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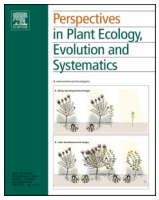

Floral nectar: Fifty years of new ecological perspectives beyond pollinator reward

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ABSTRACT

Floral nectar is central to ecology, since it mediates interactions with pollinators, flower-visiting antagonists and microbes through its chemical composition. Here we review how historical assumptions about its ecological meaning were first challenged, then modified and expanded since the discovery of secondary metabolites in nectar. We then explore the origin of specific neuroactive nectar compounds known to act as important insect neurotransmitters, and how advances in the field of bee cognition and plant-microbe-animal interactions challenge such historical views. As all actors involved in the latter interactions are under simultaneous reciprocal selective pressures, their coexistence is characterized by conflicts and trade-offs, the evolutionary interpretation of which suggests exciting new perspectives in one of the longest studied aspects of plant-pollinator interactions.

1. Fifty years of ecological perspectives

The scientific definition of nectar, first given by Linnaeus in 1735, sees nectar as the secretion of specific organs, the nectaries. Specifically, the secretion of *floral* nectar is associated with the plant reproductive structures (Nepi, 2017), and rewards animals that may perform pollination while visiting the flower (Nepi et al., 2018). Whether pollinator attraction was the primary driver leading to nectar appearance is a difficult question to answer (e.g. Sprengel, 1793; Caspary, 1848; Bonnier, 1878). Nevertheless, its centrality in mediating plant-animal interaction is nowadays undeniable, and was somehow recognized as early as the first century BC, when the poet Virgil (Georgics part IV, 149–227) used the term "nectar" to refer to the substance that honeybees collect from the fields and store in combs as honey.

By virtue of its carbohydrate and amino acid content, nectar has been considered a readily absorbable, cost-effective, alimentary reward offered by plants in exchange for the pollination service mediated by animals (Nicolson, 2007; González-Teuber and Heil, 2009; Heil, 2011). Up to 90% of its dry weight consists of sugars (Lüttge, 1977), while the other 10% includes a plethora of other components such as amino acids, inorganic ions, proteins, lipids and organic acids (Nicolson and Thornburg, 2007). Amino acids and inorganic ions are the most abundant classes after that of sugars (e.g. Lüttge, 1961; Mostowska, 1965; Göttlinger et al., 2019). Though much less abundant than in pollen, the role of amino acids in enhancing nectar nutritional value (e.g. Jervis and Boggs, 2005), contributing to its taste and attractiveness (e.g. Gardener and Gillman, 2002; Carter et al., 2006), and affecting the foraging choices of pollinators (e.g. Seo et al., 2019) is well known. A historical listing of European scientists who reported amino acids in nectar between the 1950 s and the 1970 s is given by Baker and Baker (1975). The inorganic ion content of nectar, on the contrary, is often overlooked, in spite of the fact that minerals and ions may play a crucial role in maintaining salt balance in nectar feeding animals (e.g. Hiebert and Calder, 1983; Nicolson and Fleming, 2003).

If discussion is limited to these main classes of nectar chemicals, the ecological significance of nectar could easily be confined to its being an alimentary reward involved exclusively in pollinator nutrition and attraction. However, since the early 1970 s the discovery of a series of nectar chemicals (Barberis et al., 2023a and references therein) not involved in primary metabolic pathways (Baker and Baker, 1977, 1986), and therefore considered secondary metabolites (Pichersky and Gang, 2000), has challenged this traditional view. Back then, most advances in

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nectar chemistry sprang from the pioneering work of Baker and Baker, who were also the first to speculate on the function of these so-called "unfavourable substances" (Baker and Baker, 1975). They postulated what is nowadays known as the "pollinator fidelity" hypothesis, which states that secondary metabolites in nectar discourage flower-inconstant insects from visiting the flowers, while favouring specialist visitors (Adler, 2000). This hypothesis relies on the assumption that specialists are more effective pollinators than generalists and deliver more intraspecific pollen (Rhoades and Bergdahl, 1981; Adler, 2000 and references therein). Since the 1970 s, the main traditional alimentary function of floral nectar has therefore been flanked by a second crucial role suddenly recognized by scholars: that of discouraging those nectar consumers who contribute little or nothing to the pollination service.

This hypothesis was later expanded by Janzen (1977) and Baker (1978), who proposed that nectar secondary metabolites might deter nectar thieves, such as ants. This further interpretation, which many researchers agree to consider an extension of the "pollinator fidelity" hypothesis, was later called the "nectar robber" hypothesis (Adler, 2000). The results of Stephenson (1982) showed that the floral nectar of *Catalpa speciosa,* which contains iridoid glycosides, is only moderately consumed by nectar thieves, despite its abundance and accessibility. Shortly after consuming the nectar, thieves appear intoxicated and behave abnormally (Stephenson, 1981), whereas insects acting as legitimate pollinators do not show any effect after ingestion. While some studies seem to confirm the hypothesis, others show that in most cases the deterrent effect against ants is conferred rather by mechanical adaptations (Feinsinger and Swarm, 1978; Schubart and Anderson, 1978; Guerrant and Fiedler, 1981).

In line with the idea that secondary metabolites prevent nectar exploitation by inefficient floral visitors, it was then postulated that they may prevent nectar waste by microbes (e.g. Hagler and Buchmann, 1993; Verpoorte and Schripsema, 1994). Indeed, by virtue of its sugar and amino acid composition, nectar has long been recognized as a potentially rich medium for microbial growth (e.g. Boutroux, 1884; Schuster and Ůlehla, 1913; Grüss, 1917; Schoelhorn, 1919). Curiously, nectar proteins – compounds nowadays known to protect nectar against proliferation of microorganisms and plant tissues against infection by pathogens (Carter et al., 2007; Hillwig et al., 2010; Nepi, 2017 and references therein) – were discovered more than 90 years ago (Buxbaum, 1927), but were initially thought to be nutritional, supplying nectar consumers with organic nitrogen (Lüttge, 1961; Heil, 2011).

Thus the so-called "antimicrobial" hypothesis (Adler, 2000) received more support some years later, when more experiments conducted in this direction provided evidence of it. For example, Montenegro et al. (2012) described that honey obtained from *Quillaja saponaria* exerts biological action against pathogens associated with the phenols found in the floral nectar of the plant, suggesting an antimicrobial effect of these compounds.

Initially considered "unfavourable" (Baker and Baker, 1975), nectar secondary metabolites were often later regarded as toxic. With reference to nectar non-protein amino acids, Baker and Baker were again the first to suggest that some nectar secondary metabolites could be toxic to certain flower-visitors (1977). In the same decade, a series of studies demonstrated the potential toxicity of several secondary metabolites for animal consumers in plant-herbivore interactions. Such toxicity was described to have deleterious post-ingestive effects on growth (e.g. Blau et al., 1978; Isman and Duffey, 1982), organ function (e.g. Berenbaum, 1988) and nutrient uptake (e.g. Slansky, 1992). These findings led to reconsideration of the early assumption that the benefits of nectar secondary metabolites must outweigh their cost, and the idea of direct selection of nectar toxins was momentarily obscured by the conjecture that their presence was due to prior selection pressures or pleiotropic constraints and that there were no adaptive functions in relation to pollination, or only after defense (Adler, 2000; Stevenson et al., 2017). In this pleiotropic perspective, nectar chemistry is originally determined by co-evolutionary interactions with herbivores, and the occurrence of "unfavourable" substances in floral nectar is accidental and, all things considered, a small cost to pay in exchange for defence of the plant against the major threat of herbivory. Though it cannot be excluded, this theory does not account for the significant differences in chemical composition often observed between nectar and phloem sap (Roy et al., 2017). This consideration is just one of several aspects that gradually advanced the formulation of new hypotheses.

Since the studies conducted to assess the toxicity of the secondary metabolites found in nectar were performed with concentrations equal to those found in vegetative tissues (generally greater than those found in nectar, e.g. Adler et al., 2006; Wiese et al., 2018), a new interpretation of the ecological role of these secondary metabolites emerged in the 1980 s. This new interpretation was based on the hypothesis that nectar-like concentrations of these potentially toxic secondary metabolites could indeed prove toxic for insect pathogens but not for their presumably less susceptible hosts. A series of pioneering studies conducted in this direction gave rise to the conviction that their ingestion by nectar consumers may improve the health and life expectancy of the latter (e.g. Price et al., 1980; Berenbaum, 1988). This new perspective attributing curative benefits to secondary metabolites revives the concept that the presence of these compounds in nectar must outweigh the costs associated with their consumption.

In support of this latter view, it is now well established that the putative toxicity of certain secondary metabolites in nectar often depends on the sensitivity of the nectar consumer (e.g. Tiedeken et al., 2016), or may be greater for introduced species that are not native pollinators of the plants in question (e.g. Zhang et al., 2022). Moreover, as stated above, nectar concentrations are generally lower than those found in other plant tissues (e.g. Cook et al., 2013; Palmer-Young et al., 2019), and the combined effects of two or more compounds may enhance or erase their separate effects (e.g. Muth et al., 2022). In general, pollinators may therefore benefit from consuming nectars rich in secondary metabolites which may reduce their pathogen loads, enhance their immune response or even enrich their gut microbiota (Gunasekaran et al., 2020, Baracchi et al., 2022), in line with what may be called the "medication" hypothesis. A growing number of recent studies supports this view. For example, nectar alkaloids such as gelsemine, anabasine, and nicotine benefit pollinators by increasing their resistance to parasites and pathogens (Manson et al., 2010; Richardson et al., 2015; Thorburn et al., 2015), and the idea that bees may actively seek alkaloid-enriched nectar to keep pathogens at bay (Gherman et al., 2014) has become popular. At least until recently, such active search beaviour has been explained by homeostasis, an impulse to seek a certain compound occurring when levels of the compound are low in the animal's its body (Samorini, 2013). The fact that consumption of a potentially curative compound does not produce immediate healing induced Samorini (2013) to reject the idea that ingestion of curative substances is ruled exclusively by homeostasis, but rather suggests some degree of "awareness" or "intention".

Nowadays, it has finally been established that vertebrates and invertebrates self-medicate, and a growing number of studies provide evidence of this (Hutchings et al., 2003; de Roode et al., 2013; Abbott, 2014, and references therein). Self-medication implies that the exposure of healthy animals to secondary metabolites has a cost, compensated by its beneficial effects in reducing symptoms or clearing infections (Clayton and Wolfe, 1993; Lozano, 1998; Abbott, 2014 and references therein). For example, Singer et al. (2009) found that when parasitized caterpillars of the woolly bear moth *Gramnia incorrupta* ingest plant pyrrolizidine alkaloids, it improves their survival by conferring resistance to tachinid flies, a lethal endoparasite. On the contrary, when unparasitized caterpillars consume excessive amounts of these toxins, it reduces their survival, in line with the theory.

To be fully in line with the key criteria defining self-medication, an animal must however also modify its dietary preferences, addressing its foraging to a source containing "nonnutritive" antimicrobial compounds when parasitized (Karban and English-Loeb, 1997). Again, taking the

study case of Singer et al. (2009) as example, parasitized are more likely than unparasitized caterpillars to ingest large amounts of pyrrolizidine alkaloids, indicating that infection indeed induces a change in their feeding behaviour.

Some of the secondary metabolites found in nectar share structural similarity with important neurotransmitters (Verpoorte, 2005); this observation supports the hypothesis that their presence in nectar outweighs any costs associated with their consumption. As early as the 1970 s, the idea that compounds such as alkaloids, glycosides and phenols could have a significant effect on the central nervous system of flower visitors had already been suggested (Baker and Baker, 1975, 1977). If a certain chemical can modulate neuron signal transduction, the concentrations of neurotransmitters and the activity or expression of their receptors may vary, radically changing animal behaviour (Wink, 2018). When ingestion of nectar secondary metabolites has pharmacological effects on the brain of nectar consumers, these substances have sometimes been considered drugs. For example, Wright et al. (2013), considered the nectar alkaloid caffeine in their study in this manner, providing the first evidence of its capacity to alter a pollinator's memory of reward pharmacologically. Indeed, honeybees treated with caffeine were shown to be three times more likely to remember a learned floral scent than those rewarded with sucrose alone.

It is a complex task to define a certain substance as a drug. Historically, drugs have been referred to as "nervous foods" (Mantegazza, 1871), bringing two aspects into focus: i) they often interfere with animal nervous systems at various levels, and ii) it is difficult to draw a distinct line between food and drugs. Various criteria can be used for this definition. For example, the sharpening of specific senses or the onset of addiction (Samorini, 2013), the only aspect commonly shared being changes in animal behaviour (Wink, 2018). Addiction can manifest in various ways, but generally implies that the consumer craves the chemical once its serum levels drop (Wink, 2018). Often it also implies consumption despite adverse consequences and perceptual changes in reward strength (Koob, 2015; Fattore and Diana, 2016). The key drivers of addiction are reflected in altered expressions of motivation and learning, capacities that emerged early in the Precambrian (Menzel and Benjamin, 2013), so that recent views frame addiction as a phenomenon with deep evolutionary roots, wide spread among invertebrates (van Staaden et al., 2018).

Cases in which consumption of nectar secondary metabolites implies a cost for the nectar-feeding animal – contrary to the instinct of conservation – were initially considered incidental, but observation of repeated feeding on inebriating sources raised the question of how "intentional" the ingestion of these compounds is. The hawkmoth *Manduca quinquemaculata*, for example, feeds on nectar of *Datura meteloides*, a plant belonging to the family Solanaceae, the nectar of which probably contains the hallucinogenic substances also found in the plant's other tissues (Grant, 1983). These compounds intoxicate insects, making them sluggish and disoriented (Grant, 1983). When moths remain on the ground, they are more exposed to predation (Grant, 1983). Bees exposed to hallucinogenic or narcotic substances offered by orchids such as *Epipactis helleborine* are a similar case (Jakubska et al., 2005). An ecological explanation could be that inebriating compounds in floral nectar may enhance the chance of pollination by detaining the animals (Jakubska et al., 2005).

A recent study by Galpayage Dona et al. (2022) provided first evidence that bumblebees engage in activities not directly aimed at satisfying a primary need. Despite of the absence of external incentives, bees repeatedly engaged in rolling wooden balls, suggesting that this activity – fully ascribed to play – is rewarding in itself, an aspect in line with the criteria defining play. This finding, along with a series of other studies, marks a breakthrough in the field of insect behaviour, since it provides additional evidence of the existence of a form of sentience in bumblebees (e.g. Bateson, 2014; Held and Špinka, 2011; Solvi et al., 2016; Birch, 2020). This in turn raises the question of whether the search for hallucinogenic/inebriating substances may also be rewarding in itself, and whether returning to such nectar sources may be dictated exclusively by the insurgence of physical dependence.

Other coercive mechanisms not necessarily implying intoxication are known, for instance that of offering nectar containing nicotine: after experiencing such nectar, bees keep returning to the food source even when it becomes suboptimal compared to other available rewards (e.g. Baracchi et al., 2017a, 2017b). In line with the prediction of Rhoades and Bergdahl (1981), detaining the pollinator in this case may increase mobilization of conspecific pollen, promoting the plant's reproductive fitness.

The above examples suggest potential harmful behaviour of floral visitors as a consequence of nectar ingestion. Such cases frame the concept of pollinator manipulation, a term that researchers have used since the early 2000 s (e.g. Biernaskie and Cartar, 2004; Bayleis et al., 2007), and which gained full recognition after formal introduction of the "manipulation" hypothesis by Pyke (2016), later structured by Nepi et al. (2018). However, to be fair, with reference to various nectar secondary metabolites, as early as 1981 Rhoades and Bergdahl wrote: "though at first sight the presence of these toxic substances seems incompatible with the reward function of nectar, they probably represent a mechanism to manipulate pollinator behaviour to the advantage of the plant and to exclude nectar thieves". They guessed that a combination of rewarding and defensive chemicals could model insect visiting patterns to favor plant fitness beyond the pollinator fidelity hypothesis.

Along with those secondary metabolites that have strong biological activities due to their structural relationship with animal neurotransmitters (Verpoorte, 2005), an additional case concerns nectar chemicals that are environmental sources of invertebrate neurotransmitters per se. This is the case of biogenic amines (Roeder, 1999; Blenau and Baumann, 2001; Scheiner et al., 2006; Farooqui, 2012), a class of compounds only recently reported in floral nectar (Muth et al., 2022; Barberis et al., 2023b; Barberis et al., 2023c). The two biogenic amines reported in floral nectar to date are tyramine and octopamine, the invertebrate counterparts of the vertebrate adrenergic transmitters, ruling the so-called fight or flight response, which is to say rapid adaptation to energy-demanding situations (Roeder, 2005). They are decarboxylation products of the amino acid tyrosine and although tyramine is the biological precursor of octopamine, the twoare considered to act as independent neurotransmitters (Roeder, 2005). Their consumption can modulate a plethora of behavioural traits such as motivation (e.g. Farooqui, 2012), reward-seeking (e.g. Schulz and Robinson, 2001; Peng et al., 2020), locomotion (e.g. Fussnecker et al., 2006; Hardie et al., 2007), learning (e.g. Mercer and Menzel, 1982; Hammer and Menzel, 1998) and social communication (e.g. Barron et al., 2007; Linn et al., 2020).

Regarding their effects on food-source communication and exploitation, for example, octopamine was demonstrated to increase the likelihood of honeybee dancing (Barron et al., 2007), while lowering the probability that bees heed social information from other foragers (Linn et al., 2020). This means that even if the food source is poor, bees are more likely to retain their personal information than to heed indications of a richer source. This evidence supports the hypothesis that nectar octopamine increases bee faithfulness to a plant species favouring the latter's reproductive success. Octopamine was also demonstrated to regulate foraging behaviour in honeybee colonies, inducing a change in the type of food source to which foragers direct their collection activity, with a trend towards directing their efforts to less valuable resources (Giray et al., 2003). This phenomenon may be due to the fact that consumption of octopamine and tyramine lowers the sucrose concentration necessary to elicit the proboscis extension reflex; in other words it increases bee perception of the value of a food source (e.g. Pankiw and Page, 2003; Mc Cabe et al., 2017). Regarding locomotion, an exploratory study investigating the effect of nectar-like concentrations of tyramine on bumblebee flower visits showed that bees fed tyramine-enriched solutions spent less time foraging on a single flower

than those fed control solution, suggesting that their behaviour was on the whole more dynamic (Barberis et al., 2023b).

2. Beyond pollinator reward: the role of microbial interaction

So far, nectar-mediated interactions have been described as a bipartite phenomenon between plants and floral visitors, whereas the way secondary metabolites wind up in nectar has been neglected. This aspect is in fact still largely unclear (Heil, 2011 and references therein). Along with the discovery of nectar secondary metabolites back in the 1970 s, this question has resurfaced. One of the main hypotheses explaining the presence of secondary metabolites in floral nectar stated that nectaries secrete almost unmodified substances that flow directly or indirectly via the vascular tissues by passive diffusion (Lüttge, 1977, Fahnn, 1988). Today, several examples have shown that the chemical composition of nectar is usually quite different from that of phloem (e.g. Bertazzini and Forlani, 2016), and different mechanisms of fine-tuned nectar secretion have been described (e.g. Radhika et al., 2010; Lin et al., 2014). However, most of the models of floral nectar secretion, for example the so-called apoplastic, merocrine and eccrine models, focus mainly on the alternative processes of secretion of the nectar sugar component (Roy et al., 2017 and references therein), while the specific mechanisms ruling transport and secretion of other metabolites are still largely unknown.

Beyond this aspect of the knowledge gap, it is now well established that the chemical composition of floral nectar may not only be shaped by phylogenetic constraints but also by ecological drivers (e.g. Nepi et al., 2010, Bogo et al., 2021). Among these it is worth mentioning, for example, interactions with specific guilds of pollinators that may drive selection towards convergent nectar chemistry in unrelated taxa (e.g. Pozo et al., 2015), or interactions with different habitat types (at least in species with wide ecological ranges) (e.g. Farkas et al., 2012) and the influence of human-driven landscape changes such as urbanization, habitat fragmentation and land use (e.g. Tew et al., 2021; Biella et al., 2022). As habitat type and landscape can impart specific local microclimatic characteristics and influence animal communities, both can extensively affect nectar availability and chemistry, not only at the secretion stage, but also through post-secretion modifications, presumably influenced by meteorological conditions (e.g. Corbet et al., 1979; Plowright, 1981; Chalcoff et al., 2017; Parachnowitsch et al., 2019) and interaction with floral visitors (e.g. Bogo et al., 2021).

Nowadays, animal visitors are recognized as the principal vectors of bacteria, fungi and other microorganisms to and between flowers (e.g. Brysch-Herzberg, 2004; Herrera et al., 2010; Belisle et al., 2012). However, in many cases, not even when flowers have just opened can they be considered sterile (Aleklett et al., 2014 and references therein). Even before the bud opens, floral nectar often contains bacteria and fungi (e.g. Shade et al., 2013; von Arx et al., 2019), the abundance of which increases over time in individual flowers (e.g. Pusey et al., 2009; von Arx et al., 2019, Morris et al., 2020). Besides bacteria and fungi commonly found in air, soil and other habitats – generally the first to be detected when the flower opens (e.g. Brysch-Herzberg, 2004; Morris et al., 2020) – another commonly found group of microbes is that of flower specialists, which exhibit a range of traits that may be adaptations to nectar environments (e.g. Dhami et al., 2016; Herrera et al., 2010, Pozo and Jacquemyn 2019). Moreover, irrespective of continent or habitat type, microbial colonization was recently demonstrated to occur more frequently than previously believed. For example, Herrera et al. (2009) conducted a quantitative survey to assess how frequently floral nectar contains yeasts and found them in up to 44% of samples, while even greater percentages were obtained by other authors (see Brysch-Herzberg, 2004 and references therein for a list of studies).

On colonization, microbes may therefore modify plant-provisioned nectar chemicals or impart their own by secreting metabolic by-products into the nectar (e.g. Canto and Herrera, 2012; Vannette and Fukami, 2018; Yang et al., 2019; Vannette and Fukami, 2016; Rering et al., 2020). During sugar fermentation, for instance, different volatile organic compounds are released, and additional compounds can be added to the floral olfactory bouquet (Rering et al., 2018). Even in the case mentioned above, that of *Epipactis helleborine*, one of the potentially hallucinogenic/narcotic compounds offered in its floral nectar is ethanol (Løjtnant, 1974; Müller, 1988), which is thought to be of microbial origin (Ehlers and Olesen, 1997; Kevan et al., 1998).

The influence of microbes on floral nectar is mainly regarded as detrimental for its quality (e.g. Eisikowitch et al., 1990, Herrera et al., 2008, Vannette et al., 2013), weakening or negatively interfering with plant-pollinator mutualism. For example, some studies have demonstrated that yeasts reduce the food value of floral nectar by decreasing sugar (Canto et al., 2011; de Vega and Herrera, 2013) and amino acid concentrations (Pozo et al., 2014). In general, floral microbes are rarely believed to benefit plants (Vannette, 2020 and references therein). Flower pathogens and some nectar bacteria can reduce plant fitness, either directly or by decreasing pollinator visits (e.g. Vannette et al., 2013). Other studies, however, have demonstrated that in certain cases microorganisms may enhance pollination by producing volatiles that play a role in attracting pollinators, indirectly influencing plant fitness (e.g. Pozo et al., 2009; Herrera and Pozo, 2010; Cullen et al., 2021). However, even in cases where nectar yeasts increase pollinator visits, this does not necessarily benefit plant fitness. For example, Herrera et al. (2013a) reported reduced seed set in yeast-colonized *Helleborus foetidus* plants despite increased pollinator attraction.

In addition, microbes in nectar not only alter pollinator attraction and visitation through volatile emissions or chemical modification (e.g. Raguso, 2004; Rering et al., 2018, 2020), but their very presence seems to drive a preference for yeast-containing flowers in pollinators such as bumblebees, who were demonstrated to detect them in nectar (Herrera et al., 2013b; Schaeffer et al., 2014; Schaeffer et al., 2017). In this regard, it is suggested that nectar yeast cells supplement insects with important nutritional elements such as vitamins, sterols, and minerals (Vega and Dowd, 2005; Stefanini, 2018). Dharampal et al. (2019) was the first study in this direction and provided evidence of the benefits for honeybee larvae of the diverse communities of symbiotic microbes inhabiting the pollen surface. If pollen microbes are a crucial dietary resource for larval development, it is also likely that the microbial inhabitants of floral nectar are an important nutritional component as well (Jacquemyn et al., 2021). This view is in line with emerging evidence that the nectar microbiome, like that of pollen, may influence the health of pollinators (*sensu* López-Uribe et al., 2020) by modifying their nutritional landscape, altering foraging behaviour, and interacting with their symbionts and pathogens (Martin et al., 2022 and references therein). As consumption of nectar and pollen colonized by consortia of yeasts and bacteria (Pozo et al., 2012; Ambika Manirajan et al., 2016) may provide a regular supplement of the microorganisms that are part of a bee's gastrointestinal flora, it may also affect consumer fitness, and – in the case of social insects – colony development (Dharampal et al., 2019; Pozo et al., 2018, 2020). In their experiment, Pozo et al. (2020) found that yeasts in the bumblebee diet positively affected colony growth of *Bombus terrestris*, though the intensity of the effect depended on the yeast species. Another study by Pozo et al. (2021) showed that feeding bacterial supplements to colonies of *Bombus terrestris* led to faster egg laying, greater brood size and increased production of workers, while combined yeasts and bacteria supplements induced less evident benefits. Examples such as the above provide preliminary evidence in support of the hypothesis that nectar microbes can benefit pollinator health. Further studies are needed to clarify certain aspects. For example, Pozo et al. (2020) also demonstrated that yeasts grown in vitro could suppress growth of the bumble bee gut pathogen *Crithidia bombi*; whether the same action is also exerted in the host gut remains to be assessed.

Since biogenic amines can be generated by microbial decarboxylation of free amino acids, it has been suggested that their presence in floral nectar could be imputed to yeast metabolism rather than to that of the plant (Nepi, 2017; Nepi et al., 2018). To date, however, we still lack

evidence supporting this hypothesis, so the conservative explanation that they are plant by-products stands. By virtue of endogenous enzyme production, tyramine, for instance, can be found in various plant parts or their derivatives (Vazquez y Novo et al., 1989; Preti et al., 2016; Gobbi et al., 2019), seeming ubiquitous and implicated in a number of metabolic pathways of which tyramine – precursor of many other pharmacologically active compounds – is the first product (Facchini et al., 2000). As tyramine can be the product of specific pathways activated in response to attack by various plant enemies (Servillo et al., 2017), the production of biogenic amines may be a general defensive response against pathogens or phytophages (Facchini et al., 2002; Macoy et al., 2015; Knolleberg et al., 2020; Shen et al., 2021; Płonka et al., 2022). In fermented foods and beverages of plant origin, however, its presence is associated with microbes with aminogenic activity (Trivedi et al., 2009). In addition, some biogenic amine-producing microbes found in wine have also been found in floral nectar (Landete et al., 2007; Pozo et al., 2012; Pozo et al., 2016).

3. Knowledge gaps

Today the chemical complexity of nectar is well established, despite the fact that its composition was long assumed to be a constant trait of a species. This assumption encouraged a search for patterns, and justified pooling nectar samples when volumes were insufficient for analyses (Nicolson, 2022). For decades, this approach masked the variability of nectar, its complex physiology and dependence on the environment, all of which make its study extremely challenging (Brandenburg et al., 2009 and references therein). Explanations of the ecological role of nectar in mediating plant-animal interactions are therefore less certain due to variable chemical expression (Stevenson et al., 2017). More insights into the molecular and genetic mechanisms ruling its secretion and composition are therefore needed.

A second level of complexity is represented by recent findings concerning animal cognition. Flower visiting involves perception, memory, expectation, and decision making (Waddington, 2001), all tools known to be influenced by emotional states, at least in humans (e.g. Mathews and MacLeod, 1994; Lerner and Keltner, 2000). In recent years, the scientific community seems to have recognized the existence of emotions in vertebrates such as fish and birds (e.g. Rey et al., 2015; Valance et al., 2008), but also in invertebrates such as insects, for example bees and flies, turn out to fulfil the basic requirements of emotional behaviour (Baracchi et al., 2017a, 2017b and references therein), as well as showing a form of sentience (Galpayage Dona et al., 2022). Several studies have established that insects have high levels of cognitive sophistication (e.g. Avarguès-Weber et al., 2011; Collett et al., 2013; Giurfa, 2013; Klein et al., 2017). These important breakthroughs challenge the way we have been tackling the subject of how floral visitors exploit their floral nectar-landscape (Baracchi, 2019 and references therein). Increasing evidence that insects self-medicate and engage in rewarding activities beyond their primary needs – for pleasure, one would say – is encouraging research in this direction.

Although the importance of having more information on wild pollinators for the purpose of their conservation has been acknowledged (Pegoraro et al., 2020), the effects of nectar secondary metabolites on the great majority of wild pollinators are largely unknown. In the case of wild bees, this is probably a consequence of our limited understanding of how to establish and maintain their nests in laboratory conditions (Leonard and Harmon-Threatt, 2019). So far, research has focused mainly on managed honeybees, bumblebees, and hummingbirds (e.g. Muth et al. and references therein, Stevenson et al., 2017 and references therein, Kessler et al., 2012), despite the fact that pollinators differ vastly in life cycle, sociality, dietary specialization (Muth et al., 2017) and other characteristics. For example, even a simple response, such as eliciting the proboscis extension reflex under laboratory conditions, seems profoundly influenced by the sociality of the bee species (Vorel and Pitts-Singer, 2010). This stresses the importance (when possible) of coupling laboratory work with the study of wild pollinators under natural or semi-natural conditions, also in the light of recent reports that experiments conducted under controlled conditions may not always yield a realistic picture of animal behaviour (e.g. Mujagic and Erber, 2009; Ayestaran et al., 2010).

It is even more important to fill up the knowledge gap concerning the synergic effects of the complex combinations of chemicals found in nectar on pollinator behaviour. Although foliar chemical ecology has highlighted the importance of synergistic effects (Richards et al., 2016) and recent studies have demonstrated that these can lead to unpredicted behaviours (e.g. Muth et al., 2022), nectar chemistry studies on the field of nectar chemistry generally involve the use of one substance at a time (e.g. Wright et al., 2013; Baracchi et al., 2017a, 2017b; Estravis-Barcala et al., 2021; Hernández et al., 2018; Marchi et al., 2021; Richman et al., 2022; Thorburn et al., 2015).

A better understanding of how nectar-like concentrations of combined co-occurring secondary metabolites affect animal behaviour is a prerequisite for assessing how human-induced dispersal of chemicals in the environment may affect plant-pollinator interactions. How nectar secondary metabolites interact with phytochemicals is also still largely unknown, but some first studies have shown that even a single acute exposure to a pesticide can reshape the interactions between plants and floral visitors mediated by nectar secondary metabolites (Richman et al., 2022). This highlights the importance of using realistic concentrations of chemicals, similar to those found in natural nectar.

So far pollinators have been regarded as the main source of selection leading to establishment of given concentrations of secondary metabolites in floral nectar (Stevenson et al., 2017), and current research has outlined how these can also be affected by floral microbes (e.g. McArt et al., 2014, Parachnowitsch et al., 2018, Rebolleda-Gómez et al., 2019; Rivest and Forrest, 2020). Besides circumventing plant defensive mechanisms – such as high concentrations of reactive oxygen species (Thornburg et al., 2003) or proteins with antimicrobial properties (Schmitt et al., 2021 and references therein) – nectar specialized microbes need to colonize new spaces to maintain their populations (Morris et al., 2020), as the flowers where they live generally have short lifespans (e.g. Primack, 1985). To do this, it has been suggested that microbes may affect flower traits and influence their own dispersal (Russell et al., 2019; Vannette, 2020; Francis et al., 2021 and references therein). However, a part from a few examples like that of the fungal pathogen *Fusarium moniliforme,* which enhances bird visitation for spore dispersal (Lara and Ornelas, 2003), there is little evidence that microbial species rely on floral visitors to maintain their populations. This means that further research is needed to verify what we may call the "manipulation-for-dispersal" hypothesis.

Finally, although floral microbes are rarely believed to benefit plants, a few cases of plants with adaptations to promote microbial growth in their flowers are known. For example, Wiens et al. (2008) suggest that the palm *Eugeissona tristis* may encourage the growth of ethanol-producing yeasts by selecting mammal pollinators that consume fermented nectar while discouraging less specialized ones. Though still an untested hypothesis, if floral microbes could enhance plant fitness by promoting compounds such as exogenous insect neurotransmitters in nectar, then further research should examine the potential for plant-chemical adaptation to facilitate microbe colonization of nectar. In other words: may plants show chemical adaptations of nectar that promote microbial settlement in the flower that optimizes pollinator attraction?

4. Concluding remarks

The recently established role of microorganisms as third partners in nectar-mediated, plant-animal interactions adds considerable complexity to our attempts to elucidate the ecological functions of floral nectar (Stevenson et al., 2017; Nepi, 2017). All actors involved in plant-microbe-pollinator interactions are under simultaneous reciprocal

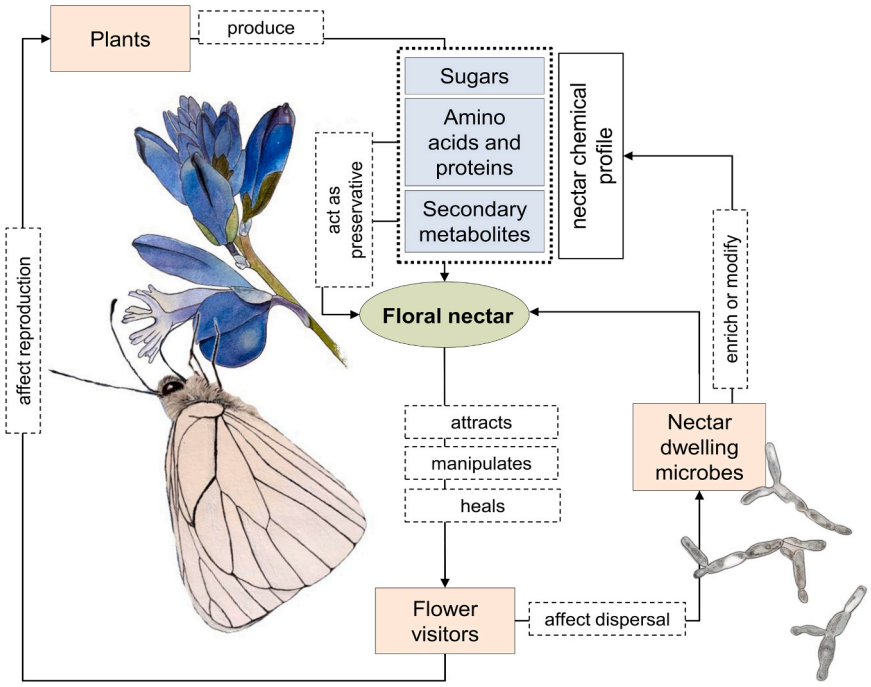

Fig. 1. Network of complex nectar-mediated plant-pollinator-microbe relationships.

selective pressures (Fig. 1). Plants must ensure pollinator visitants and protect their floral alimentary resources against microbial exploitation, while flower-specialized microbes employ nectivores to disperse between hostplants. Such conflicts and trade-offs, coupled with recent advances in chemical ecology and bioinformatics, open many exciting avenues for research in one of the longest studied aspects of plant-pollinator interactions.

Declaration of Competing Interest

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Data availability

No data was used for the research described in the article.

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