INVITED REVIEW

New perspectives in nectar evolution and ecology: simple alimentary reward or a complex multiorganism interaction?

Massimo Nepi*

Department of Life Sciences, University of Siena, Via P.A. Mattioli 4, 53100 Siena, Italy

* Email: massimo.nepi@unisi.it

Abstract

Floral and extra-floral nectars are secretions elaborated by specific organs (nectaries) that can be associated with plant reproductive structures (the so-called floral nectaries found only in angiosperms) or vegetative parts (extrafloral nectaries). These secretions are common in terrestrial vascular plants, especially angiosperms. Although gymnosperms do not seem to have true nectar, their ovular secretions may share evolutionary links with angiosperm nectar. Nectar is generally involved in interactions with animals and by virtue of its sugar and amino acid content, it has been considered a reward offered by plants to animals in exchange for benefits, mainly pollination and indirect defense against herbivores. These relationships are often cited as examples of classical mutualistic interactions. Nonetheless, recent studies dealing with compounds less abundant than sugars and amino acids challenge this view and suggest that nectar is much more complex than simply a reward in the form of food. Nectar proteins (nectarins) and nectar secondary compounds have no primary nutritious function but are involved in plant–animal relationships in other ways. Nectarins protect against proliferation of microorganisms and infection of plant tissues by pathogens. Nectar secondary compounds can be involved in modulating the behavior of nectar feeders, maximizing benefits for the plant. Nectar-dwelling microorganisms (mainly yeasts) were recently revealed to be a third partner in the scenario of plant–animal interactions mediated by nectar. There is evidence that yeast has a remarkable impact on nectar feeder behavior, although the effects on plant fitness have not yet been clearly assessed.

Keywords

nectar; plant–animal interactions; indirect defense; pollination; nectar secondary compounds; nectar proteins; microorganisms

The evolution and diversity of nectaries and nectar

According to the classical theory of plant–animal coevolution, nectar – a sugary plant secretion – is considered a trait that evolved in two types of mutualistic interactions involving plants and animals: indirect defense against herbivores and pollen dispersal.

Plants may defend against herbivores directly, through anatomical and/or chemical cues, or indirectly by attracting pugnacious ants that prey on herbivores or deter them from feeding on the plant. Indirect defense against herbivores is mediated by so-called extra-floral nectaries, i.e., nectaries generally located in vegetative parts of the plant (Fig. 1a) and never involved in pollination. On the other hand, pollen dispersal is favored by nectar close to the reproductive organs of angiosperm flowers, in so-called floral nectaries (Fig. 1b).
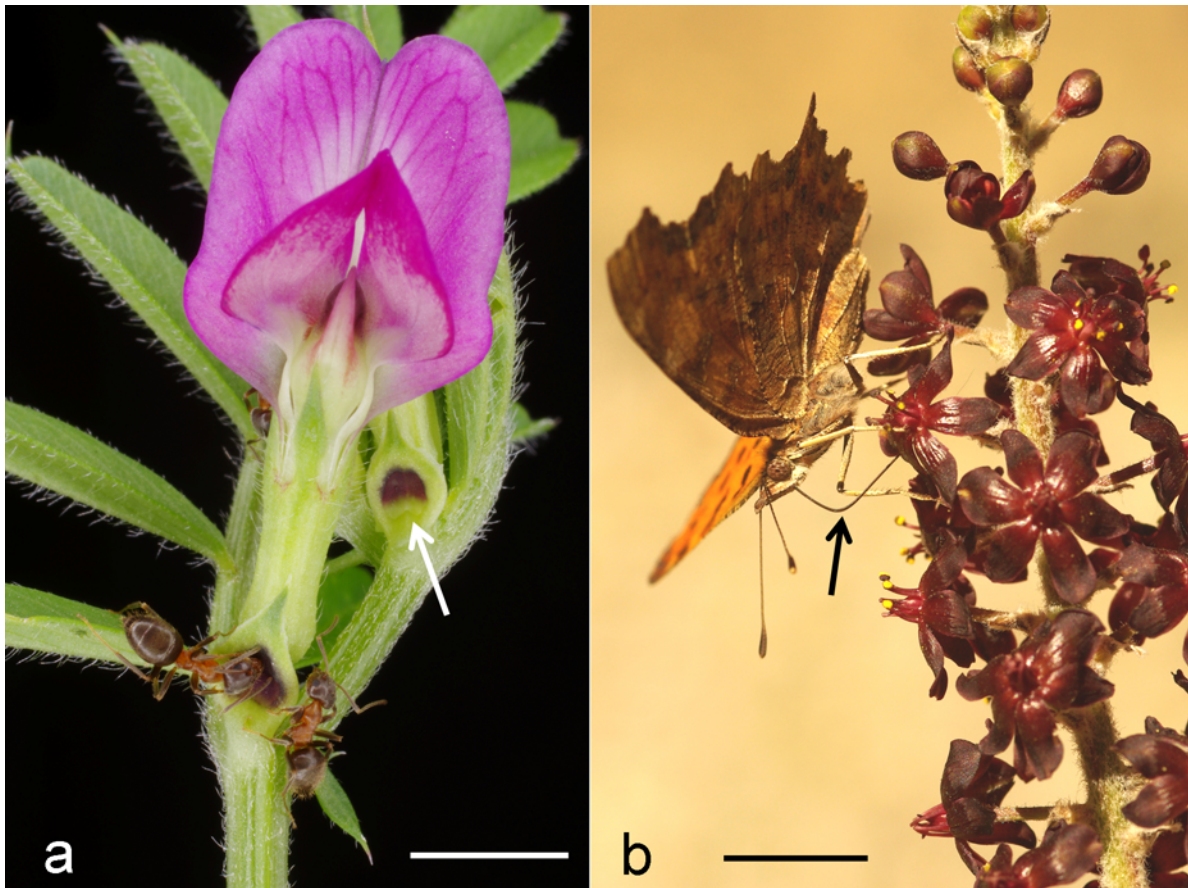
Fig. 1   Nectar and nectar feeders. **a** Ants foraging for nectar on the stipular extra-floral nectaries (arrow) of *Vicia sativa* (Fabaceae) (picture by D. Giannetti and D. A. Grasso, Myrmecology Lab, University of Parma). Scale bar: 0.5 cm. **b** *Polygonia c-album* (Lepidoptera, Nymphalidae) foraging for nectar in the flowers of *Veratrum nigrum* (Melianthaceae). Note the long proboscis (arrow) probing the flower for nectar. Scale bar: 1 cm.

In evolutionary history, extra-floral nectaries first appeared in pteridophytes. Since ferns do not have flowers, their nectaries cannot be called extra-floral but foliar, because they are always on the fronds [1]. Since ants originated in the early Cretaceous period (115–135 Ma) [2], extra-floral nectar probably initially had a function that did not involve ants [3]. De la Barrera and Nobel [4] postulated that nectar may originally have evolved independently of any interaction with animals. According to the "leaky phloem" hypothesis, nectar secretion may have originated as leakage of phloem sap through anatomical weak points in developing tissues subject to high pressure in the phloem. Interaction of extra-floral nectaries with ants may have evolved after the latter's appearance in the early Cretaceous [2]. The diversity of extra-floral nectaries is much higher in angiosperms than in ferns in terms of phenology, structure, and topography as well as in terms of the diversity and intimacy of interactions with ants [5,6]. In some angiosperms, this relationship is generalized mutualism with ants not living on the plant itself, and with extra-floral nectar as the only plant-produced nutritional reward. Other angiosperms, the so-called myrmecophytes, maintain intimate associations with individual ant colonies, providing them with food (extra-floral nectar and "food bodies", i.e., specific organs rich in lipids and proteins) as well as domatia (generally hollow stems or thorns) to house the ants [6].

Curiously, secretion of nectar-like substances is not restricted to the plant kingdom, but can also be produced by animals and fungi. The effect is to attract ants and obtain indirect defense or spore dispersal. Aphids (Hymenoptera: Aphididae) suck the phloem sap and excrete sugary droplets, called honeydew, collected by ants that defend the aphids from enemies. Caterpillars of some species of blues (Lepidoptera: Lycaenidae) secrete sugary droplets from a dorsal gland (named nectar gland because of its sugar-based chemistry, similar to true plant nectar); these secretions also attract ants that protect the caterpillar from its predators [7]. These examples demonstrate

that indirect defense mediated by sugary secretions evolved independently several times in different ecological scenarios, probably because this type of protection from predators is particularly efficient.

Floral nectaries are widely present in angiosperms, apparently much more than extra-floral nectaries. They are completely lacking only in very early branching lineages (Amborellaceae, Austrobaileyales) [8]. Angiosperm floral nectaries have enormous diversification in terms of topography, anatomy, ultrastructure, and secretion mechanisms, and they are often lost or acquired within a lineage (reviewed by Bernardello [8]). Despite this very high diversification of the secreting organ, floral nectar is always involved in interactions with pollinating animals.

The presence of nectar in Gymnosperms is debated. There are no nectaries in the vegetative parts of the plant body of any of the extant gymnosperm species [6]. Some authors (summarized by Bernardello [8]) have improperly used the term "nectar" to indicate the pollination drops of several Gnetophyta. Pollination drops, i.e., sugary secretions produced by ovules, occur in most gymnosperms and their primary functions are pollen capture and nourishment. Although most gymnosperms are wind pollinated, insect contribution to pollination is also reported in several Gnetophyta [9–13]. When insects are involved, they generally feed on the pollination drops, as they do with angiosperm floral nectar. This similar function is probably why the term "nectar" is inappropriately used to describe the pollination drop of Gnetophyta.

In some *Ephedra* and *Gnetum* species, in addition to these pollination drops, true nectaries have also been reported on the bracts enveloping the reproductive units of male and female plants and on the collars [8]. According to Jörgensen and Ridyn [14], this additional secretion in *Gnetum cuspidatum* is improbable. The liquid reported on the collars and male units of this species are probably pollination drops that have leaked from the sterile ovules associated with the male organs that secrete these drops.

According to Nepi et al. [3], although they are ontogenetically, anatomically, and physiologically distinct (not homologous), angiosperm floral nectar and pollination drops may share evolutionary links. Insect mouthpart structures and plant reproductive traits indicate that insect feeding on plant exudates, such as the pollination drops of gymnosperms, far antedates the origin of angiosperms, extending back to the early Mesozoic era [9,15]. Wind, however, has remained the main gymnosperm pollen vector. The new condition of enveloped ovules evolved by angiosperms made the primary source of sugary exudates (i.e., the pollination drop) unavailable. Insects pre-adapted to feed on sugary secretions were then "co-opted" by flowers, i.e., the more specialized reproductive structures of angiosperms, that soon exploited entomophily by producing a sugary secretion in the form of nectar. Through the impressive diversification of floral traits (comprising nectary and nectar diversification), entomophily subsequently became the main pollination mechanism in angiosperms.

It seems that angiosperms were prompter than pteridophytes and gymnosperms in exploiting animal services for indirect defense and pollen dispersal, presumably because their intrinsic adaptability favored quick adaptive evolution of new characters [16].

The traditional concept of nectar as alimentary reward in plant–animal interactions

Irrespective of the type of interactions in which it is involved (indirect defense or pollen dispersal), nectar is traditionally considered the main alimentary reward that attracts animals to plants. Nectar characteristics fit this function perfectly. Being an aqueous solution, nectar is easily ingested, digested, and absorbed by the digestive tract, and is therefore a very cost-effective alimentary resource for a wide array of animals [17,18]. Its chemical composition is dominated by high concentrations of simple sugars, a ready-to-use energy source useful for powering the flight of feeding animals. The disaccharide sucrose and the hexoses glucose and fructose are the dominant sugars in nectar and a combination of these three sugars is generally found [19]. Glucose and fructose in nectar are derived from sucrose by hydrolysis and are

therefore generally present in equal amounts. Unbalanced hexose ratios may result from different biochemical pathways of the two sugars before secretion or may be due to yeasts that alter the nectar sugar profile [20].

Floral nectars were categorized by Baker and Baker [21] according to the ratio by weight of sucrose to combined hexoses, S/(G+F): sucrose-dominant (>1.0), sucrose-rich (0.5–1.0), hexose-rich (0.1–0.5), hexose-dominant (<0.1). This categorization, although extensively used in the scientific literature, has been criticized as biased by overemphasis on sucrose. Sugar percentages are now recommended for describing the sugar profile of nectar [19].

Nectar sugar profile tends to be relatively constant between individuals of the same species [19]. This constancy is partly explained by a coevolutionary relationship between the relative percentage of sugar in nectar and the food preferences of pollinators. This implies that phylogenetically unrelated angiosperm species sharing the same type of pollinators evolved convergent floral nectar chemistry [21]. Flowers of species pollinated by Old World bats, hummingbirds, butterflies, moths, and long-tongued bees tend to produce nectar with a higher percentage of sucrose, whereas flowers pollinated by New World bats, flies, perching birds, and short-tongued bees tend to produce nectar with a higher percentages of hexoses [21]. The specific nectar sugar profile of a preferred nectar can match particular physiological traits of the pollinator. For example, hummingbirds that feed largely on nectar and sunbirds (specialized passerine nectarivores) have high invertase activity in their alimentary canal that allows them to feed preferentially on sucrose-dominant nectars [22]. On the other hand, opportunistic-generalist nectarivorous species have variable levels of invertase activity and species with the lowest activity feed primarily on hexose nectars in the wild [23].

Similarly, animal specialization appears related to sugar composition in extra-floral nectar. Obligate acacia-ant plants house mutualistic ants as an indirect defense mechanism and provide them with extra-floral nectar containing no sucrose due to its high invertase activity. Non-symbiotic ants are not attracted by this nectar because they generally prefer sucrose-rich to hexose-rich nectars [24]. On the contrary, adult *Pseudomyrmex* ants that are specialized to live on *Acacia* plants have almost no invertase activity in their digestive tracts and prefer sucrose-free extra-floral nectar [25].

The alimentary importance of nectar is complemented by the presence of free amino acids. Although they occur at 100th–1000th the concentrations of sugars, nectar amino acids contribute to the taste of nectar [19], and are also an important source of nitrogen for animals, especially those exclusively dependent on nectar for food (such as butterflies and hummingbirds) [17]. Specific preferences for different amino acids are known for floral and extra-floral nectar feeders. Proline is an amino acid preferred by bees [26] and interestingly is one of the most abundant in the floral nectar of several melittophilous plants [27]. Phenylalanine also is frequently found in floral nectar of species pollinated by bees in certain Mediterranean habitats and is a strong phagostimulant [28]. Preferences for specific amino acids or for specific mixtures of amino acids are also known for ants and these preferences vary according to ant species and specialization [24,29]. The amino acid composition of extra-floral nectar allows myrmecophyte *Acacia* species to be distinguished from non-myrmecophytes: the former have a higher proline and phenylalanine content [29]. Symbiotic ants of myrmecophytes prefer the amino acids that are abundant in the extra-floral nectar of host plants, whilst non-symbiotic ants are less selective.

Beyond the alimentary reward

An interesting aspect emerging from recent research on the chemical and biochemical characterization of nectar is that several substances are not directly related to the nutritional value of this secretion. This new information challenges the classical view of nectar as an alimentary reward for animals involved in mutualistic interactions with plants. In particular, two classes of nectar compounds have been the focus of studies this century: secondary compounds and proteins (so-called nectarins). These studies shed light on complex interactions that have hitherto remained hidden.

Secondary metabolites

To maximize plant fitness, nectar needs to repel thieves and to benefit visitors, minimizing investment in rewards. Nectar secondary compounds may serve to balance this trade-off.

Secondary metabolites such as tannins, phenols, alkaloids, and terpenes have been detected in floral nectar in several angiosperm families since the 1970s and they are considered to be toxic deterrents against predators as well as a defense against microorganisms [30,31]. Their effect on feeding animals is dose-dependent: they can be lethal in high doses, but in low doses their effects can differ, depending on the sensitivity of the interacting organism [31,32]. The concentrations of secondary compounds in nectar are lower than in other plant parts, such as leaves, stems, or flowers, where they deter herbivores [31,32]. It is not likely that secondary compounds in nectar coevolved with pollinators but rather that their presence is a pleiotropic trait, i.e., they occur in other plant organs (leaves, stems) and are transported by phloem/xylem during nectar production [31]. Pollinators presumably impose selection to lower their concentrations in nectar rather than to produce specific nectar secondary compounds [31].

Researchers have highlighted that these compounds may play an important role in modulating visitors' behavior. Kessler et al. [33] demonstrated that the repellent nicotine and the attractant benzyl acetone are both necessary for maximizing fruit production and flower visits by native pollinators, while nicotine reduces theft of nectar by non-pollinating animals. Nicotine is a typical insect-repelling alkaloid that maximizes the number of flower visitors per unit volume of floral nectar secreted by *Nicotiana attenuata*. In this way plants can minimize nectar volumes, whilst maximizing pollination efficiency and seed production [34]. Singaravelan et al. [35] demonstrated that nicotine and caffeine at low concentrations induce a significant feeding preference in honeybees. It was proposed that these psychoactive alkaloids in nectar may create dependence or addiction in pollinators as well as improving the memory capacity of honeybees [36]. Indeed, it was recently demonstrated that honeybees rewarded with solutions containing nectar-like concentrations of caffeine remembered a floral scent better than honeybees rewarded with just sucrose [37]. Although this effect can be positive for the efficiency of bee foraging activity, it also has a negative counterpart since bees tend to return to the source of caffeinated nectar when the food is no longer available [38].

There is evidence that certain secondary metabolites detected in nectar (such as gelsemine, anabasine, and nicotine) may benefit pollinators by increasing their resistance to parasites and pathogens. Most studies (reviewed in Stevenson et al. [31]) involved bumblebees infected with the gut parasite *Crithidia bombi*. Consumption of these secondary compounds by bees generally lowered their pathogen load after infection but pre-exposure of the pathogen to the same substances did not decrease its viability.

A class of secondary compounds recently highlighted in floral nectar are the non-protein amino acids, i.e., amino acids not involved in protein synthesis and therefore without primary alimentary importance. It has been hypothesized that this category of compound is also involved in modulating insect behavior, since those more common in nectar, i.e., γ-amino-butyric acid (GABA) and β-alanine, are important neuromodulators of insect nervous systems [39]. For example, in synergy with taurine, GABA is involved in limiting excessive and potentially disruptive excitation states during stress, probably acting as an antagonist of octopamine in regulating arousal pathways [40]. Since GABA-receptors in invertebrates are located peripherally in muscle tissue and neuromuscular junctions, bathed in haemolymph [41], they can be sensitive to variations in GABA levels caused by insect feeding. Non-protein amino acids may affect insect behavior in other ways as well. Phagostimulatory activity is recognized for GABA and other solutes abundant in nectar, such as sucrose, proline, and phenylalanine, and synergic effects of different stimulants seem to occur at the same insect chemoreceptor [42]. Non-protein amino acids also appear to be involved in improving muscle performance in humans [43–45]. Preliminary results seem to confirm this function in insects, since those fed with an artificial diet enriched with non-protein amino acids showed increased movement [46]. Maintenance of efficient

muscle performance in foraging insects helps plants, as it ensures pollinator movement between flowers, plants, and populations, thus promoting pollen and gene flow.

All the above considerations about secondary compounds refer to floral nectar. There has been almost no research into this class of substances in extra-floral nectar. Cardoso-Gustavson et al. [47] recently reported trace amounts of alkaloids in the extrafloral nectar of *Passiflora*. The amounts of alkaloids were not sufficient to induce deleterious effects on ant metabolism and growth and may therefore have functions other than deterrence.

Several secondary compounds are effective against microorganisms [31] and their presence in nectar may improve the efficiency of defenses composed mainly of proteins.

Nectar proteins (nectarins)

Proteins were first detected in floral nectar in the 1930s [48]. Advances in molecular biology, proteomics, and bioinformatics have only recently allowed better characterization of nectar protein profiles. Although nectarins may provide organic nitrogen to nectar consumers, their primary functions are not to attract or repel animals. Proteins found in nectar belong to two general functional classes: carbohydrate-metabolizing enzymes and proteins defending against proliferation of microorganisms (generally referred to pathogenesis-related proteins). Nectar enzymes are evidence that this secretion is not a static product, but rather a dynamic product that may undergo changes after secretion. However, little research has been dedicated to the regulation of nectar enzyme activity. Invertase, i.e., the enzyme that catalyzes hydrolysis of sucrose to glucose and fructose, thereby responsible for sugar composition, is of particular importance because it allows post-secretory transformation of the nectar sugar profile. Invertase has been known to occur in nectar since an early report in *Tilia* sp. published in the 1930s [48] but surprisingly its activity has not yet been characterized.

Although a handful of species have been analyzed by modern proteomic and bioinformatic approaches, it is clear that most of the proteins detected in floral and extra-floral nectar are proteins that defend the plant against microorganisms, and are part of a heterogeneous and widely diversified biochemical arsenal. Glucanases, chitinases, xylosidases, lipases, RNAases, peroxidases, elongation factors, desiccation-related proteins, and thaumatin-like proteins are major nectar proteins, the direct or indirect effect against microorganisms of which has been demonstrated or postulated [49–57]. A specific metabolic pathway (the nectar redox cycle) composed by five nectarins was reported in *Nicotiana* floral nectar where it produces high concentrations of hydrogen peroxide that protect nectar and nectary tissues against micro-organisms proliferation [49].

It is worth underlining that nectar proteins may have multiple biological activities that add complexity to interactions between organisms. Chitinases are a case of particular interest in plant–animal relationships. They were recognized to be part of the defense arsenal of the extra-floral nectar of myrmecophyte *Acacia* species [52] and more recently they were reported to be responsible for manipulating the digestive specialization of their ant mutualists. Until a few years ago, ant mutualists were considered to have no invertase in their digestive tract and this physiological trait was compensated by the plant through secretion of sucrose-free extra-floral nectar [25]. Astonishingly, it was recently demonstrated that ant larvae do indeed have invertase activity which is lost by the time workers eclose from the pupae and start to feed on extra-floral nectar [58]. Invertase in the ant's gut is inhibited by chitinase, a dominant extra-floral nectarin, so adult ants are obliged to feed on that specific extra-floral nectar [58]. This is a clear example of how plants can modulate the behavior of feeding animals through the biochemistry of their nectar, enhancing insect dependency on host-derived rewards, and in turn allowing more efficient feedback, in this specific case indirect defense against herbivores.

Nectar dwelling microorganisms and their impact on pollinator foraging behavior

By virtue of its sugar-based composition, nectar can be an optimal environment for the growth of microorganisms, especially when it is exposed to the environment for long time (e.g., extrafloral nectar and nectar of long-lasting flowers). The wide and varied defense "arsenal" of nectar is mirrored by a variety of microorganisms that may grow in the nectar medium. The proliferation of microorganisms depends on their ability to tolerate a wide array of nectar environments that may contain different antimicrobial compounds. Some of these microorganisms are phytopathogens deleterious for plant health that exploit nectarostomata (i.e., the specialized open stomata through which nectar is often released) to enter plant tissues [59,60]. Avoiding proliferation of these microbes in nectar greatly reduces risk of infection.

Another type of nectar-dwelling microorganism does not generally have a negative effect on plant health but may have a great impact on plant–pollinator interactions mediated by nectar. Yeasts are very common in floral nectar. About half the samples collected in tropical and temperate plant communities proved positive for yeasts and showed a preponderance of basidiomycetous and ascomycetous species of the genera *Cryptococcus*, *Metschnikowia*, and *Candida* [61,62].

Pollinators, usually insects, are the most likely candidates for transferring microbes from one flower to another [61]. Identification of yeasts isolated from nectar suggests that yeast assemblages vary among plant species, presumably as a consequence of differences in pollinator guild. Herrera et al. [61] found that yeast abundance and frequency in 22 plant species from southern Spain were significantly related to differences in the relative proportion of solitary bees and bumblebees in the pollinator assemblage: yeast incidence was more pronounced in species mainly pollinated by bumblebees. Moreover, Mittelbach et al. [63] demonstrated that basidiomycetous yeasts are significantly associated with nectar of ornithophilous flowers of the Canary Islands.

Yeasts are responsible for dramatic changes in nectar traits that may affect forager behavior. Yeast reduces the food value of nectar because it induces drastic changes in nectar chemical profile. Yeasts cause a decrease in sugar concentration and a significant reduction in sucrose percentage by fermentation [64,65]. Since sugars are the prevailing constituents of most nectars and provide a prominent energy reward for many animals, their variation may condition foraging choices. Furthermore, yeasts use amino acids as nitrogen source, lowering their level in nectars [66]. It is interesting to note that yeast proliferation is greater in pollen-contaminated nectar, probably due to diffusion of free amino acids from the pollen into the nectar [66]. As in the case of sugars, specific preferences for particular amino acid profiles are known among pollinating animals and herbivore-defending ants [26,29]. Thus, changes in nectar composition caused by yeasts may significantly alter the attractiveness of nectar to specific foragers.

Nectar fermentation by yeasts may raise ethanol concentrations to levels that are toxic to foraging animals. The fungus *Cladosporium* produces ethanol by fermenting the nectar of the orchid *Epipactis helleborine* [67]. Wasps become sluggish when they feed on this orchid and they groom their bodies less for pollinia. By limiting loss of pollinia, this behavior may enhance their transfer to stigmas and consequently orchid reproduction.

Nectar fermentation is also responsible for production of volatile organic compounds. These substances may affect plant–animal interactions by contributing to the scent of flower headspace perceived by foragers [68,69].

It is now clear that nectar-dwelling yeasts may significantly affect plant–animal interactions mediated by nectar. The extent to which this may alter the behavior of foraging animals, and in turn plant reproduction and fitness, has not yet been assessed. Herrera et al. [70] demonstrated that bumblebees can detect yeasts in artificial nectar and respond positively by preferentially visiting yeast-containing flowers and spending more time for each visit. This preference is harmful for the reproduction of *Helleborus foetidus*, probably because longer visits to yeast-containing flowers promote autogamy and therefore have a negative effect on seed production. Disentangling the details of these elaborate interactions will require further studies.

Bacteria may also have an impact on plant–pollinator mutualism. The common bacterium *Gluconobacter* sp. has a greater impact on the nectar chemistry of *Mimulus aurantiacus* than the nectar yeast *Metschnikovia reukaufii* and these changes weaken mutualism with pollinating hummingbirds [71].

Perspectives and future challenges

Considering nectar as a mere alimentary reward for pollinators is an outdated view for several reasons: (*i*) modern characterization of nectarins shows that the protein component of nectar is mainly involved in interactions with organisms other than pollinators (microbes, pathogens, yeasts); (*ii*) a third partnership in nectar-mediated flower–pollinator relationships involves microorganisms that live in nectar (such as bacteria and especially yeasts) and may impact plant–animal interactions; (*iii*) nectar contains molecules (secondary compounds) that may affect animal behavior, maximizing pollination efficiency and/or indirect defense against herbivores. A source of nectar secondary compounds can be yeast contamination itself. For example, biogenic amines (e.g., histamine, serotonin, dopamine, tyramine, tryptamine, octopamine, ephedrine) are responsible for various behavior-modifying effects on the human and insect brain [72]. They may be generated by microbial decarboxylation of free amino acids. Since they have been found in organic matrices fermented by yeasts [73], they are likely to be found in nectar. On the other hand, yeast contamination of nectar can reduce the concentration of certain secondary compounds, reducing their effect on pollinators [74]. Interactions involving microorganisms clearly make the ecological scenario linking nectar secondary compounds and pollinators more complex.

Very few of the thousands of plant secondary metabolites have been detected in nectar and a direct effect on animal brains has only been demonstrated in the case of caffeine [37]. More cases will likely be discovered in the near future by interdisciplinary research involving nectar chemistry, insect physiology and neurobiology. However, we can already glimpse a more complex web involving interactions between nectar, nectar feeders and microorganisms (Fig. 2). Foraging by nectar feeders can indirectly affect nectar chemistry through contamination with microorganisms, while modifications in nectar chemistry induced by microorganisms can influence feeder behavior. The extent to which these effects may benefit plants is unknown.

An interesting scenario proposed by Grasso et al. [75] envisages that plants evolved chemicals, such as secondary compounds, that affect animal brains. These attract insects and manipulate their behavior to the benefit of plants. Although this scenario was postulated in the context of ant–plant relationships mediated by extra-floral nectar, it can be extended to floral nectar as well. It appears that reward-mediated interactions, traditionally considered to be mutualistic, may prove to involve partner exploitation to obtain benefits.
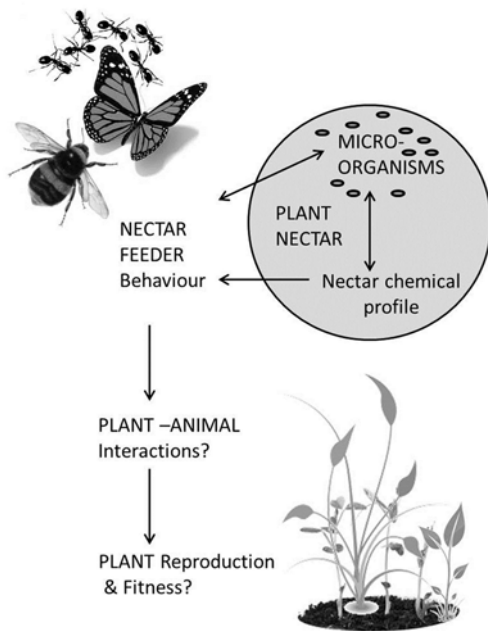

Fig. 2 Interactions between nectar feeders, microorganisms, and nectar (floral and extra-floral) secreted by plants. The extent to which these complex interactions affect plant reproduction and fitness is unknown.

References

1. Koptur S, Palacios-Rios M, Díaz-Castelazo C, Mackay WP, Rico-Gray V. Nectar secretion on fern fronds associated with lower levels of herbivore damage: field experiments with a widespread epiphyte of Mexican cloud forest remnants. Ann Bot. 2013;111:1277–1283. https://doi.org/10.1093/aob/mct063

2. Ward PS. Phylogeny, classification, and species-level taxonomy of ants (Hymenoptera:

Formicidae). Zootaxa. 2007;1668:549–563.

3. Nepi M, von Aderkas P, Wagner R, Mugnaini S, Coulter A, Pacini E. Nectar and pollination drops: how different are they? Ann Bot. 2009;104:205–219. https://doi.org/10.1093/aob/mcp180

4. De la Barreira E, Nobel PS. Nectar: properties, floral aspects, and speculations on origin. Trends Plant Sci. 2004;9:65–69. https://doi.org/10.1016/j.tplants.2003.12.003

5. Marazzi B, Bronstein JL, Koptur S. The diversity, ecology and evolution of extra-floral nectaries: current perspectives and future challenges. Ann Bot. 2013;111:1243–1250. https://doi.org/10.1093/aob/mct109

6. Weber MG, Keeler HK. The phylogenetic distribution of extra-floral nectaries in plants. Ann Bot. 2013;111:1251–1261. https://doi.org/10.1093/aob/mcs225

7. Wäckers F. It pays to be sweet: sugars in mutualistic interactions. Biologist. 2002;49:165–169.

8. Bernardello G. A systematic survey of floral nectaries. In: Nicolson SW, Nepi M, Pacini E, editors. Nectaries and nectar. Dordrecht: Springer; 2007. p. 19–128. https://doi.org/10.1007/978-1-4020-5937-7_2

9. Labandeira CC, Kvaçek J, Mostovski MB. Pollination drops, pollen, and insect pollination of Mesozoic gymnosperms. Taxon. 2007;56:663–695. https://doi.org/10.2307/25065853

10. Procheş Ş, Johnson SD. 2009. Beetle pollination of the fruit-scented cones of the South African cycad *Stangeria eriopus*. Am J Bot. 2009;96:1722–1730. https://doi.org/10.3732/ajb.0800377

11. Gong YB, Yang M, Vamosi JC, Yang HM, Mu WX, Li JK, et al. Wind or insect pollination? Ambophily in a subtropical gymnosperm *Gnetum parvifolium* (Gnetales). Plant Species Biol. 2016;31(4):271–279. https://doi.org/10.1111/1442-1984.12112

12. Rydin C, Bolinder K. Moonlight pollination in the gymnosperm *Ephedra* (Gnetales). Biol Lett. 2015;11:20140993. https://doi.org/10.1098/rsbl.2014.0993

13. Bolinder K, Humphreys AM, Ehrlén J, Alexandersson R, Ickert-Bond SM, Rydin C. From near extinction to diversification by means of a shift in pollination mechanism in the gymnosperm relict *Ephedra* (Ephedraceae, Gnetales). Bot J Linn Soc. 2016;180:461–477. https://doi.org/10.1111/boj.12380

14. Jörgensen A, Rydin C. Reproductive morphology in the *Gnetum cuspidatum* group (Gnetales) and its implications for pollination biology in the Gnetales. Plant Ecol Evol. 2015;148:387–396. https://doi.org/10.5091/plecevo.2015.1142

15. Ren D, Labandeira CC, Santiago-Blay JA, Rasnitsyn A, Shih CK, Bashkuev A, et al. A probable pollination mode before angiosperms: Eurasian, long-proboscid scorpionflies. Science. 2009;326:840–847. https://doi.org/10.1126/science.1178338

16. Crepet LM, Niklas KJ. Darwin's second "abominable mystery": why are there so many angiosperm species? Am J Bot. 2009;96:366–381. https://doi.org/10.3732/ajb.0800126

17. Nicolson SW. Nectar consumers. In: Nicolson SW, Nepi M, Pacini E, editors. Nectaries and nectar. Dordrecht: Springer; 2007. p. 289–342. https://doi.org/10.1007/978-1-4020-5937-7_7

18. González-Teuber M, Heil M. Nectar chemistry is tailored for both attraction of mutualists and protection from exploiters. Plant Signal Behav. 2009;4:809–813. https://doi.org/10.4161/psb.4.9.9393

19. Nicolson SW, Thornburg RW. Nectar chemistry. In: Nicolson SW, Nepi M, Pacini E, editors. Nectaries and nectar. Dordrecht: Springer; 2007. p. 215–264. https://doi.org/10.1007/978-1-4020-5937-7_5

20. Nepi M. Nectar: plant interface for complex interaction with biotic environment. In: Ramawat KG, Mérillon JM, Shivanna KR, editors. Reproductive biology of plants. Boca Raton, FL: CRC Press; 2012. p. 268–283. https://doi.org/10.1201/b16535-13

21. Baker HG, Baker I. Floral nectar sugar constituents in relation to pollinator type. In: Little RJ, Jones CE, editors. Handbook of pollination biology. New York, NY: Scientific and Academic Editions; 1983. p. 117–141.

22. Johnson SD, Nicholson S. Evolutionary associations between nectar properties and specificity in bird pollination systems. Biol Lett. 2008;4:49–52. https://doi.org/10.1098/rsbl.2007.0496

23. Napier KR, McWhorter TJ, Nicolson S, Fleming PA. Sugar preferences of avian nectarivores are correlated with intestinal sucrase activity. Physiol Biochem Zool.

2013;86:499–514. https://doi.org/10.1086/672013

24. Blüthgen N, Fiedler K. Preferences for sugar and amino acids and their conditionality in a diverse nectar-feeding ant community. J Anim Ecol. 2004;73:155–166. https://doi.org/10.1111/j.1365-2656.2004.00789.x

25. Heil M, Rattke J, Bolland W. Postsecretory hydrolysis of nectar sucrose and specialization in ant/plant mutualism. Science. 2005;308:560–563. https://doi.org/10.1126/science.1107536

26. Bertazzini M, Medrzycki P, Bortolotti L, Maistrello L, Forlani G. Amino acid content and nectar choice by forager honeybees (*Apis mellifera* L.). Amino Acids. 2010;39:315–318. https://doi.org/10.1007/s00726-010-0474-x

27. Nocentini D, Pacini E, Guarnieri M, Nepi M. Flower morphology, nectar traits and pollinators of *Cerinthe major* (Boraginaceae-Lithospermeae). Flora. 2012;207:186–196. https://doi.org/10.1016/j.flora.2012.01.004

28. Petanidou T. Ecological and evolutionary aspects of floral nectars in Mediterranean habitats. In: Nicolson SW, Nepi M, Pacini E, editors. Nectaries and nectar. Dordrecht: Springer; 2007. p. 343–375. https://doi.org/10.1007/978-1-4020-5937-7_8

29. González-Teuber M, Heil M. The role of extra-floral nectar amino acids for the preferences of facultative and obligate ant mutualists. J Chem Ecol. 2009;35:459–468. https://doi.org/10.1007/s10886-009-9618-4

30. Adler LS. The ecological significance of toxic nectar. Oikos. 2000;91:409–420. https://doi.org/10.1034/j.1600-0706.2000.910301.x

31. Stevenson PC, Nicolson SW, Wright JA. Plant secondary metabolites in nectar: impacts on pollinators and ecological functions. Funct Ecol. 2017;31:65–75. https://doi.org/10.1111/1365-2435.12761

32. Manson JS, Cook D, Gardner DR, Irwin RE. Dose-dependent effects of nectar alkaloids in a montane plant-pollinator community. J Ecol. 2013;101:1604–1612. https://doi.org/10.1111/1365-2745.12144

33. Kessler D, Gasse K, Baldwin IT. Field experiments with transformed plants reveal the sense of floral scents. Science. 2008;321:1200–1202. https://doi.org/10.1126/science.1160072

34. Kessler D, Baldwin IT. Making sense of nectar scents: the effects of nectar secondary metabolites on floral visitors to *Nicotiana attenuata*. Plant J. 2007;49:840–854. https://doi.org/10.1111/j.1365-313X.2006.02995.x

35. Singaravelan NG, Nee'man G, Inbar M, Izhaki I. Feeding responses of free-flying honeybees, to secondary compounds mimicking floral nectars. J Chem Ecol. 2005;31:2791–2804. https://doi.org/10.1007/s10886-005-8394-z

36. Singaravelan N. Secondary compounds in nectar: the other side of the coin. In: Zhang W, Liu H, editors. Behavioral and chemical ecology. New York, NY: Nova Publishers; 2010. p. 217–238.

37. Wright GA, Baker DD, Palmer MJ, Stabler D, Mustard JA, Power EF, et al. Caffeine in floral nectar enhances a pollinator's memory of reward. Science. 2013;339:1202–1204. https://doi.org/10.1126/science.1228806

38. Couvillon MJ, Al Toufailia H, Butterfield TM, Schrell F, Ratnieks FLW, Schurch R. Caffeinated forage tricks honeybees into increasing foraging and recruitment behaviours. Curr Biol. 2015;25:2815–2818. https://doi.org/10.1016/j.cub.2015.08.052

39. Nepi M. Beyond nectar sweetness: the hidden ecological role of non-protein amino acids in nectar. J Ecol. 2014;102:108–115. https://doi.org/10.1111/1365-2745.12170

40. Stevenson PA. Co-localisation of taurine- with transmitter-immunoreactivities in the nervous system of the migratory locust. J Comp Neurol. 1999;404:86–96. https://doi.org/10.1002/(SICI)1096-9861(19990201)404:1<86::AID-CNE7>3.0.CO;2-8

41. Bown AW, MacGregor KB Shelp BJ. Gamma-aminobutyrate: defense against invertebrate pests? Trends Plant Sci. 2006;11:424–427. https://doi.org/10.1016/j.tplants.2006.07.002

42. Shoonhoven L, van Loon JJA, Dicke M. Insect–plant biology. Oxford: Oxford University Press; 2005.

43. Watanabe M, Maemura K, Kanbara K, Tamayama T, Hayasaki H. GABA and GABA receptors in the central nervous system and other organs. Int Rev Cytol. 2002;213:1–47. https://doi.org/10.1016/S0074-7696(02)13011-7

44. Zhang M, Izumi I, Kagamimori S, Sokejima S, Yamagami T, Liu Z, et al. Role of taurine

supplementation to prevent exercise-induced oxidative stress in healthy young men. Amino Acids. 2004;26:203–207. https://doi.org/10.1007/s00726-003-0002-3

45. Hill CA, Harris RC, Kim HJ, Harris BD, Sale C, Boobis LH, et al. Influence of β-alanine supplementation on skeletal muscle carnosine concentrations and high intensity cycling capacity. Amino Acids. 2007;32:225–233. https://doi.org/10.1007/s00726-006-0364-4

46. Nepi M, Nocentini D, Guarnieri M, Bogo G, Laura B, Galloni M, et al. Non-protein amino acids in nectar: are they tools to modulate insect behavior and increase plant reproductive output? In: Book of abstracts of the International Congress on Plant Reproduction; 2014 Sep 16, Bologna, Italy. Bologna: University of Bologna; 2014. p. 12.

47. Cardoso-Gustavson P, Andreazza NL, Sawaya ACHF, de Moraes Castro M. Only attract ants? The versatility of petiolar extrafloral nectaries in Passiflora. Am J Plant Sci. 2013;4:460–469. https://doi.org/10.4236/ajps.2013.42A059

48. Beutler R. Nectar. Bee World. 1935;24:106–162.

49. Carter C, Thornburg RW. Is the nectar redox cycle a floral defense against microbial attack? Trends Plant Sci. 2004;9:320–324. https://doi.org/10.1016/j.tplants.2004.05.008

50. Carter C, Healy R, O'Tool NM, Naqvi SMS, Ren G, Park S, et al. Tobacco nectaries express a novel NADPH oxidase implicated in the defense of floral reproductive tissues against microorganisms. Plant Physiol. 2007;143:389–399. https://doi.org/10.1104/pp.106.089326

51. Kram B, Bainbridge E, Perera M, Carter C. Identification, cloning and characterization of a GDSL lipase secreted into the nectar of *Jacaranda mimosifolia*. Plant Mol Biol. 2008;68:173–183. https://doi.org/10.1007/s11103-008-9361-1

52. González-Teuber M, Pozo MJ, Muck A., Svatos A, Adame-Álvarez RM, Heil M. Glucanases and chitinases as causal agents in the protection of *Acacia* extrafloral nectar from infestation by phytopathogens. Plant Physiol. 2010;152:1705–1715. https://doi.org/10.1104/pp.109.148478

53. Hillwig MS, Kanobe C, Thornburg RW, MacIntosh GC. Identification of S-RNase and peroxidase in *Petunia nectar*. J Plant Physiol. 2011;168:734–738. https://doi.org/10.1016/j.jplph.2010.10.002

54. Nepi M, Bini L, Bianchi L, Puglia M, Abate M, Cai G. Xylan-degrading enzymes in male and female flower nectar of *Cucurbita pepo*. Ann Bot. 2011;108:521–527. https://doi.org/10.1093/aob/mcr165

55. Seo PJ, Wielsch N, Kessler D, Svatos A, Park CM, Baldwin IT, et al. Natural variation in floral nectar proteins of two *Nicotiana attenuata* accessions. BMC Plant Biol. 2013;13:101. https://doi.org/doi:10.1186/1471-2229-13-101

56. Zha HG, Liu T, Zhou JJ, Sun H. MS-desi, a desiccation-related protein in the floral nectar of the evergreen velvet bean (*Mucuna sempervirens* Hemsl): molecular identification and characterization. Planta. 2013;238:77–89. https://doi.org/10.1007/s00425-013-1876-2

57. Zhou Y, Li M, Zhao F, Zha ., Yang L, Lu, Y., et al. Floral nectary morphology and proteomic analysis of nectar of *Liriodendron tulipifera* Linn. Front Plant Sci. 2016;7:826. https://doi.org/10.3389/fpls.2016.00826

58. Heil M, Barajas-Barron A, Orona-Tamayo D, Wielsch N, Svatos A. Partner manipulation stabilises a horizontally transmitted mutualism. Ecol Lett. 2014;17:185–192. https://doi.org/10.1111/ele.12215

59. Bubán T, Orosz-Kovacs Z, Farkas A. The nectary as the primary site of infection by *Erwinia amylovora* (Burr.) Winslow et al.: a mini review. Plant Syst Evol. 2003;238:183–194. https://doi.org/10.1007/s00606-002-0266-1

60. Sasu MA, Seidl-Adams I, Wall K, Winsor JA, Stephenson AG. Floral transmission of *Erwinia tracheiphila* by cucumber beetles in a wild *Cucurbita pepo*. Environ Entomol. 2010;39:140–148. https://doi.org/10.1603/EN09190

61. Herrera CM, de Vega C, Canto A, Pozo MI. Yeasts in floral nectar: a quantitative survey. Ann Bot. 2009;103:1415–1423. https://doi.org/10.1093/aob/mcp026

62. Canto A, Herrera CM. Micro-organisms behind the pollination scenes: microbial imprint on floral nectar sugar variation in a tropical plant community. Ann Bot. 2012;110:1173–1183. https://doi.org/10.1093/aob/mcp026

63. Mittelbach M, Yurkov AM, Nocentini D, Nepi M, Weigend M, Begerow D. Nectar sugars and bird visitation define a floral niche for basidiomycetous yeast on the Canary Islands. BMC Ecol. 2015;15:2. https://doi.org/10.1186/s12898-015-0036-x

64. Canto A, Herrera CM, García IM, Pérez R, Vaz M. Intraplant variation in

nectar traits in *Helleborus foetidus* (Ranunculaceae) as related to floral phase, environmental conditions and pollinator exposure. Flora. 2011;206:668–675. https://doi.org/10.1016/j.flora.2011.02.003

65. de Vega C, Herrera CM. Micro-organisms transported by ants induce changes in floral nectar composition of an ant-pollinated plant. Am J Bot. 2013;100:792–800. https://doi.org/10.3732/ajb.1200626

66. Pozo MI, Lievens B, Jacquemin H. Impact of microorganisms on nectar chemistry, pollinator attraction and plant fitness. In: Peck RL, editor. Nectar: production, chemical composition and benefits to animals and plants. New York, NY: Nova Science Publishers; 2014. p. 1–40.

67. Ehlers BK, Olesen JM. The fruit-wasp route to toxic nectar in *Epipactis* orchids. Flora. 1996;192:223–229.

68. Raguso RA. Why are some floral nectars scented? Ecology. 2004;85:1486–1494. https://doi.org/10.1890/03-0410

69. Raguso RA. Floral scent in a whole-plant context: moving beyond pollinator attraction. Funct Ecol. 2009;23:837–840. https://doi.org/10.1111/j.1365-2435.2009.01643.x

70. Herrera CM, Pozo MI, Medrano M. Yeasts in nectar of an early-blooming herb: sought by bumble bees, detrimental to plant fecundity. Ecology. 2013;94:273–279. https://doi.org/10.1890/12-0595.1

71. Vannette RL, Gauthier M-PL, Fukami, T. Nectar bacteria, but not yeast, weaken a plant–pollinator mutualism. Proc R Soc B. 2013;280:20122601. https://doi.org/10.1098/rspb.2012.2601

72. Kennedy DO. Plants and human brain. Oxford: Oxford University Press; 2014.

73. Caruso M, Fiore C, Contursi M, Salzano G, Paparella A, Romano P. Formation of biogenic amines for the selection of wine yeasts. World J Microbiol Biotechnol. 2002;18:159–163. https://doi.org/10.1023/A:1014451728868

74. Vannette R, Fukami T. Nectar microbes can reduce secondary metabolites in nectar and alter effects on nectar consumption by pollinators. Ecology. 2016;97:1419–1419. https://doi.org/10.1890/15-0858.1

75. Grasso D, Pandolfi C, Bazihizina N, Nocentini D, Nepi M, Mancuso S. Extrafloral-nectar based partner manipulation in plant-ant relationships. AoB Plants. 2015;7:plv002. https://doi.org/10.1093/aobpla/plv002

Nowe spojrzenie na ewolucję i ekologię nektaru: prosta nagroda kwiatowa czy złożone interakcje między wieloma organizmami?

Streszczenie

Nektar kwiatowy jak i pozakwiatowy są wydzielinami produkowanymi przez specyficzne organy (nektarniki), które mogą być związane z generatywnymi strukturami kwiatowymi (tzw. nektarniki kwiatowe występujące wyłącznie u okrytozalążkowych) lub występujące na częściach wegetatywnych (nektarniki pozakwiatowe). Wydzieliny te są powszechne u naczyniowych roślin lądowych, zwłaszcza u okrytozalążkowych. Chociaż wydaje się, że nagozalążkowe nie wytwarzają prawdziwego nektaru, to ich wydzieliny produkowane przez zalążki mogą być ewolucyjnie powiązane z nektarem okrytozalążkowych. Generalnie, nektar uczestniczy w interakcjach ze zwierzętami i ze względu na zawartość cukrów oraz aminokwasów jest uważany za nagrodę oferowaną przez rośliny dla zwierząt w zamian za korzyści, głównie zapylenie, a także pośrednio, za ochronę przed szkodnikami. Powyższe związki między roślinami i zwierzętami są podawane jako przykłady klasycznych interakcji mutualistycznych. Jednakże obecne badania innych składników nektaru, które występują w mniejszych ilościach niż cukry i aminokwasy zakwestionowały ten pogląd, a na ich podstawie sugeruje się, że nektar jest bardziej kompleksową wydzieliną niż tylko prostą nagrodą w formie pożywienia. Białka zawarte w nektarze (nektaryny) i wtórne metabolity nie pełnią pierwotnej funkcji odżywczej lecz w zupełnie inny sposób uczestniczą w interakcji roślina–zwierzę. Nektaryny pełnią funkcję ochronną zapobiegając namnażaniu się mikroorganizmów i infekcji tkanek roślinnych przez patogeny. Metabolity wtórne zawarte w nektarze mogą modulować zachowanie zwierząt żerujących na nektarze wpływając na zwiększenie korzyści dla rośliny. Mikroorganizmy zasiedlające nektar (głównie drożdże) obecnie uznano za trzeciego partnera interakcji między roślinami i zwierzętami, które odbywają się za pośrednictwem nektaru. Istnieją dowody, że drożdże mają istotny wpływ na zachowanie zwierząt odżywiających się nektarem, chociaż korzystny wpływ na roślinę nie został dotychczas oszacowany.