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Honey as a Functional Food for *Apis mellifera*

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Abstract

Although nectar is consumed, primarily as a supplemental food, by a broad range of insects spanning at least five orders, it is processed and stored by only a small number of species, most of which are bees and wasps in the superfamily Apoidea. Within this group, *Apis mellifera* has evolved remarkable adaptations facilitating nectar processing and storage; in doing so, this species utilizes the end product, honey, for diverse functions with few if any equivalents in other phytophagous insects. Honey and its phytochemical constituents, some of which likely derive from propolis, have functional significance in protecting honey bees against microbial pathogens, toxins, and cold stress, as well as in regulating development and adult longevity. The distinctive properties of *A. mellifera* honey appear to have arisen in multiple ways, including genome modification; partnerships with microbial symbionts; and evolution of specialized behaviors, including foraging for substances other than nectar. That honey making by *A. mellifera* involves incorporation of exogenous material other than nectar, as well as endogenous products such as antimicrobial peptides and royal jelly, suggests that regarding honey as little more than a source of carbohydrates for bees is a concept in need of revision.

INTRODUCTION

Among the 200,000 species of animals that serve as pollinators (101), only a tiny fraction feed exclusively on floral resources throughout their entire life cycles, due to the ecological and physiological challenges associated with consuming these foods. Specialization for consumption of floral foods, particularly nectar, has reached a pinnacle in *Apis mellifera*, the western honey bee, achieved with a suite of extraordinary adaptations that involve both food processing and storage. Biologically active constituents of honey, beyond sugars, appear to contribute substantively to bee health in diverse contexts that, by virtue of the unique nature of perennial eusociality and associated food processing and storage, have no equivalent in conventional herbivores or even in other florivorous (flower-feeding) hymenopterans. How this suite of adaptations evolved, however, remains an open question.

PHYSIOLOGICAL AND ECOLOGICAL CHALLENGES OF NECTARIVORY

Nectar is a plant tissue with no known function other than to reward mutualists; as such, it has long been regarded in the literature as chemically innocuous, attractive to the broadest range of consumers among floral rewards (60). Lacking appreciable protein, floral nectar is not constituted by plants to serve as a complete source of nutrition for mutualists, and there are no known nectar-feeding animals that do not also consume other protein- or lipid-rich materials during their life cycle. Pollen is often the principal protein source for nectarivores, but alternate protein sources include plant tissues, fungi, other arthropods (particularly during larval stages), and vertebrate blood during adult stages.

Beyond nutrition, a major challenge to organisms that depend on nectar is that, as a food source, it is unpredictable—temporally ephemeral, spatially variable, and phytochemically idiosyncratic (1, 108). Phytochemicals mediate plant interactions with other organisms; with respect to nectar and pollen, phytochemicals influence interactions with both mutualists (e.g., pollinators) and antagonists (e.g., nectar thieves). In a comparative analysis of nectar and pollen phytochemicals, Palmer-Young et al. (115) found that both tissue types frequently contain flavonoids (with quercetin and kaempferol glycosides predominating), phenolics (particularly phenylpropanoids), terpenoids, and alkaloids. Nectar and pollen chemistries differ both qualitatively, with chemical richness measured as 63% higher in pollen, and quantitatively, with pollens containing concentrations from 24-fold to 235-fold higher than in nectar. Nectar chemistry exhibited greater within-site and within-cultivar variability than did pollen chemistry.

As a highly digestible source of carbohydrate energy, nectar is vulnerable to theft by inappropriate floral visitors that consume nectar without performing pollination services or by microbes that preempt its use by pollinators by degrading its quality. Honey bees, for example, avoid nectar containing certain bacterial communities (61, 74). Many nectar phytochemicals, particularly phenolics and alkaloids, are antimicrobial (1, 108) and may protect against microbial alterations in quality. In addition, alkaloids, phenolics, and other phytochemicals can defend nectar against inappropriate animal visitors by acting as feeding deterrents (143). Beyond repelling nectar thieves or robbers, limiting meal size of even effective pollinators can benefit the plant in some cases by inducing them to depart and to resume foraging on other conspecific flowers, increasing cross-fertilization opportunities (105).

HONEY BEE SOLUTIONS TO CONSUMING NECTAR

Creation of honey by *A. mellifera* as a storage form for nectar circumvents or minimizes the problems of unpredictability, ephemerality, susceptibility to microbial contamination, and

phytochemical variability. Several distinctive behaviors, some of which may be unique to *Apis* species, have evolved that facilitate the transformation of nectar (and other carbohydrate-rich foods) into honey. As a eusocial species, *A. mellifera* utilizes a workforce of highly mobile adults to collect nectar as it becomes available across the landscape, utilizing sophisticated communication to inform nestmates about the location and quality of nectar sources (35). Individuals typically begin foraging two to three weeks postemergence, primarily for pollen and nectar. Nectar, typically a dilute solution of sugars, is the fundamental raw ingredient of honey, although other carbohydrate-rich foods, including homopteran honeydew and, more recently, candy, soda, and other human foods (121) are also collected and used. Returning foragers transport nectar from the field in their crop (honey stomach), during which time enzymatic processing begins (107, 147), and, when they return to the hive, they discharge the contents to waiting nestmates. A cadre of nest workers (nectar-receiver or food-storer bees) continues nectar processing by promoting water removal, each individual sucking up and regurgitating nectar onto her proboscis, thereby increasing its surface area and expediting evaporation. Other workers further promote evaporation by wing-fanning to increase circulation within the hive (35).

Active evaporation reduces the water content of nectar from approximately 80–90% to 50–60%; this more concentrated solution is then placed in cells and moved intermittently, dehydrating passively until a final concentration of 18–25% is reached, whereupon the cell is capped. The high sugar concentration renders ripe honey hygroscopic, and as a supersaturated sugar solution, it is inimical to microbial growth (39). Among *Apis* species, average honey moisture content is characteristically somewhat lower for *A. mellifera* than for its sympatric congeners. Similarly, honey of stingless bees (Meliponini) is generally found to have higher moisture content than honey made by sympatric *A. mellifera* (**Table 1**).

Beyond behavior, biochemical processing of nectar into honey involves a distinctive suite of enzymes. As they concentrate nectar, bees add enzymes to metabolize nectar components. Sucrase activity—cleavage of the disaccharide sucrose into its component monosaccharides glucose and fructose—allows bees to produce a supersaturated solution by increasing the number of solutes per liquid volume. This in turn increases honey's osmolarity and its microbial toxicity; microbes that are not osmotolerant can die from plasmolysis and exosmosis. Also involved in processing nectar into honey is glucose oxidase (GOX), which, by oxidizing glucose to produce gluconic acid and

Table 1 Water content of honeys of *Apis* species and meliponine species

	Species	Water content (%)	Reference
Apidae: *Apis*	*Apis dorsata*	21.0	70
		22.7	20
	Apis cerana	20.1	70
		21.2	20
	Apis florea	20.1	20
	Apis mellifera	17.1	70
		18.8	20
		20.2	13
Apidae: Meliponini	*Plebeia tobagoensis*	42	13
	Melipona favosa	30.2	13
	Frieseomelitta aff. varia	19.9	40
	Melipona quadrifasciata anthidioides	41.9	40
	Scaptotrigona postica	27.0 to 40.2	40

hydrogen peroxide, protects honey from microbial degradation by lowering pH and sterilizing the medium via free hydroxyl radical production and subsequent oxidation of bacterial DNA, membrane lipids, and proteins (85).

Because nectars, and thus honeys, can contain substantial quantities of potentially toxic phytochemicals, enzyme-mediated detoxification of phytochemicals is a prerequisite for utilizing nectar-based food. In *A. mellifera*, the principal Phase 1 enzymes involved in xenobiotic detoxification are the cytochrome P450 monooxygenases (P450) (11). Some detoxification, however, may occur passively. As honey ripens in the hive, it is exposed to hive temperatures maintained in the range of 35°C; the phenolic content of nectar of *Aloe littoralis* is significantly reduced from 0.65% to 0.49% after exposure within an *Apis cerana* hive to these temperatures for 24 h (86), suggesting that *Apis* species may have some capacity to cook honey to render it less chemically challenging.

Water and plant resins are also involved in honey production and utilization. A subset of foragers collect water and store it temporarily for multiple uses, including not only evaporative cooling (113) but also honey dilution; due to its high viscosity, honey generally must be diluted by nurse bees to be fed to larvae and adults, particularly in winter. As for resins, some foragers collect them for processing into propolis, an antimicrobial mixture that, in wild colonies, lines virtually all interior surfaces in the nest (forming a propolis envelope) (141). Although plant resin sources vary widely, honey bees display distinct preferences for particular plant species (and even chemotypes within species) (44). Propolis has long been regarded as a structural agent, providing strength to cell walls, as well as an antimicrobial agent (125), but the chemical selectivity of resin foragers suggests that bees differentiate among resin sources based on their biological activity (44).

Among the main phytochemicals reported in honey in northern latitudes—pinobanksin, pinocembrin, quercetin, chrysin, and galangin (72)—few if any occur in floral nectars, but all occur widely in propolis. Resins do not appear to be consumed directly [Simone-Finstrom et al. (138, p. 8) state, "To our knowledge, honey bees do not naturally consume propolis. . .the mode of action of a therapeutic effect of propolis on colony pathogens is probably via volatile compounds. . .or direct contact"], but resin phytochemicals may be available for consumption by virtue of the ability of honey, particularly in early stages of ripening in cells while still dilute, to absorb them. Aqueous extracts of propolis typically contain many phenolic acids, including cinnamic acid derivatives (159), that occur broadly in honeys.

Processing food prior to storage to prolong its shelf life is exceptionally unusual among animals. Humans are perhaps unique in engaging in diverse food-processing behaviors that increase food suitability and consistency for consumption, ensure its availability during periods of scarcity, remove toxins, facilitate transport and distribution, and deactivate spoilage microbes (67). These functions all have, to some degree, parallels in the lives of honey-making bees. Although honey has enormous nutritional significance as the principal energy source for flight, thermoregulation, and wax production, its phytochemicals, with their diverse biological properties, including but not limited to antimicrobial activity, make it well-suited to serve as a functional food.

HONEY AS A FUNCTIONAL FOOD

That honey making by *A. mellifera* involves incorporation of multiple materials other than nectar from environmental and internal sources is inconsistent with the longstanding conviction that honey serves as little more than a carbohydrate source for bees (10, 136). As Erler & Moritz (47, p. 391) remarked, "It is the ability to store the huge variety of foraged antimicrobial substances that lends the honeybee colony an enormous advantage not just within the bee pollinators but also over many other social insects that require animal protein in their diet. The capacity to store

food provides the honeybees with an opportunity to selectively choose among the variety of stored products in an adaptive way dependent on their own or the colony's health status." Honey bees, however, do more than just store food—bees process both nectar and pollen extensively before storing them.

Functional foods are defined as those that "provide essential nutrients often beyond quantities necessary for normal maintenance, growth and development and/or other biologically active components that impart health benefits or desirable physiological effects" (148, p. 52). The concept, introduced into the human nutrition literature over 30 years ago, is rarely applied to nonhuman species, but, in view of "the exceptional capacity of the honeybee colony to store foraged plant products over extended periods of time" (47, p. 391), it provides a theoretical framework for understanding effects of honey on bee health that are not readily explained by its nutritional content, much as the terms self-medication, pharmacophagy, and pharmacophory have been variously used to describe the medicinal use of nonfood plant material as a response to parasitic infection or other diseases (47, 52). As a functional food, honey differs from plant material consumed only in response to specific stresses in that it is a regular diet item that promotes health due to its content of nutraceuticals, a term coined in 1989 to describe a "food, or parts of a food, that provide medical or health benefits, including the prevention and treatment of disease" (22). Nutraceuticals are regarded as "a toolbox for the prevention of disease" (37, p. 876) or as "[f]ood products to be taken as part of the usual diet in order to have beneficial effects that go beyond basic nutritional function" (29, p. 4).

Ironically, Sato & Miyata (134) recognized honey as a functional food and source of nutraceuticals for humans long before its multifarious roles in honey bee health were suspected. Many of the same functional properties of honey that are operative in humans (3) may well have evolved in the context of enhancing bee health.

EVOLUTIONARY ORIGINS OF *APIS* HONEY MAKING

The adaptive value of food processing and storage has likely exerted selection pressure on the genome of honey bees, as it has in humans (155)—dramatic diet shifts can remodel gene families for more specialized functions (e.g., 59). Among adaptations for honey processing are genome-encoded enzyme activities, so comparative genomics (71) may provide insights not only into reconstructing the evolutionary history of honey making, but also into determining whether the putative health-promoting properties of honey are acquired from foraged environmental sources, from genome-encoded biosynthesis, or from symbiotic microbial associates.

Honey bees belong to the superfamily Apoidea, which includes apoid wasps and the monophyletic lineage Anthophila, comprising the world's 20,000 bee species (131). Apoid wasps are primarily insect predators, although pollen-feeding occurs in multiple lineages, and at least one sphecid (*Krombeinictus* sp.) provisions larvae with pollen and nectar (153). With very few exceptions, however, all bees are specialized consumers of pollen and nectar as larvae and adults and store these foods in some form for future use by larvae (131).

Although approximately 85% of bee species are solitary, the remaining 15% display levels of group living from communal nesting to complex eusocial societies with tens of thousands of individuals. Storage of nectar-based food evolved concurrently with pollen storage. In solitary bees, mixing pollen and nectar improves the efficiency of transport in the field and enhances storage in the nest; along with glandular secretions, nectar facilitates formation of coherent masses of pollen grains differing in texture and size (106). Thus, storing pollen necessarily involves storing nectar. Nearly half of the provisions of the solitary alfalfa leaf-cutting bee *Megachile rotundata* are sugars derived from nectar (25), and because the 20% water content of provisions derives from nectar,

the larval diet is effectively 2:1 nectar:pollen. Storing pollen with nectar may also increase the digestibility of pollen; pollen grains can actively release most proteins and free amino acids almost immediately upon incubation in sugar solutions (55). The near absence of sucrose in the alfalfa leaf-cutting bee provisions (despite its presence in alfalfa nectar) suggests that invertase or sucrase activity may be involved in preparing provisions, but the identity and source of the enzyme(s) responsible for this conversion are unknown.

The transition to storing nectar independently of storing pollen required additional key innovations, notably, the ability to construct storage containers. Because water promotes microbial growth, reducing water content and/or protecting receptacles with antimicrobial substances increases prospects for long-term storage of liquid or semiliquid materials. Lining receptacles with waterproof materials provides a mechanism for liquid storage, reducing risks of leakage and concomitant spoilage, promoting high humidity in the nest environment.

Resin-collecting behavior, which appears in multiple lineages of both social and solitary bees, may also have increased the efficacy of pollen storage, both by waterproofing and by preventing microbial colonization of provisions. The resin bees in the genus *Megachile* (Megachilidae) are solitary species that collect plant resins to line nest entrances and larval chambers; some species nesting in hollow stems also use resins to construct partitions between cells and to close off the nest cavity, as do several solitary *Hylaeus* species (Colletidae). Resin collecting for waterproofing (or possibly disinfection) may have been a preadaptation for constructing liquid storage containers by social species.

Production of honey or honey-like nectar storage products is restricted to eusocial species. Cardinal & Danforth (26) determined that "eusociality evolved once in the common ancestor of the corbiculate Apidae, advanced eusociality evolved independently in the honey and stingless bees, and. . . eusociality was lost in the orchid bees." By extension, then, this phylogeny suggests that storage of substantial quantities of nectar-based food as honey may have evolved independently in honey bees and stingless bees. Indeed, although orchid bees do collect nectar and pollen (e.g., 15, 120), they are not known to make honey or store food at all. *Bombus* species have an annual life cycle and thus do not process or store food for overwintering, although they construct wax honey pots for short-term food storage. In pollen-storing bumble bees, workers store pollen and nectar in separate wax pots and feed larvae a regurgitated blend of these foods (34). While the fluid in nectar pots can be very thick, whether it constitutes honey is unclear. Behavioral processing of nectar is minimal; returning foragers deposit nectar directly into pots, rather than offloading to nestmates for active evaporation (118), and the thickened liquid in honey pots may simply result from passive evaporation.

Apines and meliponines display similarities in processing nectar for long-term storage—e.g., apines store processed nectar in wax cells often lined with propolis, and meliponines store processed nectar in pots of beeswax combined with plant resins. Meliponine honeys are biologically active, with significant antioxidant and antimicrobial properties; as in *A. mellifera* honey, the antioxidant capacity of meliponine honey is correlated with phenolic content (158). Moreover, sucrase activity has been detected in the hypopharyngeal glands of at least one meliponine species (33). The higher water content and lower sugar content of meliponine honeys, however, make them prone to more rapid deterioration than is *A. mellifera* honey, particularly to overgrowth by filamentous fungi (9). Thus, they are less suitable as a source of stored foraged materials to promote colony health. That said, substantially less attention has been paid to food processing and storage by the 500 or more species of meliponines than has been accorded to food processing and storage by *A. mellifera* (alone among the seven *Apis* species), and the seemingly unique nature of *A. mellifera*'s honey may simply reflect a knowledge gap that will be logistically challenging to close.

ENDOGENOUS ENZYMES AND NECTAR PROCESSING BY *APIS MELLIFERA*

Detoxification Genes in Bee Genomes

Consuming pollen, nectar, and resin phytochemicals requires the biochemical capacity to process the phytochemicals in all of these materials. Pollen grains all contain flavonoids, particularly quercetin and kaempferol, plant signaling substances that mediate both pollen germination and fertilization (98). Many nectars contain these flavonols, and both pollen and nectar can contribute to the flavonol content of honey. Nectar of rosemary, *Rosmarinus officinalis*, for example, is the major source of kaempferol in honey bee honey (54). As for propolis, although resin sources vary in chemistry, flavonoids, phenolic acids, and phenolic aldehydes occur frequently and abundantly; less frequently encountered are coumarins, stilbenes, and lignans (133). Crude propolis can contain upwards of 17% flavonoids. Whereas temperate zone propolis, primarily from poplars and their relatives, is high in flavanones and flavones, propolis in tropical areas tends to have a different profile, including prenylated flavonoids, prenylated derivatives of *p*-coumaric acid, caffeoylquinic acid derivatives, and lignans.

Among the P450 genes in the *A. mellifera* genome, encoded by 46 genes in the honey bee genome, those in the CYP6 and CYP9 families are involved in detoxification of phytochemicals (11). As determined by bioassay and/or molecular modeling and in silico docking, CYP6AS enzymes metabolize flavonols, including quercetin, a ubiquitous constituent of nectar, honey, pollen, beebread, plant resins, and propolis (90). CYP6AS subfamily size varies across bee species that differ in degree of sociality: Within-family diversity increases with degree of sociality, ranging from 7 in the solitary southeastern blueberry bee *Habropoda laboriosa* and the facultatively eusocial *Lasioglossum albipes* to 16 in *Apis* species and 17 in the perennial eusocial *Melipona quadrifasciatus* (68). This pattern mirrors the transition from short-term storage of ephemeral low-quality foods to long-term storage of concentrated processed food. Diversification of dietary sources of flavonoids in a more concentrated form over evolutionary time may have selected for subfamily expansion and possible subfunctionalization to provide more specialized phytochemical detoxification.

Glucose Oxidase/Glucose Dehydrogenase in Bee Genomes

In both nectar and pollen, conversion of glucose into gluconic acid and hydrogen peroxide discourages microbial overgrowth in two ways—by lowering pH and by generating hydrogen peroxide. GOXs (EC 1.1.3.4) are oxidoreductase enzymes that act on the first hydroxyl group of glucose molecules. These enzymes are closely related and virtually identical in structure to glucose dehydrogenases (GDHs), which are also oxidoreductase enzymes acting on the first hydroxyl groups of glucose and other sugar molecules. In contrast to GDHs, however, GOXs are unique in using oxygen as an electron acceptor, whereas GDHs utilize a wide array of electron acceptors as cofactors, including NAD, FAD, and pyrroloquinoline quinone (PQQ), but not oxygen (48). Thus, GOXs are responsible for H_2O_2 production.

Initially found in fungi, GOX was first found from an animal source in the hypopharyngeal glands of *A. mellifera* (51). This enzyme was later linked to the antibacterial activity of honey via production of H_2O_2 and D-gluconic acid from glucose (152). Burgett (24) reported the activity of GOX in nine social Hymenoptera species across three superfamilies—Formicoidea, Vespoidea, and Apoidea—hypothesizing that this enzyme might occur in most honey-storing social insects. The *A. mellifera* genome contains one functionally recognized GOX gene (*GB44549* in Amel_HAv3.1) that has been isolated and characterized (111) and is located just adjacent to *GB44548*, a gene predictively annotated as GDH. GOX may have evolved from an ancestral

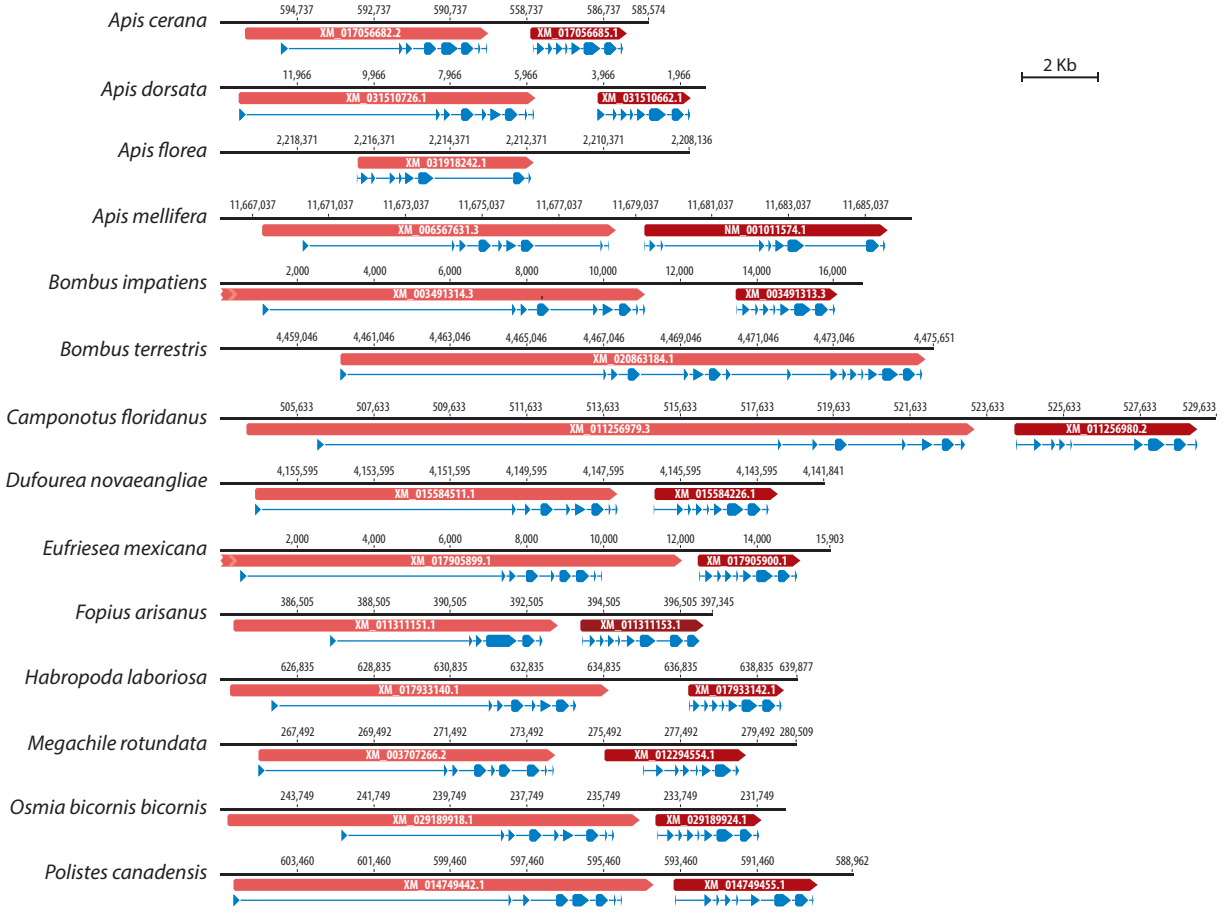

Figure 1

The GDH/GOX tandem in 14 representative Hymenoptera. The black line and numbers show the genomic region within a scaffold in the respective assembly. Pink arrows indicate GDH homologs, red arrows indicate GOX homologs, and blue arrows indicate intron/exon structure. All sequences were obtained from the National Center for Biotechnology Information. Abbreviations: GDH, glucose dehydrogenase; GOX, glucose oxidase.

oxidoreductase that produced a tandem of two GDHs, one of which further changed and functionalized into GOX (79).

Analysis of the genomes of 14 representative Hymenoptera species, including two wasps, two ants, and four bees, reveals that the tandem of two oxidoreductase genes has probably been present since the last common ancestor of these species, with these two genes always occurring within the same genomic context (**Figure 1**). Except for those from *A. mellifera*, however, the two tandem genes from every genome are both predictively annotated as GDHs—by the National Center for Biotechnology Information automatic annotation pipeline—and no GOXs are predicted in any other Hymenoptera. A maximum likelihood tree of the amino acid sequences of these enzymes from the 14 species, however, neatly separates the downstream from the upstream sequences in all of the genomes with high confidence, grouping all of the downstream sequences with *A. mellifera* GOX and all of the upstream sequences with GDH, suggesting that GOX might be present in all of these genomes (**Figure 2**). This suggestion aligns with the prediction (24) that GOX function

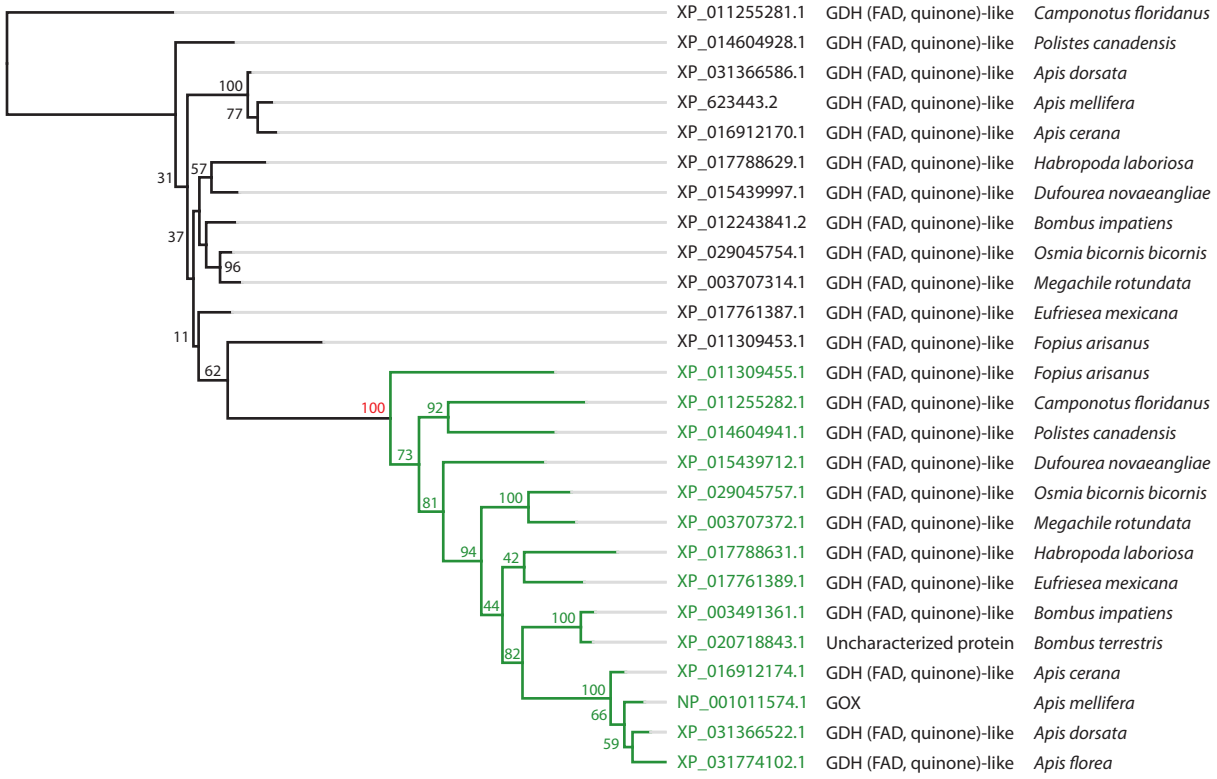

Figure 2

Maximum likelihood tree with 100 bootstrap replications of the 26 GDH and GOX genes in 14 Hymenoptera species. Branch lengths are proportional to amino acid replacements, and the numbers are the bootstrap significances. Branch labels are NCBI RefSeq protein identifiers, followed by NCBI annotation and species. The tree was created with RaxML v.7.2.8 (142). Abbreviations: GDH, glucose dehydrogenase; GOX, glucose oxidase; NCBI, National Center for Biotechnology Information.

is possessed by most, if not all, food- and nectar-storing insects. In fact, Sommeijer et al. (140) detected H_2O_2 in the provisions of the mining bee *Colletes halophilus*, an oligolectic solitary species; the absence of H_2O_2 in dietary pollen suggested to these authors that GOX is produced by the bees and added to the larval provisions as a preservative that is necessary due to their semiliquid nature. *A. cerana* and *Bombus terrestris* are the only two species with a single gene in this region, and their genes both fall in the GOX lineage. This discrepancy could be attributed to either a missed stop codon during gene prediction (particularly in *B. terrestris*, where the gene is unusually long) or a loss of the GDH gene (a possible scenario for *A. cerana*).

GOX production by honey bees is apparently restricted to worker hypopharyngeal glands and is highly expressed across a range of developmental stages. GOX mRNA expression increases progressively with age, beginning two days posteclosion (cleaners and nurses) and reaching its highest level in nectar processors and foragers. GOX is very highly expressed in hypopharyngeal glands of randomly selected hive bees, but no expression of this enzyme was detected in mandibular glands, head salivary glands, or thorax salivary glands (21), nor was GOX expressed in fat body from nurses three or eight days after eclosion (30), in midguts (137), or in whole digestive tracts in nurses and foragers. In contrast with previous findings (79), these studies determined that expression patterns of GOX and its neighboring GDH transcripts were not comparable.

Another perspective on the production of gluconic acid in honey (128) is that gluconic acid–producing bacteria contribute gluconic acid to honey directly; to date, no studies have estimated relative contributions of endogenous and exogenous sources to gluconic acid levels in honey.

Invertase

Among the enzymes added to nectar as it is processed into honey by workers are sucrases that metabolize sucrose into fructose and glucose. As sucrose is the main sugar in most nectars, this transformation is critical both for honey production and for honey functional properties. Converting sucrose into fructose and glucose increases the osmotic potential of honey, which increases its antimicrobial properties and protects it from spoilage (thereby prolonging its storage life). Sucrose is different from other disaccharides in having an α1-β2-glycosidic bond between the two sugar monomers, making it both an α-D-glucoside and a β-D-fructofuranoside, with the consequence that it is a substrate of both α-glucosidases (EC 3.2.1.20, also known as maltases or α-glucosidases) and β-fructosidases (EC 3.2.1.26, also known as invertases). The main mechanistic difference between these two types of enzymes is that the disaccharide is split on opposite sides of the glycosidic oxygen atom (43). Because of this subtle difference, both enzyme names (i.e., invertase and α-glucosidase) have been used interchangeably for the sucrase activity of honey bee glandular secretions.

The α-glucosidases (EC 3.2.1.20) are widely distributed across organisms, including plants, fungi, bacteria, mammals, and insects. In insects, α-glucosidases are ubiquitously present in midguts (145). Mature nurse honey bees have hypopharyngeal glands that occupy much of their heads. As they transition into foragers, the glands shrink (154), and sucrase activity has been detected in the shrunken hypopharyngeal glands (18). Kubo et al. (76) isolated an approximately 70-Kd protein selectively synthesized in hypopharyngeal glands of older workers and identified it as α-glucosidase; they used cloning, sequencing, and RT-PCR to establish that this protein is encoded by a honey bee gene (*GB43247*) expressed specifically in forager glands and with homology to a possible maltase from *Aedes aegypti* and *Drosophila melanogaster* (112). Three types of α-glucosidase were later purified from *A. mellifera* and from ripe honey (type I, type II, and type III), and immunoblotting detection on tissues detected α-glucosidase type I in the ventriculus, α-glucosidase type II in the ventriculus and hemolymph, and α-glucosidase type III only in hypopharyngeal glands and in honey. These three types also had different substrate preferences, with α-glucosidase type III being the only one with a kinetic profile consistent with sucrose degradation (77). Mutational analyses of *A. mellifera* type III α-glucosidase using an in silico built protein model demonstrated its preference for sucrose and the mutations necessary to change that preference to maltose (99). Active α-glucosidase has also been purified and characterized from *A. cerana indica* (27).

Despite the widespread use of the term invertase in the honey bee literature (e.g., 139), and in contrast with α-glucosidases, there is no evidence of an invertase-coding gene in the genome of *A. mellifera* or its relatives (45). There are also no reports of the kinetic parameters of sucrase activity during honey ripening, which differs between invertases and α-glucosidases. Invertases (EC 3.2.1.26), also called β-fructofuranosidases, were long considered to be exclusive to microorganisms and plants. This notion was challenged after the discovery of invertase genes in the genome of the silk moth *Bombyx mori* (36) and subsequently in other Lepidoptera (64, 119). In *B. mori*, this gene was likely transferred horizontally from a microorganism and circumvents toxicity of alkaloid sugar-mimicking glucosidase inhibitors from *Morus alba* leaves, which act on midgut α-glucosidases but not on invertases (36). Invertase genes were later found in two other species, both Coleoptera: the mountain pine beetle (*Dendoctronus ponderosa*) (73) and the emerald ash borer

(*Agrilus planipennis*) (160). That invertase genes in insects are typically intron-less and are not present in all immediately phylogenetically related species supports the horizontal gene transfer theory, although a definite eukaryotic source of insect invertases was identified in *Manduca sexta* (119) by demonstrating that gene sequences for insect invertases have a 3′ untranslated region and poly-A tails.

Because invertase occurs in beebread (75) and in proteomic analyses of honey (14), the possibility exists that honey ripening is aided by the forager gland microbiome in a mutualistic interaction that could increase the efficiency with which nectar is processed. Harpel et al. (64) identified an invertase in a proteomic survey of saliva of another nectar-feeder, the butterfly *Heliconius melpomene*. In addition, just as α-glucosidases are widespread in nature, so are their inhibitors, which include not just alkaloids but also plant, yeast, and propolis flavonoids (78, 83, 122).

Beyond GOX and α-glucosidase, other enzymes are routinely detected in honey. Amylase has long been known to occur (111), as have proteases, serine protease inhibitors, and glucose dehydrogenase isoforms (82).

ENZYMES THOUGHT TO BE PLANT DERIVED: ACID PHOSPHATASE AND CATALASE

Catalase (EC 1.11.1.6), in nearly all living organisms, metabolizes hydrogen peroxide (H_2O_2) to water and molecular oxygen. The combined action of catalase with other H_2O_2-scavenging enzymes, such as peroxidases and superoxide dismutase, is critical for protecting cells from oxidative damage caused by elevated H_2O_2 levels. Catalase activity in honey was documented over 100 years ago (8, 56, 62), and its presence has been attributed to pollen in the honey and yeasts associated with honey during ripening (56). Catalase activity in different honeys (16, 66, 135) is presumed to keep H_2O_2 levels—produced by GOX activity—below toxic concentrations. Thus, H_2O_2 activity in honey relates directly to levels of GOX versus levels of catalase. Nonperoxide phytochemical components are thought to contribute to antimicrobial activity in many honeys, most notably manuka (*Leptospermum scoparium*: Myrtaceae), which ostensibly owes its very high antimicrobial activity to the conversion of nectar dihydroxyacetone to methylglyoxal during processing (95). Weston (149), however, suggested that manuka's antimicrobial activity originates with an excess of H_2O_2 resulting from the low content of catalase in manuka pollen.

The first genome assembly of *A. mellifera* identified a catalase protein (GB11518, AAN76688.1), but the predicted enzyme does not have a detectable secretory signal (32). The *A. mellifera* catalase predicted in the most recent *A. mellifera* genome assembly, however, is 597 amino acids long and does contain a potential secretory signal as predicted by the EMBOSS 6.5.7 sigcleave tool. This feature is not, however, definitive evidence that the enzyme is secreted, as in silico signal cleavage predictions with this tool are only 75–80% accurate for eukaryote signal peptides.

Acid phosphatases (EC 3.1.3.2) comprise a family of ubiquitous hydrolase enzymes that catalyze hydrolysis of orthophosphate monoesters under acidic conditions (23). Acid phosphatase activity was detected in honey decades ago (57); although activity assays in pollen, nectar, true honey, and artificial honey suggest that the activity arises from enzymes of plant origin (5, 151), insufficient information is available to identify unequivocally the origin of honey acid phosphatases. No study has yet isolated or characterized this enzyme at the amino acid level. Moreover, Flanjak et al. (49) found no significant differences in median acid phosphatase activity between black locust, chestnut, and honeydew honeys, the latter of which derives from neither nectar nor pollen. Activities of diastase and GOX, known to be bee derived, were also indistinguishable between the two monofloral honeys and the honeydew honey. Protein separation and identification studies

have not produced clear evidence of acid phosphatases that could provide a basis for characterization (16, 17, 42).

There are at least seven acid phosphatases in the genome of *A. mellifera*, with more than 24 predicted isoforms. As in humans, these acid phosphatases have different chromosomal origins and share different amino acid homology. Four groups of acid phosphatases are distinguishable in honey bees: venom acid phosphatase, venom acid phosphatase–like, lysosomal acid phosphatase, and acid phosphatase type 7 homolog. How the acid phosphatases in honey align with these categories is as yet undetermined.

ORIGINS OF ANTIOXIDANTS OF HONEYS AND LONGEVITY ENHANCEMENT

The phytochemical content of honey may enhance longevity through its antioxidant activity. Among theories of aging in social insects, particularly *A. mellifera* (7, 41), most relevant to differential longevity in workers is the oxidative stress theory of aging, which posits that irreversible accumulation of oxidative damage leads to senescence. Whereas order-of-magnitude differences in longevity of queens relative to workers appear to be independent of antioxidant gene expression (31), differential longevity of workers conforms to tenets of the oxidative stress hypothesis. Winter bees live up to 24 weeks as adults, whereas summer foragers have a life expectancy of three to four weeks (7). In summer workers, the transition from hive tasks to foraging is the central variable for honey bee aging, although chronological age, irrespective of behavioral state, contributes to aging as well (127). Associated with lifespan extension is a reduction in the abundance of peroxidizable (polyunsaturated) fatty acids and, concomitantly, a reduction in oxidative damage (63).

The influence of honey constituents on longevity occurs via regulation of genes associated with lifespan. In several studies (81, 93), *p*-coumaric acid ingestion by honey bee larvae upregulated forkhead (FOXO) 2.38-fold; FOXO has been implicated in bee longevity because its homolog in *Caenorhabditis elegans* downregulates life-shortening genes and upregulates antioxidant enzymes (catalase, superoxide dismutase) in *Culex pipiens*. In addition, *D. melanogaster* FOXO, when overexpressed in fat body, increased female lifespan (53). Chrysin, found in both honey and propolis (88), added to the diet of adult *D. melanogaster* increased the median lifespan of females by up to 12% and the maximum lifespan by up to 22% but had no longevity-enhancing effects on males (80). In female flies consuming diets to which chrysin was added, *Hsp70* expression levels were reduced by up to 82% compared with flies on unamended diets. Although *Hsp70* is thought to be geroprotective, the lower level of *Hsp70* expression after chrysin consumption could be a biomarker of younger biological age given that flies with lower levels of Hsp70 reported live longer than do flies with higher levels.

Honey constituents may also enhance longevity directly through their antioxidant activity. Many antioxidant phenolics in honey may be capable of neutralizing reactive oxygen species that damage proteins, DNA, and fatty acids, leading to cell death. Honey antioxidant capacity depends on nectar source; in the first study to examine this relationship, Frankel et al. (50) found 20-fold variation in antioxidant capacity across 14 monofloral honeys. Interest in honey as a functional food for humans increased markedly with recognition of its antioxidant content; a search of the Web of Science core collection (March 3, 2020) yielded nearly 400 papers with titles including the words "antioxidant" and "honey." Of these, however, only 38 included "*Apis mellifera*" as a topic; the preponderance concern human health and nutrition (e.g., 2), and the significance of antioxidant activity in honey to honey bee longevity (or any other health benefits) is not well known.

Curiously, studies examining whether honey itself, rather than isolated constituents, can enhance longevity are more often conducted on parasitoid wasps than on the honey bees that

produce it; most such studies are aimed at improving mass rearing of parasitoids for biological control. Harvey et al. (65) compared life history attributes of *Gelis agilis* (Ichneumonidae) reared on honey, a honey-sugar mimic, or glucose. *G. agilis* females consuming honey produced twice as many offspring as those reared on other diets; female longevity was reduced only on the honey-sugar mimic diet, suggesting contributions of constituents of honey other than sugars in increasing longevity and reproduction.

Effects of individual honey constituents with high antioxidant capacity on lifespan have been more frequently evaluated in adult honey bees than has intact honey. Liao et al. (84) conducted a series of longevity assays with bees on a sugar-casein protein diet formulated with *p*-coumaric acid, quercetin, and the two phytochemicals together. Diets with *p*-coumaric acid increased longevity by 17.6%, and those with quercetin increased longevity by 6.2%; bees consuming the two phytochemicals together did not, however, experience longevity enhancement. Similar effects were reported by Wong et al. (156) with the same phytochemicals; bees consuming *p*-coumaric acid lived longer than bees consuming the control diet, although bees consuming diets containing both *p*-coumaric acid and quercetin experienced reduced longevity. Bernklau et al. (12) demonstrated that four phytochemicals—caffeine, gallic acid, kaempferol, and *p*-coumaric acid—enhanced longevity in adult bees at ecologically appropriate concentrations. Although all of these phytochemicals occur in honey, caffeine is less widely distributed and is known primarily from nectar of *Citrus* and *Coffea* (157). Few if any studies exist on the colony-level effects of honey antioxidant content on worker longevity or colony health.

NONPEROXIDE ANTIMICROBIAL ACTIVITY OF HONEY

In the context of human health, honey has long been associated with antibacterial activity, and activity against human pathogens is well documented. Inhibitory effects have been documented against planktonic bacteria, and honeys (particularly manuka honey) can also interfere with bacterial quorum sensing and damage both single-species and multi-species biofilms (110). For decades, the antimicrobial activity of honey was attributed to its H_2O_2 content. In the past 30 years, however, components of honeys, from foraged phytochemicals and from endogenous secretions, have been identified that confer antimicrobial properties beyond those attributable to H_2O_2. Among bee-derived constituents are five major royal jelly proteins (82), the antimicrobial peptide (AMP) hymenoptaecin (46) and the AMP defensin-1 (42). Defensin-1, produced by nurse bee hypopharyngeal glands, is active against *Bacillus subtilis*, *Staphylococcus aureus*, and *Paenibacillus larvae* (American foulbrood) (144). The royal jelly proteins, also produced by the hypopharyngeal gland, comprise most of the proteins found in honey (42, 126). The most abundant of these, MRJP1, yields three jelleins, AMPs that cause cell wall lysis and death in bacteria and are thus likely responsible for much of the bactericidal activity of honeys (19).

With respect to bee health, assays of antimicrobial activity of honeys against ecologically relevant bee pathogens are few in number. Erler & Moritz (47), for example, demonstrated inhibition by polyfloral honey of both American foulbrood (*P. larvae*) and European foulbrood (*Melissococcus plutonius*) bacterial strains, as well as strain-specific inhibition by sunflower and black locust honeys. Nafea et al. (100) tested four monofloral honeys [*Citrus* spp., a clover (*Trifolium alexandrium*), a cotton (*Gossypin* [sic] *barbadens*), and camphor] against American foulbrood and found variation in efficacy, with clover and cotton showing the highest inhibitory activity.

The antimicrobial activity of honey phytochemicals has historically been documented in assays with human pathogens (144), so its ecological relevance to honey bee health is not always clear. Among the phytochemicals associated with antimicrobial activity are phenolic acids, flavonols, flavanones, flavones, and isoflavones (110). In terms of activity of honey phytochemicals against

bee pathogens, Bernklau et al. (12) examined the effects of caffeine, gallic acid, kaempferol, and *p*-coumaric acid on adult bees infected with the microsporidian *Nosema ceranae* and reported that, with the exception of gallic acid, all decreased spore loads relative to controls, particularly at low concentrations.

Because, over the course of a season, bees produce and store honeys from a wide range of nectars, the potential exists for bees to self-select optimal honey types to manage microbial challenges. Nurse bees infected with the microsporidian parasite *N. ceranae* presented with a choice of single-source honeys (linden, black locust, honeydew, sunflower) selectively consumed sunflower honey, which had the highest antimicrobial activity and which, if consumed exclusively, reduced the prevalence of the parasite after only six days (52). Although the specific constituents responsible for the antifungal properties were not identified, Gherman et al. (52, p. 1782) ruled out sugar profile and viscosity and speculated that "it may well be the diversity of honey stores that facilitates colony-level immunity against the full spectrum of pathogens the colony is exposed to." Among the flavonoids found in sunflower honey are pinocembrin; pinobanksin; chrysin; galangin; quercetin; and, in smaller quantities, tectochrysin and kaempferol (129). Several of these compounds, alone and in combination, exhibit pronounced antifungal and antibacterial activity (132) and may contribute to the efficacy of this honey against *Nosema* infection.

In general, antifungal activity of honey is associated with phytochemicals that likely derive from propolis. Of the main phytochemicals reported in honey in northern latitudes—pinobanksin, pinocembrin, quercetin, chrysin, and galangin (72)—few if any occur in floral nectars. All, however, are common in propolis (130). The major phenolic compounds identified in honey are flavonoids and include flavonols (quercetin, kaempferol, galangin), flavanones (pinocembrin, pinobanksin), and flavones (chrysin, luteolin) (72, 123). These compounds are typical of temperate zone propolis and are associated with fungistatic properties (133). Propolis from tropical regions differs in composition, containing primarily prenylated derivatives of *p*-coumaric acid and flavonoids, as well as caffeoylquinic acid derivatives, also associated with fungistatic properties (87).

Although propolis is not known to be ingested per se (138), the fact that common constituents of propolis are shared among a wide variety of honeys across geographic regions (146) suggests that ingesting honey may represent a major route of consumption by bees of propolis-derived compounds. Many of the most common propolis-derived constituents of honey are water soluble; in addition, although propolis is itself deterrent to bees, when an extract is added to sugar candy, palatability increases markedly, and it is "avidly consumed" (38).

IMMUNITY-BOOSTING ACTIVITY OF HONEY CONSTITUENTS

Central to insect humoral immunity are the AMPs, regulated by the IMD and Toll pathways, which protect against a diversity of pathogens, including viruses, bacteria, fungi, and protozoa (96, 116). Several honey constituents upregulate multiple AMPs; *p*-coumaric acid, for example, upregulates apidaecin in adult honey bees (92) and five additional AMPs in three-day-old larval bees up to 25-fold (93). Among other immunity genes upregulated by *p*-coumaric acid were *LYZ*, induced 1.75-fold; *b-1,3-glucan recognition protein*, upregulated 1.68-fold; and *peptidoglycan recognition protein S2*, upregulated 1.87-fold.

Palmer-Young et al. (116) similarly showed that six phytochemicals—amygdalin (cyanogenic glycoside), anabasine, nicotine (alkaloid), aucubin, catalpol (iridoid glycoside), and thymol (terpene)—increased expression of AMP genes 12.9- to 61-fold in older bees after one week of consuming them, with most upregulating hymenoptaecin. The functional significance of this upregulation is illustrated by up to 99.8% reductions in deformed wing virus after less than a day of phytochemical consumption.

Abscisic acid, a plant hormone, is found in many honeys. Heather honey (*Erica* spp.) contains two isomers, *cis*,*trans*-abscisic acid and *trans*,*trans*-abscisic acid, with total concentrations ranging from 2.5 to 16.6 mg/100 g honey. Both isomers are abundant in *Erica* flowers (102). Consumption of abscisic acid contributes to colony health in several ways, including by elevating endogenous levels of ABA in the bodies of bees, by stimulating hemocyte response to nonself recognition, by enhancing wound healing and activation of granulocytes and plasmatocytes, and by increasing pesticide resistance.

HONEY CONSTITUENTS AND TOXIN TOLERANCE

Honey consumption by bees has been demonstrated to enhance tolerance of ingested natural and synthetic toxins. Relative to consuming high-fructose corn syrup or sucrose, consuming honey enhanced survival of adult bees in the presence of aflatoxin B1, a mycotoxin produced by *Aspergillus* (109). Ingestion of extracts of honey, pollen, or propolis upregulated *CYP6AS* genes encoding enzymes that metabolize quercetin (69, 90) and *CYP9Q* genes encoding enzymes that metabolize quercetin, the acaricides coumaphos and bifenthrin (91), and neonicotinoid insecticides (89).

The specific constituents in honey extract that induce CYP9Q3 expression (92) include three phenolic acids (caffeic, cinnamic, and *p*-coumaric acids), a flavone (chrysin), a flavonol (galangin), and two flavanones (naringenin, pinocembrin). The phenolic acid *p*-coumaric acid is known not only from a variety of honeys, often as a major constituent [e.g., buckwheat (117)], but also from propolis (4). RNA sequencing (RNA-Seq) analysis of adult workers (92) and larvae (93) revealed that *p*-coumaric acid upregulates a suite of detoxification genes, including multiple CYP6 and CYP9 genes in both larvae and adults, as well as esterase, transferase, and transporter genes in adults. In nurse midguts, CYP6AS genes were upregulated 1.90- to 3.11-fold by *p*-coumaric acid, and CYP9Q3 was upregulated 2.55-fold; in three-day-old larvae, six CYP6AS genes were upregulated 1.9- to 45-fold, and six CYP9 genes were upregulated 1.46- to 3.12-fold. In a separate study (94), RNA-Seq analysis of gene expression in larvae reared for three days on diets with low and high levels of quercetin revealed that, among the 28 P450 genes in the CYP3 Clan (to which the principal detoxifying P450s belong), seven were upregulated by both levels of quercetin, two (CYP6AS17 and CYP9R1) were upregulated by the low quercetin treatment, and four (CYP6AS1, CYP9Q1, CYP9Q2, and CYP9Q3) were upregulated by the high quercetin treatment.

That phytochemical upregulation of these P450s has functional significance in enhancing metabolism of pesticides has been determined in several studies. Consumption of *p*-coumaric acid in a sucrose-based diet increased midgut metabolism of coumaphos by approximately 60% (91). Liao et al. (84) showed enhanced longevity of adult bees consuming two pyrethroid pesticides in combination with either *p*-coumaric acid or quercetin, and Wong et al. (156) demonstrated that both compounds enhance longevity of adult bees consuming imidacloprid, although the response was biphasic, with a negative effect at higher concentrations. Mitton et al. (97) supplemented bee diet with *p*-coumaric acid and indole-3-acetic acid to determine the impact of these acids on adult survival and capacity of adult bees to tolerate tau-fluvalinate. Supplementation with either compound led to an approximate 20% increase in survival of bees exposed to tau-fluvalinate, with *p*-coumaric acid increasing activity of both cytochrome P450s and glutathione reductase in treated and control bees.

In addition to upregulating immunity genes, ingested abscisic acid (ABA) can increase tolerance of adult honey bees to carvacrol, a monoterpenoid phenolic used as an antibacterial agent by some beekeepers, and oxalic acid, used by beekeepers as an acaricide (103). Consumption of ABA by newly emerged and three-day-old nurse bees raised LC_{50} values for oxalic acid twofold to

10-fold. Because supplementation with ABA increased catalase activity by 40%, Negri et al. (103, 104) suggested that ABA acts to reduce toxicity via enhancing catalase activity, which may counteract reactive oxygen species generated by toxins.

HONEY CONSTITUENTS AS REGULATORS OF DEVELOPMENT

Caste determination in honey bees is influenced by diet; whereas queen-destined female larvae receive no food other than royal jelly (a blend of glandular secretions) from nurse bees, worker-destined larvae are fed a form of jelly for their first three days of life and subsequently receive jelly mixed with honey and beebread. Phytochemicals in these plant foods thus have the potential to influence developmental pathways. Adding *p*-coumaric acid to a royal jelly diet during in vitro larval rearing yields female adults with incomplete ovary development (93). This inhibition of ovary development may result from alteration of gene expression by *p*-coumaric acid. In the hippo signaling pathway, which is involved in organ size regulation, more than half of the 46 pathway genes were differentially regulated in larvae consuming *p*-coumaric acid, with 7 of 21 genes upregulated by more than twofold. Also upregulated from 1.3- to 2.7-fold were 14 genes involved in worker–queen caste differentiation; major royal jelly proteins were downregulated by *p*-coumaric acid ingestion relative to control diet (with up to 6.6-fold downregulation of the MRJP1, or royalactin, gene).

In addition to *p*-coumaric acid derived from plants, honeys also contain plant microRNAs (58). Zhu et al. (161) reported that consumption of plant microRNAs by larvae reduced development rate, ovary size, and body size and thereby promoted development into workers rather than queens via a form of RNAi castration. Although the principal source of dietary microRNAs appears to be the pollen in beebread, some contribution to developmental regulation of microRNAs present in the honey incorporated into beebread has not been definitively ruled out.

HONEY CONSTITUENTS AND OVERWINTERING SURVIVAL

Stored honey is essential to the survival of overwintering honey bees in temperate climates, and there is some evidence that honey constituents other than carbohydrates promote cold tolerance. Supplemental ABA increases both the innate immune response and overwintering survival of honey bee colonies at cold temperatures (25° C) that otherwise reduce survival by almost half relative to standard temperatures (34° C) (102). Supplemental ABA also accelerated development in larvae experiencing cold stress, possibly by elevating transcription of Hex7b, as well as vitellogenin (vg) and heat shock protein 70 (hsp70), both of which are cold stress–responsive (124). Negri et al. (103) suggested that ABA coordinates stress responses, including cold exposure and wounding, through the Toll pathway.

CONCLUSIONS AND FUTURE RESEARCH OPPORTUNITIES

Based on the nature of the honey that they produce, honey bees are unique among animals in their ability to process nectar and package it for long-term storage. That said, identifying a genomic signature of honey making remains an elusive goal, as does understanding the evolution of the distinctive behaviors and physiological adaptations associated with the process. Moreover, much of the received wisdom about honey is not well supported by an abundance of literature. An evaluation of bee genomes indicates that activity historically attributed to invertase in honey making may actually be produced by α-glucosidase and that to some extent acid phosphatase and catalase activity might be due to endogenous, rather than exogenous, enzymes. Moreover, although

honey constituents can have striking impacts on individual bee behaviors, including learning and memory (114), colony-level impacts of such behavioral effects are rarely assessed, and mechanisms underlying short-term behavioral responses are not easily elucidated with genomic tools.

A limitation of the honey chemistry literature is that it is dominated by efforts to identify unique constituents for authenticating floral origin and by attempts to characterize individual constituents responsible for a particular type of biological activity. Thus, little attention has been paid to interactions among the hundreds of honey constituents already identified and to potential synergistic or antagonistic effects among constituents, which may derive not only from flowers but also from the bees themselves, from plant-derived resins in propolis, and possibly from bee-associated microbiomes. The diversity of the biological activities of honey depends not only on the diversity of the phytochemicals that bees collect from the environment, but also on the interactions among those phytochemicals and chemicals of non-plant origin, and virtually no studies have tested for interactions among these hundreds of co-occurring substances.

Understanding how phytochemical diversity affects the beneficial effects of honeys on the health of both individual bees and the colony as a whole has implications for the future of apiculture. Due to intensification of agricultural monocultures, urbanization, and other forms of habitat degradation, honey bees are often unable to find sufficient plant resources to thrive and, when pressed, will collect and consume a variety of human-produced substances that differ dramatically in composition from their natural foods (28, 121). Moreover, beekeeping practices that substitute sucrose or high-fructose corn syrup for honey in times of nectar dearth may affect bee health by altering expression patterns of multiple genes involved in protein metabolism and oxidation reduction relative to honey (150). Even if sugars are similar between natural and human foods, the absence of a honey phytochemical profile may have consequences with respect to maintaining immunity, detoxification, and thermoregulatory capabilities. Because resin collection is limited to a narrower range of plant sources than is nectar, the absence of suitable sources can also lead to potentially maladaptive behavior, including collecting asphalt to incorporate into propolis (6); the effects of the presence of asphalt constituents and the absence of resin constituents that normally are incorporated into honey have not yet been assessed.

In summary, honey is integrated into virtually all aspects of the lives of honey bees; more importantly, its composition has the potential to influence or ameliorate the most persistent problems that have afflicted contemporary apiculture for the past three decades—namely, pesticides, pathogens, parasites, and poor nutrition. Understanding how honey bees utilize honey as a functional food can have significant dividends in improving honey bee health and provide new insights into the importance of food storage in social evolution.

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