent fats (Human and Nicolson, 2006; Nicolson and Human, 2013), whereas pollen lipids can range to up to 20 per cent of the dry weight (Roulston *et al*., 2000). The carbohydrate content is between 25 and 50 per centof dry weight, which includes the added carbohydrates of honey/nectar (Nicolson and Human *et al*., 2013).

Lactic acid bacteria from the crop are added to pollen during collection, and microbial activity could lead to predigestion or the addition of essential nutrients (Gilliam, 1997).The bee gut hosts a unique microbial community consisting of around nine bacterial species clusters. These bacteria are adapted to their host, with each cluster occupying specific niches and spatial locations within the gut. This microbial community is passed between bees through social interactions, similar to how gut microbes are transmitted in mammals. Gut bacteria of honey bees are known to synthesize B vitamins (Kwong and Moran, 2016) and they could potentially enrich B vitamins in bee bread or degrade complex polysaccharides in the exine (Lee *et al*., 2014).

4) BEE NUTRITION

Honey bees transition through different behavioural castes throughout their adult life. As a worker bee shifts from being a nurse to becoming a forager, its physiology undergoes significant changes, and its nutritional requirements adapt accordingly. Unlike other insect species, adult nurse bees produce glandular secretions known as royal jelly, which is fed to larvae. Similar to mammalian milk, royal jelly supplies the necessary nutrients for the growth and development of larval bees.

a) Nutritional Processes: Pollen Digestion by Nurse Bees and Larval Nourishment

Bee species feed their larvae by mass provisioning or by progressive feeding (Michener, 2000). The larvae of solitary bee species develop without their mother (or other adult bees) around to feed them; instead, they rely on nectar and pollen that the mother had mass-provisioned in their cell when she laid the egg. Thus, except for solitary bees, which of course must mass provision, there is

no strong correlation between level of sociality and mode of larval feeding. However, progressive feeding in honey bees has influenced their nutrition.

The nurse caste of honey bee workers is responsible for feeding all other members of the colony. They accomplish this by directly providing honey and bee bread or by producing glandular secretions, such as royal jelly, after consuming honey and bee bread themselves. Consuming pollen stimulates the development of the mandibular and hypopharyngeal glands in young nurse bees. This development, often measured by the size of the acini or protein content, reaches its peak between six and ten days after the bees emerge from their cells (Pernal and Currie, 2000). Hypopharyngeal food glands are specific to the Hymenoptera, but only in honey bees are they enlarged in a nurse bee caste such that they can secrete jelly to feed the larvae (Moritz and Crailsheim, 1987). Gut bacteria are also transferred between bees in both honey bee (only partially by trophallaxis) and bumble bee colonies and play a role in digestion and defence against parasites and pathogens (Billiet *et al*., 2017). Honey bee larval and adult nutrition is thus governed by how food is collected, stored, and distributed within the colony.

Because they feed all other bees, nurse bees are the main pollen consumers in the colony, showing much higher pollen consumption and midgut protease activity than foragers (Crailsheim*et al*., 1992). Indeed, the growth of their hypopharyngeal glands is correlated with the protease activity of the midgut (Moritz and Crailsheim, 1987). The pollen wall exine is a physical barrier to the extraction of nutrients. In addition, the size of pollen grains varies enormously and the surface area to volume ratio may affect both nutritional value and the ease of nutrient extraction (Rourke and Buchmann, 1991). Osmotic shock has been suggested as a mechanism of pollen digestion in bees: Exposure to lower osmotic concentrations in the midgut than the crop could cause the grains to rupture and release the cytoplasm (Kroon *et al*., 1974).

b) Royal Jelly

Honey bees are distinct among bee species because they transform the food, they consume into a nutritious substance called royal jelly, which is fed to their brood. This royal jelly is produced by the mandibular and hypopharyngeal glands located in the heads of the bees. Nurse bees place royal jelly into the cells of worker larvae and queen larvae. Queen larvae receive an abundance of jelly, while worker larvae are given much less food and are fed on demand during the later stages of their development. (Haydak, 1970). Furthermore, workers are fed with jelly for the first three days and then fed with a combination of glandular secretions, honey, and pollen (Crailsheim, 1990).Larval starvation, alone or in combination with other stressors, can weaken colonies.If worker larvae are fed with insufficient food during development, they develop into smaller, weaker adults; young larvae can also be cannibalized by adult workers (Brodschneider and Crailsheim, 2010).

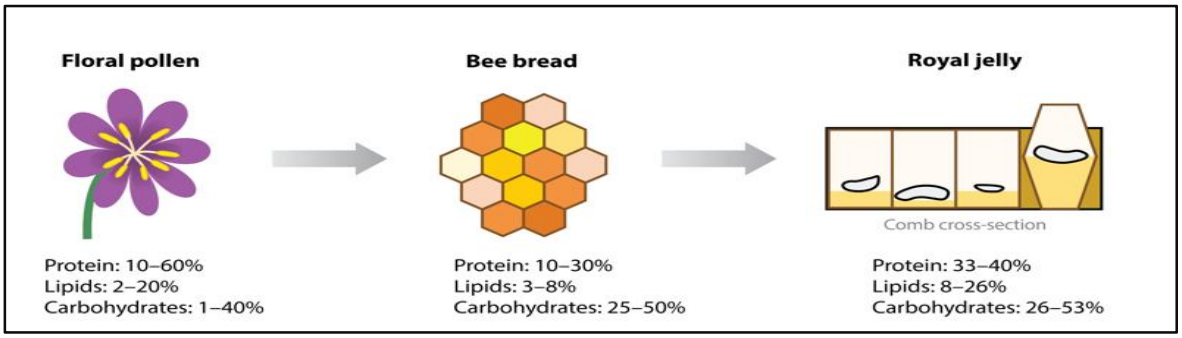


Fig. 7 The refinement of floral pollen into royal jelly by adult worker honey bees

Royal jelly is a nutritional refinement of the honey and bee bread consumed by nurse honey bees (Fig. 7). Its chemical composition is remarkably similar even when collected from colonies in geographically distinct locations.

Royal jelly typically consists of approximately 63% water, 14% protein, 18% carbohydrates, and 6% fats. The carbohydrates present in royal jelly are similar to those in honey, primarily glucose and fructose in nearly equal proportions, with small or trace amounts of other sugars, including sucrose (Wang *et al*., 2016). By comparison, the proteins of royal jelly, called the major royal jelly proteins (*MRJPs*) are specifically produced by nurse bees. They are secreted by the nurse bees' hypopharyngeal and mandibular glands and are the growing larva's sole source of essential amino acids. *MRJP's* are produced by other hymenopterans, but honey bees produce nine specific *MRJP*'s. Rather than having metabolic targets, it is more likely that when they are consumed, *MRJP*'s are digested and then reassembled into corporeal proteins. In fact, one might predict that these proteins have the optimal amino acid composition for growing honey bees, as they are the sole source of essential amino acids bees receive for much of their development (Buttstedt*et al*., 2014).

Royal jelly also contains a significant amount of fat (as much as 10 per cent of the wet weight). Up to 90 per cent of the fats in royal jelly are uncommon free fatty acids (*i.e*., hydroxyl and dicarboxylic fatty acids) (Plettner *et al*., 1996). Significantly, the fats of royal jelly are characterized by the very large component of two compounds, 10-HDA [(E)-10-hydroxy-2-decenoic acid] and 10-HDAA (10-hydroxy-decanoic acid). These compounds are related to components of queen mandibular pheromone but arise from different oxidation pathways (Plettner *et al*., 1996). Interestingly, 10-HDA, which is up to 73 per cent of the fatty acid profile of royal jelly (and up to 2–4 per cent of royal jelly's total wet weight), is an epigenetic modulator of gene expression, functioning as a histone deacetylase inhibitor (Spannhoff*et al*., 2011) and thus could play a role in caste determination.

In addition to containing macronutrients, royal jelly also contains 2 per cent micronutrients, including compounds such as sterols, vitamins, minerals, and other substances like phenolic compounds (Ciulu*et al*., 2013). The most common sterol found in royal jelly is 24-methylene cholesterol. Other sterols derived from pollen have also been reported from royal jelly, including cholesterol, stigmasterol, and isofucosterol (Lercker*et al*., 1982). Honey bees cannot synthesize sterols, so all sterols found in royal jelly are acquired from the specific plant sources of the pollen

bees collect.

How specific compounds found in royal jelly contribute to caste differentiation in honey bees is still unknown. Floral pollen fed to worker larvae could play a role in caste differentiation; a recent study identified that floral pollen contains microRNA molecules that interfere with primary metabolic pathways. When larvae were fed with pollen-specific microRNAs in royal jelly, honey bee larvae developed into adults with worker-like traits (*i.e.*, smaller, fewer ovarioles; slower development time) (Zhu *et al*., 2017) (Fig. 8).

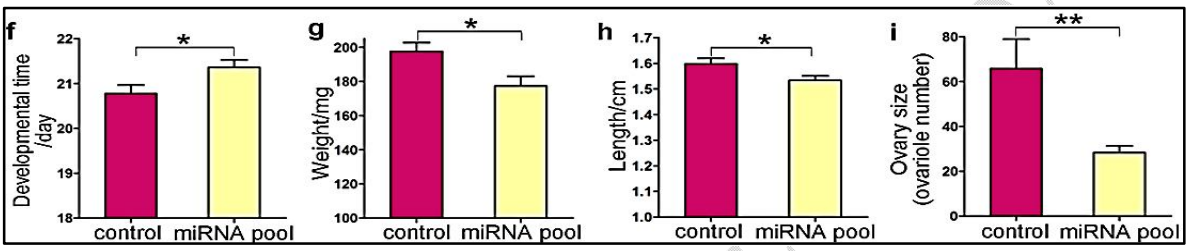


Fig. 8 Effect of plant miRNA pool on honey bee phenotypes

c) Nutritional Needs of Adult Workers Honey Bees

In honey bees, intake targets (expressed as P:C ratios) are biased toward carbohydrates, not surprisingly because of their nectar-rich diet (Stevenson *et al*., 2017). Intake targets also vary with worker age: When essential amino acids (EAA) were used as the protein source, the EAA:C ratio shifted from 1:50 to 1:75 over the first two weeks of adult life and was 1:250 in caged foragers (Paoli *et al*., 2014).Studies demonstrate that survival is significantly reduced when diets are restricted to high protein or essential amino acids. This confirms the broader pattern observed in other animals, where high protein diets generally shorten lifespan (Simpson and Raubenheimer, 2012). Honeybees prefer salty water sources, especially those containing sodium (Lau and Nieh, 2016). Bee microbes could potentially synthesize essential dietary vitamins not provided by pollen. In addition, sterols are important for most organisms and are essential dietary components for insects. The sterol requirements of bees are mostly unknown, but it is proved that honey bees require diets containing the pollen-derived sterol, 24-methylene cholesterol (Svoboda and Lusby, 1986).

Foraging requires high cognitive and flight abilities. Nurse bees undergo a major shift in their physiology when they transition through behavioural castes from nurse to forager. This radical alteration in behaviour is accompanied by a reduction in the hypopharyngeal glands; by changes in brain morphology, neurochemistry, and gene expression, and by changes in flight muscle biochemistry and gene expression, which generate increased metabolic and aerodynamic power (Roberts and Elekonich, 2005). Nurse bees have more highly developed hypopharyngeal glands, increased blood proteins, and greater lipid reserves in their fat bodies, compared with foragers. The

depletion of lipid reserves occurs just prior to initiation of foraging and can be induced by severe starvation of the colony (Toth *et al*., 2005). The onset of foraging is delayed by the presence of foragers, regardless of the colony's nutritional state, so foragers are thought to exert a social effect and not directly affect the nutritional state of nurse bees by trophallaxis.

d) Colony-Level Nutrition

Studies indicate that feeding colonies for several months on either of two commercial artificial diets or pollen did not alter hemolymph protein concentrations in nurse bees or the overall colony population size. However, colonies fed with artificial diets experienced compromised health, with higher incidences of queen losses and *Nosema* infection (DeGrandi-Hoffman *et al*., 2016). A polyfloral diet generally provides better nutrition than a monofloral diet and supports better bee health, immunocompetence, and longevity, but the composition of the particular pollen constituents also matters **(**Alaux*et al*., 2010).At the colony level, foraging effort is biased toward sources that complement nutritional deficiencies (Hendriksma and Shafir, 2016).Zarchin*et al*. (2017) found that foragers danced more vigorously to recruit pollen that complemented colony essential fatty acid deficiency.

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