Chapter

Geographical, Entomological and Botanical Origins of Honey

Robin E. Owen

Abstract

The Codex Alimentarius Commission defines honey as: "... the natural sweet substance produced by honey bees from the nectar of plants ... which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature". Honey, produced in all regions of the world varies widely in its chemical and physical properties, which depend on the plants the bees visit and on the species of *Apis* themselves. The Codex sets standards for the composition of honeys, levels of contaminants permitted, and the correct labelling according to floral source and geographic origin. The growth of stingless bee (Meliponidae) domestication in Central and South America, Asia and Australia has led to another significant source of honey, which is very variable in its properties. Here I review of the properties of honeys and the techniques used to analyze the geographical, entomological and botanical origins of honey, discuss some of the properties and features of the honeys made by the stingless bees, and discuss unusual honeys, the so-called "mad honeys", made from nectar containing toxic compounds, and the effect of toxic nectar on bees (bumble bees) and humans.

Keywords: honey, honey composition, honeybees, *Apis*, stingless bees, meliponid bees, toxic honey

1. Introduction

All bees (Apoidea) collect pollen and nectar from plants for food for themselves and their brood, and the eusocial bees in particular the honeybees (Apini, *Apis*), and the stingless bees (Meliponini) often store considerable quantities of nectar in their hives. This nectar has been processed by the bees, by addition of enzymes, etc. and when stored is now classified as honey. Bumble bees (Bombini, *Bombus*) also store nectar in their colonies, but in much smaller amounts which they generally use quite rapidly, and although it does thicken it is not processed and does not count as honey as such. Both honeybees and stingless bees produce honey in amounts that can, and have been, profitably harvested by humans of many different societies for thousands of years [1–3]. The Codex [4] standard for honey adopted by the Codex Alimentarius Commission in 1981, and revised in 1987 and 2001 and amended in 2019 defines honey as:

"... the natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature".

Therefore, strictly speaking honey, as such, is a product of honeybees (*Apis*). Honey is produced in all regions and in most countries of the world, and thus varies widely in its chemical and physical properties, which depend on the plants the bees visit and on the species of *Apis* themselves. The Codex sets standards for the composition of honeys, levels of contaminants permitted, and the correct labelling according to floral source and geographic origin. Recently the growth of stingless bee (Meliponid) domestication in Central and South America, Asia and Australia has led to another significant source of honey, but which is not regulated to the same extent as *Apis* honey and is much more variable in its physiochemical properties.

I will (1) provide an overview of the physicochemical and biochemical properties of honeys and the techniques used to analyze the geographical, entomological and botanical origins of honey, (2) discuss some of the properties and features of the honeys made by the stingless bees – the Meliponidae, and (3) discuss unusual honeys, the so-called "mad honeys", made from nectar containing toxic compounds, and the effect of toxic nectar on bees (bumble bees) and humans.

2. Characterization of honey and international standards

The recognized standards are that of the International Honey Commission (IHC) [5] and the Malaysian Standards (MS) [6]. Although most honey consumed worldwide is undoubtedly *Apis* honey (and mainly from *A. mellifera*) the Codex does differentiate between Blossom or Nectar Honey which comes from the nectars of plants, and Honeydew Honey which comes from the excretions of plant sucking insects (Hemiptera) or the secretions of living parts of plants [4]. The standards cover both sources of honey. The standards set by the IHC and the European Union (EU) are given in **Table 1**. However, there is considerable inconsistency between legislation national in the legislation of many countries[1] applying to the Codex and the standards (**Table 1**) [7]. Many countries maintain out of date quality criteria, in particular there is variation regarding moisture content, HMF, diastase activity, electrical conductivity, and sugars [7].

In recent years there has been a considerable increase in the consumption and commercial production of honey by stingless bees (Meliponini) in the Neotropical countries such as Mexico [2] and in tropical parts of Asia, particularly Malaysia [8]. This has led the Malaysian Department of Standards to set standards for stingless bee honey produced in Malaysia. The Malaysian Standard [6] defines *kelulut* or stingless bee honey as:

"A natural sweet with certain acidity substance produced by stingless bees of Meliponini tribe from the nectar of plants or from secretions of living parts of plants, which the stingless bees collect, transform by combining with the specific substances of their own, deposit, dehydrate, store and leave in the natural honey pots to ripen and mature."

[1] Argentina, Belgium, Brazil, Canada, China, Colombia, Czech Republic, Ethiopia, India, Germany, Greece, Japan, Poland, Russia, Serbia, Slovakia, Turkey [7].

	Component	Determination method	Standard		Use	Refs.
			IHC	MS		
1	Moisture, M (g/100 *g* i.e. %)	Refractometer	≤21	≤35	QA/QC	[6]
2	Free acidity, FA (meq/100 g)	Titration	≤50	n/a	QA/QC	[10]
3	pH	pH meter	n/a	2.5–3.8	QA/QC	[6]
4	Ash content (g/100 g)	Heated to 600°C, residue weighed	≤0.5	≤1.0	QA/QC	[6]
5	HMF content (mg/kg)	SPEC-UV/HPLC	≤40	≤30	QA/QC	[10]
6	Diastase activity, DN	Schade/phadebas	≥ 8	n/a	QA/QC	[10]
7	Sugars (g/100 g)					
	(Fructose + glucose) = TRS	GC, HPLC-RID	≥60	≤85	QA/QC	[10]
	Sucrose	GC, HPLC-RID	≤5	≤7.5	QA/QC	[10]
	Maltose	GC, HPLC-RID	n/a	≤9.5	QA/QC	[10]
	Others (e.g. erlose)	GC, HPLC-RID	—	—	Characterization	[11]
8	Plant phenolics	FCM/$AlCl_3$/HPLC/UHPLC	n/a	Present	QA/QC	[9]
9	EC (mS/cm)	Conductimeter, (lower range 10^{-7}S)	≤0.8 *	—	QA/QC	[10]
10	Amino acids + proteins	HPLC, UHPLC	—	—	Characterization	[12]
11	Vitamins	SPEC/COL, HPLC	—	—	Characterization	[12]
12	Lipids	GC-MS	—	—	Characterization	[12]
13	Minerals	AAS/OES/ICP/MS	—	—	Characterization	[12]
14	Organic acids	HPLC/IC/^{1}H-NMR	—	—	Authentication	[12]
15	Hydrocarbon composition	HPLC/^{1}H-NMR/GC	—	—	Toxin ID; Bee ID	[13, 14]
16	C_3/C_4 sugar ratio	$\Delta\delta^{13}$ protein/honey & C_{14}%	—	—	Authentication	[15]
17	DNA	DNA metabarcoding	—	—	Plant ID, bee ID	[16]

Table 1.
*Some components and properties measured in raw honey from **Apis** and meliponid bees.*

Notes: TRS = total reducing sugars; QA/QC = required to meet standard; *EU standard; HFM hydroxymethylfurfurnal; FCM/$AlCl_3$ = Folin–Ciocalteu method, AlCl3 colorimetric assay; BSWhite = bisulfite White method, Winkler = Winkler method; HPLC/UHPLC = high-performance liquid chromatography, ultra-high-performance liquid chromatography; GC = gas chromatography, HPLC-RID = HPLC coupled to a refractive index detector; SPEC/COL = spectrophotometric/colorimetric analysis; GC–MS = GC & mass spectrometry; ^{1}H-NMR = nuclear magnetic resonance; DNA metabarcoding = sequencing of plastid rbcLa, mt COI, nuclear internal transcribed spacer 2 (ITS2) region of nuclear ribosomal DNA.

Furthermore, the MS defines raw *kelulut* honey as that collected from natural sealed honey pots, while processed *kelulut* honey is raw honey which undergoes drying at a temperature not more than 40°C to reduce moisture content, to not more than 22.0% [6]. The Malaysian standards are also given in **Table 1**. A notable difference between *Apis mellifera* honey and stingless bees honey is that the latter is more acidic contributing to its unique sour taste [9] and the requirement that plant phenolic compounds must be present [6].

The various components of honey, are given in **Table 1**, together with the most common chemical and biological methods used to analyze these. The first nine are requirements of international or national standards to certify honey as genuine, and these consist of physicochemical and biochemical properties of the honey. Moisture, free-acidity, pH and ash content (1–4, **Table 1**) are all basic physicochemical properties of honey specified by the standards to fall within prescribed limits. The next two following, Hydroxymethylfurfurnal (5-(hydroxymetyl-)furan-2-carbaldehyde) or HFM content and diastase activity (5–6, **Table 1**) indicate if the honey has been subject to undue heating and/or improper storage [17].

Honey, composed of 60–80% monosaccharides and disaccharides, is the most concentrated sugar source found in nature, and so sugars from the nectar collected by the bees are, of course, the essential components of honey. Both the standards specify the sum of Fructose and Glucose to be at a specified minimum or maximum, and sucrose to be at a specified maximum (7, **Table 1**). To further characterize other sugars are often also measured (see **Table 2**) the honey, but are not required by the standards.

Next, phenolic compounds (8, **Table 1**) produced by plants, and present in nectar are required to be present in Meliponid honey but not in *Apis* honey. Phenolic compounds are made up of either one or more aromatic rings with hydroxyl groups,

Plant	*n*	EC	pH	FA	F	G	S	M	I	E
Acacia	36	0.185	3.9	14.5	34.65	21.60	8.8	3.6	1.2	2.8
Rhododendron	29	0.300	4.1	15.0	37.60	30.65	2.6	8.6	2.5	3.7
Chestnut	60	1.160	5.4	17.0	40.60	25.70	3.6	5.6	2.4	4.3
Dandelion	31	0.505	4.6	10.0	35.90	32.60	0.3	5.7	1.7	0.8
Heather	22	0.860	4.5	28.0	37.95	28.85	0.6	1.9	1.3	0.2
Lime	39	0.665	5.1	12.5	37.25	34.55	4.5	5.7	2.2	0.9
Rapeseed	36	0.210	4.1	12.0	37.05	35.75	2.0	2.2	1.5	1.4
Fir honeydew	132	1.015	4.7	31.5	30.15	23.15	2.7	4.9	3.4	4.5
Metcalfa honeydew	14	2.025	5.2	31.0	29.95	23.55	0.1	6.2	2.65	0.6

Note: n = sample size, EC = electrical conductivity (mScm^{-1}), pH = pH-Value, FA = free acidity (meq/kg), F = fructose (g/100 g), G = glucose (g/100 g), S = sucrose (g/100 g), M = maltose (g/100 g), I = isomaltose (g/100 g), E = erlose (g/100 g).

Table 2.
Summary of physical characteristics of honey or honeydew from nine species of plants. The data are derived from Ruoff et al. [22], and are the midpoint of the ranges of values given in their **Table 1***. A generalized distance matrix was calculated using these data to give* **Figure 2***.*

and are classified as either flavonoids (e.g. flavonols, anthocyanidins, flavanones) or others (e.g. phenolic acids) [12]. They work as primary antioxidants of free radical scavengers [9] with supposed health benefits. The phenolic composition in honey depends on many factors, including plant preferences by bees, geographic origins or weather conditions [12]. Ramly et al. [9] compared total phenolic content (TPH) and total flavonoid content (TFC) in honey from four species of meliponid bees from Banggol Peradong, Malaysia: *Heterotrigona itama, Geniotrigona thoracica, Lepidotrigona terminata* and *Heterotrigona erythrogastra*. As shown in **Figure 1** from Ramly et al. [9] there is considerable difference between species for both TPC and TFC, and this can probably results from different floral preferences of the bees foraging in the same geographical area [9]. Electrical Conductivity (9, **Table 1**) is positively correlated with ash and acid content of the honey, is technically easy, and is a good indication of the botanical origin of the honey [10].

The next set of components (10–13), Amino acids & Proteins, Vitamins, Lipids and Minerals, have all been measured in a wide variety of honeys to further characterize the honey for specific purposes, often related to benefits for human health, and using an extensive variety of methods [12].

Organic acids (14, **Table 1**) are often metabolic intermediates or result from microbial metabolism, and some have antidiabetic, antimicrobial, or antioxidant activity, and they can also be good markers of honey authenticity [12]. For example, Seraglio et al. [18] used aliphatic organic acids to differentiate Brazilian *Mimosa scabrella* or bracatinga honeydew honey from blossom honeys and adulterated honeydew honey.

Two types of hydrocarbons (15, **Table 1**) have been isolated from various honeys.

Grayanotoxins are a class of diterpenoids produced as secondary metabolites produced by plants and occur in the nectar of some species. When honey made from this nectar is ingested by humans these can have extreme toxic effects (see Section 5 below). Grayanotoxins can be detected and identified by reversed phase HPLC column using a water–methanol gradient [13]. The entomological origin of honey can be determined by the presence and the analysis of fragments of beeswax, which consist of various hydrocarbons and other organic compounds that differ between species of bees. Zuccato et al. [19] differentiated between various stingless bee honeys on thjis basis using nuclear magnetic resonance, similarly Zhang et al. [14] used gas

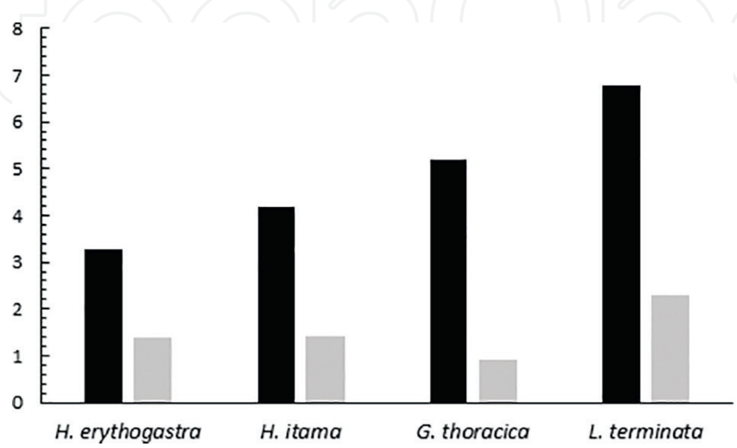

Figure 1.
Total phenolic content, (TPC; mg of gallic acid equivalents (GAEs) per gram of honey) and total flavonoid content (TFC; mg catechin equivalents (CE) per gram of honey) in honey of four stingless bee species (Heterotrigona itama, Geniotrigona thoracica, Lepidotrigona terminata and Heterotrigona erythrogastra). TPC = unshaded, TFC = shaded. From: Ramly et al. [9].

chromatography to distinguished species specific hydrocarbon profiles in honey from *A. mellifera* from *A. cerana*.

Pure honey which meets the standards (**Table 1**) tends to be relatively expensive, and there is a temptation to produce and sell fraudulent honey, and this is often produced by adulterating pure honey by adding extra sugar solution. One cheap way of doing this is to add commercially available syrups such as High Fructose Corn Syrup 55, etc. [15]. However, although these are readily available, these syrups generally are derived from C_4 plants, which use the carbon isotopes ^{13}C and ^{12}C in a different ratio than C_3 plants from which most nectar and honey is derived by bees [15]. The difference in the proportions of the C_3 and C_4 sugars (16, **Table 1**), and in relation to total protein the honey, thus indicates honey adulterated by this means [15].

Finally (17, **Table 1**) DNA in the honey coming from the plants or the insect visitor, can be used to identify in many instances exact species of each [16]. The method involves the sequencing of the barcoding regions of the nuclear, internal transcribed spacer 2 (ITS2) region of nuclear ribosomal DNA, and the chloroplast Ribulose-1,5-bisphosphate carboxylase-oxygenase gene (rbcLa) to identify plant species, and the barcoding region mitochondrial Cytochrome Oxidase I gene (mt COI) to identify bee visitors [16]. Prosser and Hebert [16] analyzed seven different honeys (Produced in Canada - Light, Dark, Blended, Pasteurized, Medium; France – Creamed; Mexico - Meliponine). They detected a total of 72 botanical sources in the Light honey, 16 of which could be identified as to species, and 63 botanical sources in the meliponine honey, but only two of these could be identified to species, the rest only to Family level [16]. The bees were *Apis mellifera* and *Melipona beechii* for the Canadian and Mexican samples respectively [16].

As can be seen honey is an extremely variable biological product, and its composition depends on many factors – the plant from which the nectar was gathered and its geographical location, the type of bee, how the honey has been collected and stored, being the most obvious ones. Some components may be present in very small quantities (minerals for example), and some are only relevant for identification and are not relevant to the person consuming the honey.

3. Characterization of pure honey

Although the IHC and Malaysian Standards ensure consistence among honeys, producers and regions, there can still be considerable variation among unifloral honeys. Most honey is polyfloral with the nectar being a mixture gathered by bees from many different plant species, but unifloral honeys are those where the nectar has come from a single plant species [22]. Unifloral honey is produced by placing the honeybee hives for a limited amount of time by a single crop species, ideally somewhat isolated to force the bees to forage solely from that crop. Ruoff et al. [22] obtained and analyzed honey from nine different species of plant, and in **Table 2**, I have given the main components they measured which I have summarized from their **Table 1**. As an easy way to visualize the similarities and differences between the honeys I performed an Unweighted Pair Group Mathematical Average (UPGMA) cluster analysis on the data in **Table 2** using Mesquite [20], and the results are shown in **Figure 2**. Unsurprisingly the two honeydew honeys are very similar and cluster together, but what is interesting and perhaps surprising is that honeys from three very different

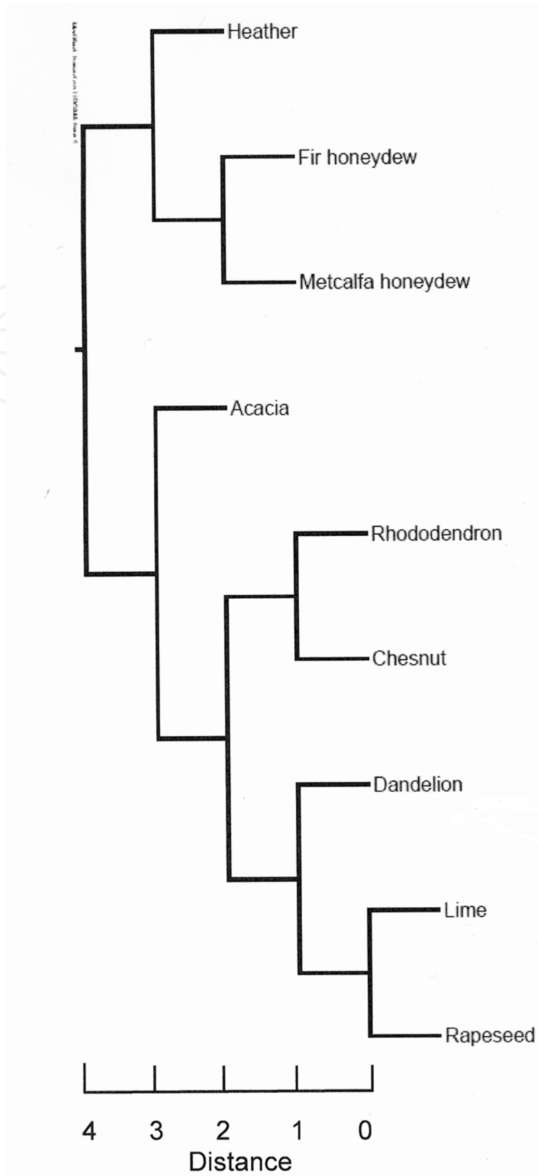

Figure 2.
Result of the Unweighted Pair Group Mathematical Average (UPGMA) cluster analysis on. the data in Table 2 using Mesquite [20].

plants Dandelion (Taraxacum officinale, Asteraceae), Lime (Tilia spp., Malvaceae), and rapeseed (Brassica napus var. oleifera, Brassicaceae) form a distinct cluster (**Figure 2**). Of course, these honeys do different in appearance, taste and other qualities even though they are very similar in sugar composition (**Table 2**). Ruoff et al. [22] also derived more detailed discriminant functions that allow the honeys to be classified and distinguished by their physicochemical values and sugar concentrations.

4. Meliponid bee honey

The Meliponini, or stingless bees are one of the four tribes of corbiculate bees comprising the subfamily Apinae in the bee family Apidae; the others being the Apini

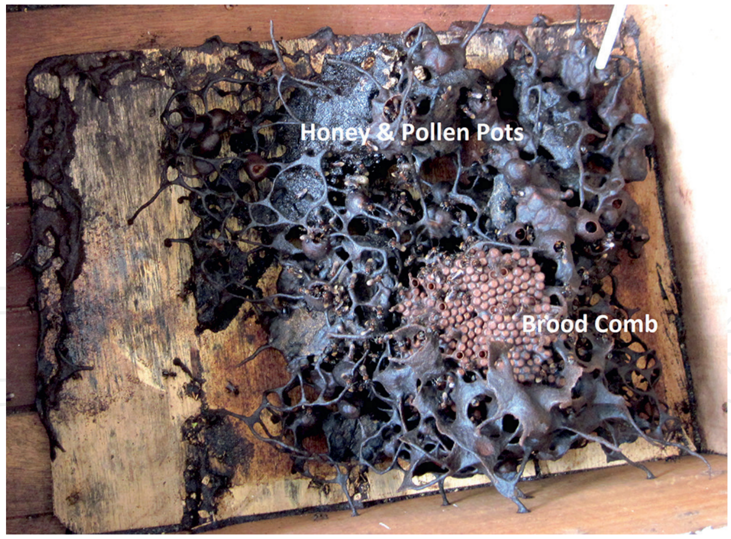

Figure 3.
A colony of Trigona hypogea in Malaysia. Image by Mohamad Izham M.A. used from Wikimedia under the Commons Creative Commons Attribution-Share Alike 3.0 Unported license.

(honeybees), Bombini (bumble bees) and the Euglossini (orchid bees) [21]. The meliponids are a very diverse tropical group of bees with over 500 species identified and of these there are over 400 species in the Neotropics, 50 in the Paleotropics, 60 in the Indo-Malaysian region and 11 in northern Australia [3, 8, 21]. The diversity is the highest in the Neotropics, but some of the most important species economically and culturally in Latin America are in the large genus *Melipona* (74 species) which only found in the Neotropics [2]. *Trigona* is the other large genus which is global in distribution occurring from the Neotropics to the Indo-Malaysian region. **Figure 3** shows a typical colony of *Trigona hypogea* in Malaysia. The honey is stored in extensive wax pots surrounding the brood comb.

Like *Apis* honey, stingless bee honey has been extensively measured for its physicochemical and biochemical properties, but there are far more species used for producing honey than with honeybees. One would expect much more variation and this is what has prompted the Malaysian government to implement standards [6]. In **Table 3** I have complied comparable data where available from the sources given in Nordin et al. [8] for the Malaysian Standards for stingless bees, and I have also included comparative data from five populations of honeybees, *A. mellifera*, from different geographical regions. Again as a way to visualize the similarities and differences between the honeys produced by different species of bees, I performed an Unweighted Pair UPGMA cluster analysis on the data in **Table 3** using Mesquite [20], and the results are shown in **Figure 4**. As can be seen, in some cases there is clustering of closely related species, i.e. those within the genus. Also with *A. mellifera*, the populations from different geographical locations tend to cluster together, i.e. make similar honey suggesting a possible phylogenetic effect for this species. Turning now to the stingless bees, **Figure 5** shows the same analysis now run just with the meliponid species from **Table 3**, and without *Apis*. Superficially there does appear to be some clustering according to genus however it appears that geographical location is of primary importance. Species of the different genera have produce very similar honeys when in the same region, whereas species of the same genus produce very different honeys when in different regions. This is what one would expect given that stingless bees are mostly generalist foragers.

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Taxon	Ref.	M	FA	pH	HMF	Ash	EC	TRS	F	G	DN
Homomtrigona fimbriata	[23]	41.0	52.0	3.30	46.00	1.00	2.60	22.00	7.4	15.0	?
Lepidotrigona doipaensis	[23]	31.5	197.5	3.50	2.30	0.51	1.20	38.50	12.0	11.9	1.60
Lepiodtrigona flavibasis	[23]	28.0	168.0	3.70	8.50	0.51	1.30	68.00	16.0	13.0	3.10
Lisotrigona furva	[23]	28.0	53.0	3.60	0.21	0.18	0.34	62.50	33.5	26.5	?
Melipona beecheii	[24]	28.6	41.5	3.20	9.23	0.46	0.58	69.21	?	?	1.30
Melipona paraensis	[25]	26.4	30.4	4.29	3.40	0.14	1.37	60.80	?	?	2.90
Melipona quadrifasciata 1	[26]	32.5	42.5	3.71	5.20	?	0.33	61.77	34.7	27.4	23.00
Melipona quadrifasciata 2	[26]	30.0	28.0	3.74	1.45	0.15	0.22	60.24	?	?	1.72
Melipona scutellaris	[27]	28.0	40.4	3.55	1.77	0.18	0.27	55.45	?	?	2.16
Melipona scutellaris latrelle	[28]	25.5	42.7	3.83	?	0.16	0.52	67.38	54.3	42.4	?
Melipona sp.	[29]	38.7	35.7	3.60	8.60	0.38	0.39	49.40	?	?	15.63
Melipona subnitida	[30]	24.8	32.5	?	7.56	0.02	0.10	50.97	29.2	21.8	0.0
Scaptotrigona mexicana	[31]	23.9	?	3.75	12.61	0.49	0.28	56.48	?	?	?
Tetragonisca angustula	[32]	24.4	45.2	4.10	9.93	0.39	0.13	55.46	?	?	32.28
Tetragona carbonaria	[33]	26.5	128.9	4.00	1.20	0.48	1.64	?	17.5	24.5	0.40
Tetragonilla collina	[23]	28.0	25.0	3.90	5.90	0.24	0.43	52.00	26.0	26.0	0.40
Tetragonula fuscobalteata	[23]	26.0	96.5	3.70	22.0	0.67	1.35	32.50	21.0	31.5	?
Tetragonula laeviceps	[34]	27.0	81.4	3.62	1.07	0.27	0.62	47.87	27.1	20.8	?
Tetragonula laeviceps-pagdeni	[23]	28.0	76.0	3.60	5.40	0.22	0.59	29.00	17.0	12.0	0.63
Tetragonula testaceitarsis	[23]	30.5	70.5	3.60	2.95	0.20	0.59	41.00	22.0	19.0	0.22
Tetrigona apicalis	[23]	42.0	495.0	3.20	0.26	1.40	2.60	12.50	6.7	5.90	4.90
Tetyrigona melanoleuca	[23]	43.0	592.0	3.40	28.0	3.10	2.80	15.00	6.0	8.90	0.15
Trigona angustula latreille	[35]	24.3	39.2	4.20	1.30	0.20	0.66	?	23.5	30.1	16.70

Taxon	Ref.	M	FA	pH	HMF	Ash	EC	TRS	F	G	DN
Trigona sp.	[36]	13.0	78.1	3.35	3.18	0.20	0.57	29.34	?	?	16.67
Trigona laevipceps	[36]	15.7	50.8	?	3.32	0.14	0.57	27.37	?	?	13.64
Trigona pagdenis	[36]	14.7	20.0	4.01	3.97	0.22	0.45	41.64	?	?	11.11
Apis mellifera 1	[37]	18.3	26.5	?	10.82	0.18	0.28	62.28	23.5	38.8	42.87
Apis mellifera 2	[38]	20.1	17.6	4.28	0.58	0.23	0.26	75.92	41.0	34.9	10.89
Apis mellifera 3	[39]	16.7	41.3	4.52	2.01	?	0.95	61.92	349	27.0	38.53
Apis mellifera 4	[39]	16.9	29.2	3.48	13.12	?	0.25	66.56	37.2	29.3	35.24
A. mellifera 5	[39]	13.6	23.6	4.18	1.98	?	0.62	60.28	36.6	23.7	15.78

*Note: Categories as defined in **Tables 1** and **2**;? = missing data.*

Table 3.
*Data from the sources given in Nordin et al. [8] for the Malaysian Standards for stingless bees. Also included are data from five populations of honeybees, **A. mellifera**, from different geographical regions.*

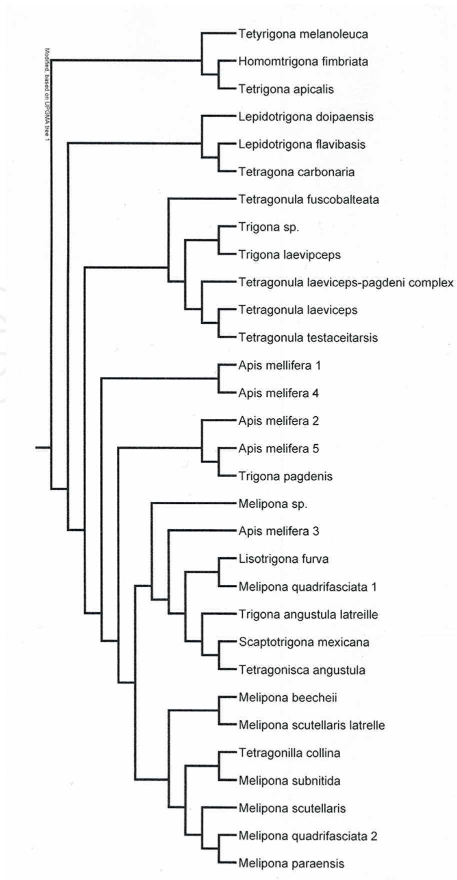

Figure 4.
Result of the Unweighted Pair Group Mathematical Average (UPGMA) cluster analysis on all the data in **Table 3** *using Mesquite [20]. Geographic origins of A. mellifera; 1 = Brazil; 2 = Thailand; 3, 4, 5 = Spain.*

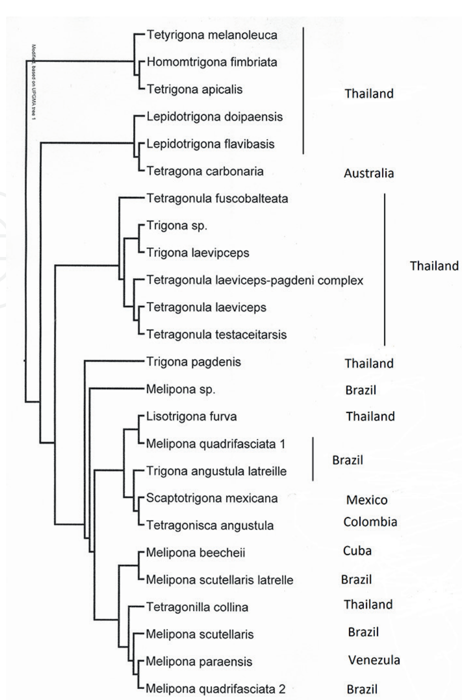

Figure 5.
Result of the Unweighted Pair Group Mathematical Average (UPGMA) cluster analysis on only the data in **Table 3** *from the melipoind species using Mesquite [20]. The geographical origin of each species is also indicated.*

5. Nectar and toxic honey

Not all nectar contains only beneficial compounds. Some plants secrete nectar containing toxic compounds to which pollinators may or may not be tolerant [40]. These toxins are secondary metabolites of plants and include various alkaloids (e.g. caffeine), diterpenoids (e.g. grayanotoxins), and cyanogenic glycosides, all of which are involved in plant defense [41]. Thus it is appears paradoxical as to why they are found in nectar (and pollen in a few species) which is a reward for pollinators [41, 42]. Many of these compounds are moved via the phloem and may just diffuse into the nectar rather than being actively transported in, as are sugars [42, 43]. Thus it is most reasonable to suppose that these metabolites are initially "unintentionally" deposited in the nectar and then subsequently selection and co-evolution with pollinators occurs [41]. This may be for reduced metabolite concentrations in nectar in some cases or in others for the retention of these metabolites [41]. For example caffeine, which occurs naturally at low concentrations in *Coffea* and *Citrus* species, improves the memory of honeybees when presented with a sucrose reward containing caffeine [44], therefore one would expect that bees would return more often to these plants.

Honeybees have fascinated humans for millennia, originally perhaps, just as a source of food as prehistoric cave paintings suggest, and then taking on a mystical and a religious significance. Indeed the Kulung and Gurung people of eastern Nepal still collect honey in this ancient precarious fashion by climbing down bamboo rope ladders hung from the top of granite cliffs to reach the colonies of the giant black honeybee, *Apis laboriosa*[2] [1]. These bees build single combed nests in aggregations sometimes of 50 or more under rock ledges high on cliffs in deep river valleys [45]. The honey is especially prized at certain times of the year because of its intoxicating properties; this is the so-called "Mad Honey" [1]. One class of toxins which has been extensively studied are the grayanotoxins, which are the active ingredient in the notorious "mad honey" mainly produced in the region around the Black Sea especially in Turkey [46], and also in Nepal [47]. In humans, low doses of mad honey cause hypotension, dizziness, nausea, excessive sweating and vomiting, but at high doses can cause serious cardiac problems including atrioventricular block, syncope and asystole (cardiac arrest), however there are no reported human deaths [48]. Grayanotoxins are polyhydroxylated cyclic hydrocarbons neurotoxins which block sodium channels in cell membranes thus nerve and muscle cells remain in a state of depolarization [48]. Although grayanotoxins (GTX) occur in a variety of Ericaceae, *Rhododendron ponticum* is of particular interest due to its invasive nature and it has been extensively studied [49–51]. In *Rhododendron* GTX I, II and III are the types of GTX (of the 25 different kinds) which commonly occur [48]. Tiedeken *et al*. [50] tested the toxicity of GTX I and GTX III on three native species of bees in Ireland where *R. ponticum* is invasive: A*pis mellifera*, a solitary bee *Andrena carantonica* and the bumble bee *B. terrestris audax*. Honeybee mortality increased by 20% when fed solutions containing GTX I, although the survival of the solitary bees and the bumble bees was not affected, however solitary bees did exhibit sublethal toxic effects including aversion to feeding and abnormal behaviors.

[2] This species is now recognized as a high altitude cliff bee endemic to the pan-Himalayan region and distinct from the giant honeybee *Apis dorsata* [45]. These bees build single combed nests in aggregations of 50 or more under rock ledges high on cliffs in deep river valleys [45].

Also neither bumble bee survival nor behavior was affected after consumption of GTX I even when the bees were subjected to the additional stressors of parasite infection or food reduction [50]. Previously Tiedeken et al. [49] had found that in paired-choice experiments *B. terrestris dalmatinus* did not preferentially avoid the toxic compounds nicotine, amygdalin, caffeine and GTX when presented at naturally occurring concentrations in sucrose solution. Again this subspecies did not suffer increased mortality when fed GTX but experience significant mortality with amygdalin and caffeine [49]. Some bumble bees are adapted to plants with toxic nectar. For example *Bombus gerstaeckeri* exclusively visits *Aconitum* spp. in Europe and is tolerant to aconitine in the nectar [51]. Egan et al. [52] examined geographic variation in the concentrations of GTXI and GTX III in nectar of *Rhododendron ponticum* in its native (Spain & Portugal) and introduced populations (Ireland). Interestingly, the nontoxic (to bees) GTXIII occurred at only low levels (~ 0.2 $\mu g\ mg^{-1}$) in both populations but GTXI was on average just more than half the concentration in the introduced populations in Ireland as compared to that in its native range (mean 0.81 vs. 1.46 μg mg−1 respectively) [52]. Egan et al. [52] found that 13 environmental and climatic variables did not significantly affect GTX levels in nectar, and suggest that pollinator-mediated selection for lower GTX concentration and/or release from nectar robbers in the invasive populations could account for this difference.

Toxic nectar and honey is certainly somewhat of an oddity, but nevertheless of great interest and importance for human health and behavior, and for understanding patterns of selection and coevolution between bees and plants.

6. Conclusions

Honey has been consumed in all regions of the world for millennia and continues to be an important component of many peoples' diets and used as a sweetener. However, as a sweetener it is relatively expensive [53] but is often preferred because of its health benefits and because honey is generally perceived as being "environmentally friendly" [53]. Even in medieval China (circa 400 BCE) honey was expensive, although apiculture was quite well developed, honey production was limited [54]. Honey, along with honeydew and cane sugar, was one of the most prestigious sweeteners that the wealthy could afford, as opposed to the malt sugars consumed by most of the population [54].

Most honey was and still is, *Apis* honey, with the exception of stingless bee honey produced by the Mayans [2], and by Australian aboriginal groups [3]. As analytical techniques have improved our understanding of the physicochemical and biochemical components and properties of honey have increased tremendously. This has led to the more exact characterization of the chemical, physical and biological properties of honeys. Some of these techniques are also used forensically. Since many specialized honeys are relatively expensive there is documented fraud whereby, for instance genuine C_3 honey can be differentiated from C_3 honey that has been diluted by the addition of sugars from C_4 plants. The techniques are also extensively used to characterize the different types of honey produced by stingless bees. The domestication of meliponid bees has undergone a resurgence in Mexico [2] and Australia [3] and has been extensively developed in Malaysia since 2012 as an alternative to honeybee keeping [8]. Many species of stingless bees have been domesticated to produce honey, which therefore varies much more than *Apis* honey which comes from only a few species, and mostly the western honeybee *Apis mellifera mellifera.* The Malaysian government has taken the very progressive step of setting out their own set of standards for meliponid honey [6], and what is particularly significantly different from the honeybee standards [4] is that the Malaysian standard [6] includes plant phenolic content to be required. This emphasizes the potential health benefits of using stingless bee honey.

Honey is generally regarded as an "ethical" sweetener as compared to refined sugar, etc. and so some work has been done using life cycle assessment (LCA) to estimate the carbon footprint and greenhouse gas (GHG) emissions in the production of honey [55–57]. Depending on the size of the operation the emissions are quite variable [55], also large honey producers the main used of their hives is to provide pollination services [55]. When pollination services are also accounted for by an LCA then it appears production has the greatest impact (e.g. use of glass for the honey jars and electricity used for refrigeration), and then distribution the next [56]. In fact, the study by Arzoumanidis et al. [56] found that transport of the orange-blossom honey from Italy to the U.S.A. had the most impact in the distribution phase. However, in many countries (e.g. Romania, [53]) people generally prefer to buy and consume locally produced honey which is obviously the most environmentally sustainable strategy. Nonetheless, some countries export most of their honey, an example being Argentina where almost 95% of all honey produced is exported adding to the carbon footprint [57], although within Argentina GHG emissions are comparable to those in other countries [57].

Honey is, and always will be, one of the most important naturally produced foods used by humans. It is essential that we continue to learn more about it, continually develop better methods of analysis and to promote environmentally friendly ways of production.

Author details

Robin E. Owen
Department of Biology, Mount Royal University, Calgary, Alberta, Canada

*Address all correspondence to: rowen@mtroyal.ca

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