Israel Journal of Plant Sciences *Vol. 57* *2009* *pp. 253–261*
DOI: 10.1560/IJPS.57.3.253

The components that determine honeybee (*Apis mellifera*) preference between Israeli unifloral honeys and the implications for nectar attractiveness

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(Received 15 January 2009; accepted in revised form 13 May 2009)

ABSTRACT

Unifloral honeys are honeys that are dominated by a single nectar source. Several samples of Israeli honeys were analyzed for their physicochemical characteristics and tested by pollen analysis for their botanical source. Based on pollen content, unifloral honeys were harvested only from landscapes of planted forests including: aethel (*Tamarix* sp.), carob (*Ceratonia siliqua*), and eucalyptus (*Eucalyptus* sp.). However, honeys extracted from agricultural landscapes should also be considered as unifloral due to pollen underrepresentation. No evidence for unifloral honeys from natural landscapes was found. Later, honeybee preference between different honeys and sucrose solution was tested and these preferences were correlated with honey traits. The preference experiment revealed that bees tend to prefer sucrose solution rather than any honey source. Among honeys, bees showed the highest preference for citrus honey and the lowest preference for avocado honey. Preference for aethel, cotton, and eucalyptus was intermediate. The electrical conductivity value of the honeys was negatively correlated with honey preference, indicating that the mineral content of honey, and probably of nectar, affects the attractiveness to bees.

Keywords: electrical conductivity, minerals, physicochemical analysis, pollen, preference, unifloral honey

INTRODUCTION

Honey is a unique outcome of the prolonged process of coevolution between plants and honeybees (*Apis mellifera*). During seasons of floral abundance, honeybees gather more nectar than they are able to consume and they convert it into honey that is stored in the nest for times of shortage. This behavior enables the honeybee colony to survive all year round, in contrast to seasonal species. The process by which bees convert nectar to honey includes two cardinal changes: water evaporation and enzymatic inversion of sucrose into glucose and fructose. However, additional minor changes also occur (Crane, 1980). As a result, honey composition depends both on nectar composition, which is the raw material, and on the contribution of the bees. Since honeybees are generalist pollinators and gather nectar from a wide diversity of flowers, honey is usually a mixture of different nectars (Maurizio, 1975). This situation is especially true for countries like Israel, where vegetation patches are relatively small and heterogeneous (Médail and Quézel, 1997). However, in certain conditions in which nectar sources are dominated by a single plant species, unifloral honeys are available (Oddo and Piro, 2004).

Many studies motivated by an interest in the nutritive

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value of honey and by commercial needs have identified characteristics and composition of unifloral honeys (Anklam, 1998; Oddo and Piro, 2004; Cuevas-Glory et al., 2007). Unifloral honeys may also be a useful tool for the ecological research of plant–pollinator interactions. Nectar attractiveness to pollinators is dominated by its sugar content, but an increasing number of publications provide evidence for the contribution of minor nectar components in addition to sugars (Adler, 2000; Raguso, 2008). In order to analyze the composition of minor components in nectars and to learn about their contribution to pollinator attraction, nectar is often collected manually (Kearns and Inouye, 1993; Dafni et al., 2005). Even though nectar collection is a common procedure, in some plant species it is a highly laborious and time-consuming task, and the amount of nectar collected is usually limited and seldom enough to perform biological tests. Unifloral honeys, on the other hand, are relatively simple to extract in large amounts and they can be used as a nectar substitute for chemical analyses (Alissandrakis et al., 2003; Naef et al., 2004; Afik et al., 2006a) or for biological assays (Hagler and Buchmann, 1993; Afik et al., 2006b, 2008; Tan et al., 2007). Using honey as a nectar substitute should, however, be done with caution due to our limited ability to identify honey sources and due to the changes in nectar composition that occur during the process of honey ripening. Any results achieved using honey still remain to be verified through nectar analysis (Alissandrakis et al., 2003; Naef et al., 2004).

The purpose of the current study was to test the feasibility of harvesting unifloral honeys in Israel from three types of landscapes: (1) natural vegetation represented by jujube (*Ziziphus spina-christi*) and Jaffa scabious (*Cephalaria joppensis*); (2) planted forests represented by aethel (*Tamarix* sp.), carob (*Ceratonia siliqua*), and eucalyptus (*Eucalyptus* sp.), though the first two species are also part of the natural vegetation; and (3) agricultural landscapes represented by avocado (*Persea americana*), citrus (*Citrus* sp.), and cotton (*Gossypium* sp.). The preference of honeybees among the different honey sources was tested in order to find the honey traits that most influenced preference. We discuss the relevance of these findings for foraging behavior in the field.

METHODS

Honey samples

Honey samples were collected from beekeepers, and their botanical origin was estimated by the beekeepers according to the harvesting site and season. We analyzed six samples each of citrus, cotton, eucalyptus, and jujube honeys; four samples of aethel honey; and a single sample each of avocado, carob, and Jaffa scabious honeys.

Pollen analysis

Pollen analysis was performed for verification of the botanical origin of the honeys. An aliquot of 10 g of honey was diluted in 10 ml of double distilled water (DDW) and centrifuged for 5 min at 3,000 rpm. The supernatant liquid was poured off, leaving about 2 ml of sediment and water. A 2-µl drop of sediment and water was tapped on a glass slide for microscope observation at a magnitude of ×400. A total of one hundred pollen grains was counted and the percentage of the pollen grains belonging to the estimated botanical origin was measured (Maurizio, 1975). This count was replicated three times for each honey sample and the percentages of the respective pollen were averaged.

Physicochemical analysis

Moisture: Water content was measured by a hand-refractometer (REF 116, brix units, 58-92 ATC). This measure was taken with fresh honey samples to avoid honey crystallization.

pH and free acidity: Honey pH was measured with a pH meter (Orion 420 m) after dissolving 10 g of honey in 75 ml of DDW. The same honey solution was later used to measure free acidity by adding 0.1M NaOH until the pH value reached 8.3. Titration volume was multiplied by 10 to present free acidity in units of millimoles acid per kg honey (Bogdanov, 2002).

Electrical conductivity (E.C.): Honey E.C. was measured by conductivity meter (Cyberscan 500) after dissolving 25 g of honey in 75 ml of DDW (Bogdanov, 2002).

Minerals: Analysis was performed by inductively coupled plasma atomic emission spectroscopy (ICP-AES). 0.5 g of each honey sample was dissolved in 2 ml of 65% nitric acid and shaken for 3 h in a bath of warm water. Afterward, each sample was mixed with 8 ml DDW. The content of 30 minerals was examined with a flame-photometer (Spectro, Kleve, Germany).

Carbohydrates: Sugar analysis was performed by HPLC. The following sugars were used as standards for calibration: fructose, glucose, sucrose, and raffinose. Sucrose was used as a standard for total disaccharides, whereas raffinose was used as a standard for total trisaccharides. For further details, see Dag et al. (2006).

Honey preference

Seven colonies were tested for their honey preferences.

Each colony populated five frames of a nucleus hive and was kept in a separate 5 × 2.5 × 2 m (20-mesh) screened enclosure. The bees had ad libitum access to a water source and were provided with a pollen patty once a week. Honey preference was studied using a cafeteria-style choice paradigm, in which each colony could choose from six available feeders. Five feeders were filled with honey solutions of the following sources: aethel, avocado, citrus, cotton, and eucalyptus. One additional feeder contained sucrose solution. Three more honey sources (carob, scabious, and jujube) that were analyzed in this study were not tested in the preference experiment due to their limited amounts available. The solutions were prepared by diluting honey or sucrose with DDW to reach 50% (w/w) total dissolved solids, measured by a hand-refractometer. Even though the tested honeys contain mainly glucose and fructose, their refractive index is similar to that of sucrose (Kearns and Inouye, 1993).

Solutions were presented to the bees in 200-ml bird feeders with a 4 × 4.7 cm^2 pool at the bottom, allowing a few tens of bees to feed from a single feeder simultaneously. The six feeders were placed in a circle, 10 cm apart, on a carousel that rotated at a velocity of 2 rpm to prevent a potential location bias. The experiment ended when 130 ml was consumed from one of the honey feeders. Whenever the first consumed solution was the sucrose solution, measurements were taken but the experiment continued until 130 ml was consumed from one of the honey feeders as well. The volume of the solution consumed from each feeder was measured at the end of the experiment. The solution from which bees consumed the highest volume was set to represent 100% consumption, and the consumed volumes from the rest of the feeders were compared with this highest volume. The results are presented as relative percent consumption of the different solutions.

Statistics

Differences between honey sources in the various parameters were tested by one-way ANOVA, followed by a Tukey–Kramer test that was used to identify which of the honey sources were different. Honey sources with only a single sample (aocado, carob, and scabious) were not included in this analysis. Differences in the preferences between honey sources were also tested by one-way ANOVA. The arcsine square root transformation was employed on the percentage honey consumption data prior to analysis (Sokal and Rohlf, 1995). Pearson product–moment correlation coefficient was calculated to test correlations between honey preference and the various honey parameters, using the average values and the average consumption for each honey source. All statistical analyses were performed using JMP 8.0 software (SAS Institute).

RESULTS

Pollen analysis

Pronounced differences in the percentages of the respective pollens were found among the different honey sources. Eucalyptus, carob, and half of the aethel honey samples contained the respective pollen as the predominant pollen (>45%, von Der Ohe et al., 2004), whereas cotton and scabious honeys had only 1% of the respective pollen out of their total pollen content. The pollen analysis results are summarized in Table 1.

Physicochemical analysis

Moisture: The water content of most honey samples ranged between 15% and 17.5%; only the carob honey sample had higher water content. Cotton and aethel, which were harvested during the summer, had a higher percentage of moisture than jujube and citrus, which

Table 1
Percent of pollen grains belonging to the assumed botanical source of the honey samples

Honey source		No. of	Percent assumed source,
Common name	Scientific name	samples	mean (SD)
Aethel	*Tamarix* spp.	4	46.4 (19.2)
Avocado	*Persea americana*	1	14.3
Carob	*Ceratonia siliqua*	1	62.4
Citrus	*Citrus* spp.	6	29.6 (13.5)
Cotton	*Gossypium* spp.	6	0.8 (0.9)
Eucalyptus	*Eucalyptus* spp.	6	59.5 (10.6)
Jujube	*Ziziphus spina-christi*	6	10.6 (6.1)
Scabious	*Cephalaria joppensis*	1	1.3

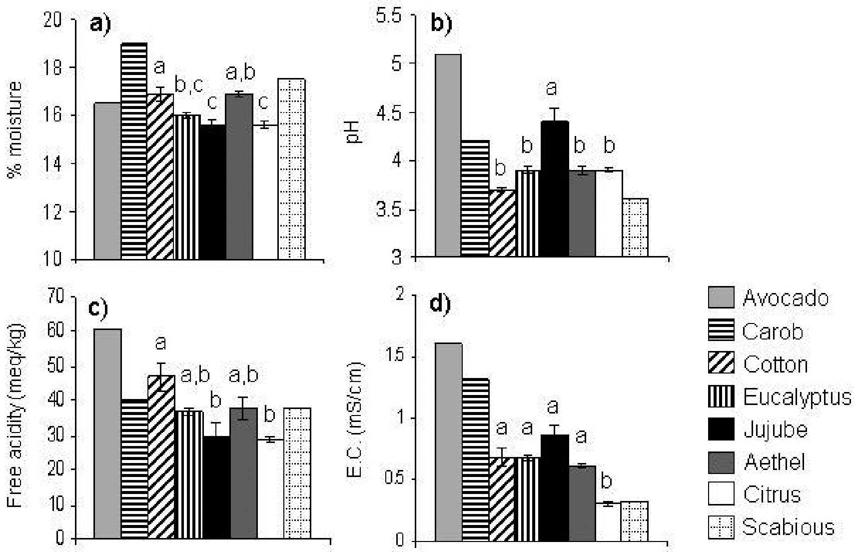

Fig. 1. Various honey measurements: (a) moisture, (b) pH, (c) free acidity, and (d) electrical conductivity. Different letters above columns indicate significant differences between honey sources among the five sources that included more than a single sample (Tukey–Kramer test, $p < 0.05$).

were harvested during the spring (Tukey–Kramer test, $p < 0.05$; Fig. 1a).

pH: The pH of most honey samples was around 4. The pH of the avocado sample was exceptionally high, at above 5. Jujube honey also had relatively high pH, which was significantly higher than the other honey sources (Tukey–Kramer test, $p < 0.05$; Fig. 1b).

Free acidity: The highest values of free acidity of around 60 meq/kg were measured in the avocado honey and one sample of cotton honey. The lowest values of around 20 meq/kg were measured in two samples of jujube honey. Significant differences were found between cotton honey and jujube and citrus honeys (Tukey–Kramer test, $p < 0.05$; Fig. 1c). Free acidity and pH were negatively correlated (Pearson product-moment, $r = -0.58$, $p = 0.001$), after excluding the avocado honey. This honey differed from the others in this aspect and had the highest values of both free acidity and pH.

Electrical conductivity: Extremely high E.C. values were measured in the avocado and carob honeys whereas citrus and scabious honeys had the lowest values. The E.C. of citrus honey was significantly lower than that of the other honeys (Tukey–Kramer test, $p < 0.05$; Fig. 1d).

Minerals: Honey minerals were split into major (>20 mg/kg) and minor (<10 mg/kg) minerals. Silicon concentrations were intermediate between the major and minor minerals, with a unique bimodal distribution, which was not correlated to the honey source. Most honey samples had silicon concentration lower than 10 mg/kg but in a few of them concentrations around 70 mg/kg were measured. The group of the major minerals includes potassium, calcium, sodium, phosphorus, sulfur, and magnesium. Potassium was the dominant mineral in all honeys, and its concentrations significantly correlated with the E.C., which strongly depends on total mineral content (Pearson product-moment, $r = 0.9$, $p < 0.0001$). Honeys differed in the composition of all major minerals (Tukey–Kramer test, $p < 0.05$; Fig. 2), except phosphorus. A general profile was found for the major mineral concentrations, except sodium, with high values in avocado and carob honeys, low value in citrus honey, and intermediate values for the rest of the honeys in the following order: cotton > eucalyptus > jujube > aethel. There were only a few exceptions from this profile, such as high potassium in jujube honey and low calcium in avocado honey. Several minor minerals had extreme values in the single avocado or single carob honey sample (Table 2). Eucalyptus honey was significantly richer than other honeys in manganese, and cotton honey was significantly richer in strontium (Tukey–Kramer test, $p < 0.05$).

Carbohydrates: The sugar composition of the different honeys was similar (Fig. 3). The mean values were 43% glucose, 42% fructose, 12% disaccharides, and 3% trisaccharides. The only significant difference was that glucose concentration of cotton honey was greater than that of jujube (Tukey–Kramer test, $p < 0.05$).

Honey preference

When honeybees were given a choice between five dif-

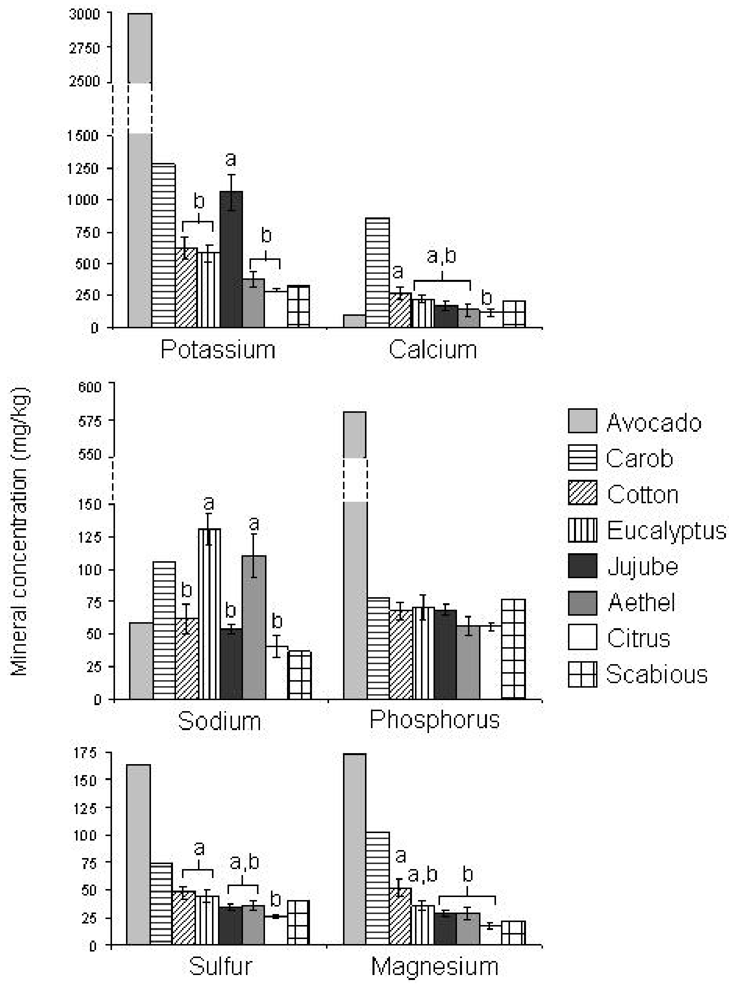

Fig. 2. Concentrations of major minerals in the different honey sources. Different letters above columns indicate significant differences between honey sources among the five sources that included more than a single sample (Tukey–Kramer test, $p < 0.05$).

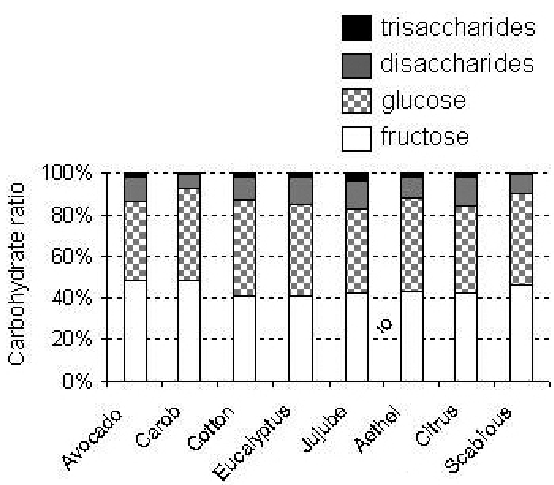

Fig. 3. The composition of the major carbohydrates of the tested honeys. The percentages are averages for each honey source and calculated from the total sugar content of the honey.

Table 2
The mean concentrations (mg/kg) of minor minerals that were detected in the honey samples

Mineral	Mean (SD)	Extreme values*
Al	2.7 (2.6)	Carob—13.3
B	6.5 (2.0)	
Ba	0.8 (0.5)	
Cd	0.5 (1.2)	Carob—6.2
Cu	0.7 (1.0)	Avocado—4.8
Fe	3.7 (2.1)	Carob—12.9
Mn	0.8 (0.6)	Carob—2.7
Sr	0.6 (0.3)	
Zn	2.1 (2.5)	

*The avocado or carob honey samples were considered as extreme values whenever their mineral concentration was higher than the mean by 2.5 standard deviations.

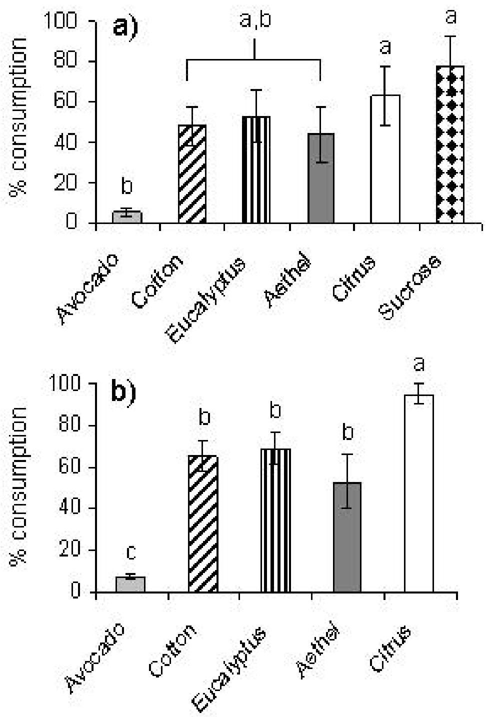

Fig. 4. Percent consumption of the different honey sources by bee colonies (n = 7). (a) Relative consumption including the sucrose feeder. (b) Relative consumption among honey feeders only. Different letters above columns indicate significant differences between honey sources (Tukey–Kramer test, $p < 0.05$) after arcsine square root transformation of percent consumption.

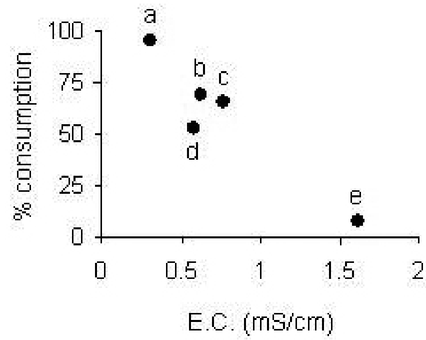

Fig. 5. The correlation between the average values of electrical conductivity of each honey and the average consumption of these honeys: (a) citrus, (b) eucalyptus, (c) cotton, (d) aethel, and (e) avocado.

ferent honey feeders and a sucrose feeder, the greatest consumption was from the sucrose feeder, but this was significantly different only from the consumption of the avocado honey (Tukey–Kramer test, $p < 0.05$, Fig. 4a). When consumption was compared only between honey feeders, the consumption of citrus honey was significantly greater and that of avocado honey lower than that of the other honeys (Fig. 4b).

Several correlations between honey consumption and honey parameters were tested. The E.C. was the only parameter that significantly correlated with honey consumption (Pearson product-moment, r = 0.94, p = 0.017). Figure 5 demonstrates the average E.C. and the average consumption of the different honeys.

DISCUSSION

This study illustrates some of the difficulties involved in defining Israeli honeys as unifloral. Based on pollen analysis, honey is usually considered as unifloral if the relative frequency of the pollen of that species exceeds 45% (von Der Ohe et al., 2004). According to our results, only honeys that originated from planted forests (aethel, carob, and eucalyptus) could be considered as unifloral honeys. The pollen content of honey, however, does not necessarily accurately reveal the botanical source of the honey. The proportion of the respective pollen may under- or overrepresent the relative contribution of the respective nectar. Eucalyptus pollen, for example, falls into the category of overrepresentation (Oddo and Piro, 2004). Therefore, a honey classified by its pollen content as being unifloral eucalyptus honey may in fact be derived from less than 45% eucalyptus nectar. On the other hand, all the agricultural honey sources that we tested are considered to have underrepresentation of their respective pollen: avocado (Terrab et al., 2004; Dag et al., 2006), citrus (Oddo and Piro, 2004), and cotton (Tsigouri et al., 2004). The extremely low percent of cotton pollen in cotton honey may be the result of consuming nectar from extrafloral nectaries of cotton plants. With such honeys, it is difficult to determine whether they are unifloral or not, as there are no standard methods to determine honey source other than pollen analysis.

Less is known about pollen content of honeys from natural vegetation, such as jujube and scabious. There is a record of jujube honey containing more than 40% of the respective pollen (al-Khalifa and al-Arify, 1999), but it is not clear what pollen percentage of this plant is necessary for classifying its honey as unifloral. We are not familiar with any literature concerning scabious honey, but when Ne'eman et al. (1999) tested bumblebees foraging on scabious flowers they observed that only 4% of their pollen load was scabious pollen and suggested that nectar thieving may occurs in these flowers. The same robbing behavior may also be true for honeybees and can explain the low scabious pollen count in scabious honey. Therefore, we cannot determine whether our jujube and scabious honeys were not unifloral, or whether in these honeys the proportion of the respective pollen also underrepresents the relative contribution of the respective nectar.

Due to the low accuracy of using pollen analysis in order to identify the nectar sources of the honey, more reliable markers should be considered. Markers should be specific to a single plant or a limited group of plants and they should originate directly from the nectar. Two examples of such markers are hesperetin for citrus honey (Ferreres et al., 1994) and perseitol for avocado honey (Dvash et al., 2002). Since honey is rarely produced from a single nectar source, by detecting the concentration of these compounds in the honey it is possible to estimate the contribution of the relevant nectar source to the tested honey. Our study suggests two additional markers. We found relatively high manganese concentrations in eucalyptus honey, which is consistent with values from other studies (Forte et al., 2001; Terrab et al., 2003; Fernandez-Torres et al., 2005). We therefore suggest that manganese may be a marker for eucalyptus honey. We also found high strontium concentrations in cotton honey, and suggest it as a marker for cotton honey. Manganese, strontium, and any other mineral, however, are not produced exclusively by a specific plant and may be found in some additional nectar sources and indeed both were also detected in carob honey. Reliable markers, even if not unique, could at least be used to reject a sample as being of a particular source. Therefore, using these minerals as honey markers is only partially reliable, but they should be an important part of a set of honey analyses.

Being aware of the limitations of using honey as a nectar substitute, this study also demonstrates advantages of this method. Avocado is an example of an agricultural crop that depends on honeybee pollination to set high yields. It is unattractive to bees (Ish-Am and Eisikowitch, 1998), but the reason for the low attractiveness was not clear. The extremely low consumption of avocado honey by bees in choice tests indicates that it tastes bad to bees. Avocado honey and nectar composition had to be analyzed in order to isolate possible repelling components. Further research revealed that the high mineral content of avocado nectar, mainly potassium, is probably the reason for its low attractiveness (Afik et al., 2006a).

A second demonstration of what can be learned by using honey as a nectar substitute relates to the high consumption of sucrose solution during the preference experiment. Nectar is the natural feeding source of many bee species and serves to attract them to the flowers to perform pollination. Therefore, it is assumed that nectar composition is well adjusted to the taste of potential pollinators in order to increase attractiveness (Proctor et al., 1996). Honey, being a product of the nectar, is also expected to be highly attractive, especially for the honeybees that produce it and feed on it. The results of this study demonstrate that none of the tested honeys was more attractive than a simple sucrose solution, and actually most of them even tended to be less attractive (Fig. 4). This may suggest that repulsive nectar components are not exceptional but rather highly common (Adler, 2000). Honey, in contrast to sucrose solution, contains mainly fructose and glucose, but it is unlikely to be the reason for the high preference for the sugar solution since bees tend to be indifferent between sucrose and hexose mixtures (Southwick et al., 1981; Afik et al., 2006a). Moreover, it may even be argued that bees should prefer a hexose solution since it saves them the energy that has to be invested in order to invert sucrose into fructose and glucose (Harborne, 1993). A previous study (Afik et al., 2006a) demonstrated that bees locate honey sources faster than sugar solution, probably due to the odor of the honey, which is missing in sugar solution. Hence, the tendency of bees to prefer sucrose solution rather than honey, based only on the trait of taste, is even greater than the current results, which were compensated by the odor effect.

Several honey parameters were tested for their correlation with honey consumption, but only the E.C. had a significant correlation. This indicates that the total mineral content of the honey is an important factor in determining honey consumption. Furthermore, assuming that the mineral content of the sucrose solution is negligible, the high preference for this solution is consistent with the negative contribution of minerals. The K/Na ratio that was hypothesized to affect nectar attractiveness did not significantly correlate with preference. In fact, there is only anecdotal evidence in support of this hypothesis (Petanidou, 2007). Previous work (Afik et al., 2006a) demonstrated that the mineral content of the honey reflects the mineral content of the nectar, therefore our conclusions for honey most likely also apply to nectar. All nectars contain some minerals (Waller et al., 1972; Hiebert and Calder, 1983; Nicolson and Worswick, 1990), and the mineral concentration and composition affects plant–pollinator interactions. These important interactions have been addressed by a few studies (Waller et al., 1972; Bouchard et al., 2000; Afik et al., 2007), but probably deserve much more attention (Petanidou, 2007).

Minerals, however, are not the only honey and nectar components that may have a deterring effect on pollinators. Some other components such as alkaloids (Singaravelan et al., 2005), phenolics (Hagler and Buchmann, 1993), and even certain sugars (Allsopp et al., 1998) were found to repel bees. Several explanations have been suggested for the adaptive role of repelling components in nectar (Rhoades and Bergdahl, 1981; Adler, 2000; Irwin and Adler, 2008). The prevalence of this phenomenon, according to the current results, may indicate that we have to look for a general explanation rather than a specific one for each case. Therefore, the hypothesis that repelling nectar components provide no benefit for the plant and may be a pleiotropic consequence of its production in other plant tissues, seems to best explain our findings (Adler and Irwin, 2005). Similarly, Nicolson and Thornburg (2007) concluded that phylogenetic history is the primary determinant of nectar sugar composition, whereas pollinators have only a secondary effect. Our study was addressed to deal with the issue of unifloral Israeli honey and the most common honey sources were tested. By selecting different honey sources, similar methods may be used to answer evolutionary questions such as whether honey and nectar from plants that evolved in the natural distribution area of honeybees are more attractive for them.

Even though honeybees are generalists and forage for nectar from a variety of flower species, unifloral honeys can be harvested even in the high plant diversity of Israel. This is possible mainly in managed landscapes such as planted forests or agricultural plots. We hope that this study will expose the advantages of using unifloral honeys as a tool for ecological research that may enhance our understanding of plant–pollinator relationships.

ACKNOWLEDGMENTS

We wish to thank Haim Efrat, Yossi Slabezki, and many Israeli beekeepers for providing the honey samples. We thank Erez Tsur, Orit Amar, and Yulia Sobotin for their help and Ari Schaffer and Yelena Yeselson for the carbohydrate analysis. This research was funded by a research grant from the Israeli Honey Commission.

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