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Nectar chemistry is tailored for both attraction of mutualists and protection from exploiters

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Plants produce nectar to attract pollinators in the case of floral nectar (FN) and defenders in the case of extrafloral nectar (EFN). Whereas nectars must function in the context of plant-animal mutualisms, their chemical composition makes them also attractive for non-mutualistic, exploiting organisms: nectar robbers and nectar-infesting microorganisms. We reviewed the chemical composition of both FNs and EFNs and found that nectar composition appears tailored to fulfil these ambivalent roles. Carbohydrates and amino acids usually function in the attraction of mutualists and appear adapted to the physiological needs of the respective mutualists. Volatiles are a further group of compounds that serves in the attractive function of nectars. By contrast, secondary compounds such as alkaloids and phenols serve the protection from nectar robbers, and most nectar proteins that have been characterised to date protect FN and EFN from microbial infestation. Nectar components serve both in attraction and the protection of nectar.

Introduction

Nectars are aqueous solutions that are secreted by plants to attract and reward animal mutualists. The two major functional groups of these mutualists comprise pollinators such as insects, birds and bats, which are attracted to floral nectar (FN), and defending arthropods such as ants and parasitoids, which are attracted to extrafloral nectar (EFN). Floral nectar is considered the most common means by which animal-pollinated plants reward their pollen vectors.[1] By contrast, EFN is not involved in pollination[2,3] but serves plant indirect defence against herbivores.[4] EFN has been described for more than 300 plant genera[2-5] and, thus, also represents an important means by which plants ensure and control mutualistic interactions with animals.

The fraction of soluble solids that can be found in nectars mainly comprises mono- and disaccharides and amino acids. However, other compound classes such as proteins, lipids, phenols, alkaloids and volatile organic compounds (VOCs) have also been reported from various nectars.[6,7] The main function of nectar compounds is related to the attraction of mutualistic animals. For example, sugars, amino acids and lipids have been described as nutritionally valuable nectar components[8,9] that contribute to its attractiveness for various mutualistic animals, although VOCs recently have been identified as further attractive constituents.[7]

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Due to its high concentration of nutritionally valuable compounds, nectar is also attractive to other consumers, which do not render a mutualistic service to the plant[10-12] and thus act as exploiters: nectar robbers[13] or nectar-infesting microorganisms.[14,15] Whereas nectar robbers can significantly reduce the defensive efficacy of EFN or the pollinating efficiency of FN by competing with defenders[16,17] or pollinators,[18,19] pathogenic microorganisms could use nectar, and thus the nectaries, as an entry to infect the plant.[20] Yeasts and fungi have been identified in nectar[14,21] and were found to affect nectar sugar composition,[15] thereby reducing the control of the plant over this important nectar trait. In order to fend off those non-mutualistic organisms, nectar possesses various chemical compounds that are related to its protection from exploiters rather than to its attractive function. For example, proteins, secondary metabolites, and VOCs can play a role as repellents to nectar robbers or as inhibitors of pathogen growth. In this review we summarize the main chemical compounds that have been found in nectar in the context of their ecological functions in shaping plant-animal interactions and in protecting nectar from exploitation.

Sugars and Amino Acids

Sugars and amino acids (AAs) are the compounds mainly associated with the attractive function of nectar. Concentration and composition of nectar sugars has been often correlated with specific responses of nectar visitors.[8,9,22-24] The attractiveness of sugars and AAs is usually highly correlated with the nutritive value of these compounds for respective consumers. Because different groups of animals strongly differ in their physiology and, thus, in their nutritive requirements, most authors have assumed that the pollination syndrome of plants is strongly correlated with the composition and concentration of constituents that can be found in floral nectar.[8,9,22,23] For example, hummingbirds, butterflies, moths and long-tongued bees usually prefer sucrose-rich nectars, whereas short-tongued bees and flies prefer nectar rich in hexoses, that is, fructose and glucose.[23,25-29] However, some nectarivorous birds lack the sucrose-cleaving enzyme, invertase and are, thus, not able to assimilate sucrose.[30] Although some studies have failed[31,32] to find this supposed relationship between nectar sugar content and pollination syndrome, Krömer et al.[29] supported the general hypothesis with a large-scale study comprising 111 species of the plant family Bromeliaceae, suggesting that nectar sugars in this plant family might represent a putative adaptation to their pollinator visitors. Less information is available for the role of sugars in EFN, but many ants have been reported to strongly prefer sucrose over hexoses.[33-37] By contrast, workers of obligate plant-ants have been found to lack invertase[38,39] and consequently preferred sucrose-free over sucrose-containing nectars. These observations highlight a role of carbohydrates in the interaction of EFN with its consuming plant defenders that is similar to their role in FN.

Similarly, amino acids in FN were found to be related with the pollinator syndrome of the respective plant species. For example, birds and bats do not exclusively feed on nectar and can also gain nitrogen by consuming pollen, insects and fruits,[40,41] whereas many adult insects feed only on FN. This physiological difference leads to the general expectation that nectar AA concentration should be higher in insect—than vertebrate—pollinated flowers.[42-44] Indeed, high AA concentrations have been reported for FNs from flowers that are adapted to pollinators such as butterflies,[8,9] flies[40] or bees,[45] and specific behavioral responses to AAs have been demonstrated for insect pollinators: butterflies and flies can detect single AAs in nectar sugar solutions[40,41] or showed preferences for nectar mimics containing specific mixtures of amino acids.[46] By contrast, nectarivorous birds were not found to prefer specific AAs in nectar solutions.[8,47,48]

Variations in behavioral responses of nectar visitors to different AAs have also been shown for EFN. Ants in general prefer artificial mixtures of sugars plus amino acids over pure sugar solutions[33,49-52] and certain ants responded to single AAs and to specific amino acid-sugar solutions.[33,53] The preferences for different AAs in nectar can vary among ant species[33] and different responses have been found to be associated with different life histories of ants.[53] Cafeteria-style experiments, in which ants could freely choose among different solutions containing various combinations of sugars and AAs, demonstrated that generalist ants were less selective to certain AAs than highly specialized plant-ants, which feed exclusively on plant-derived food rewards.[53] For example, the obligate plant-ant, *Pseudomyrmex ferrugineus* (a species that obligatorily inhabits *Acacia* host species and that is nutritionally dependent on the host-derived food[54]) showed specific preferences for solutions containing those four amino acids (phenylalanine, proline, valine and leucine) that are highly concentrated in the EFN of their host plant.[53] Unfortunately, little is known about the importance of AAs in the metabolism of ants and the detailed functions of these four AAs in the nutrition of *P. ferrugineus* remain to be uncovered. However, phenylalanine and proline were also found at high concentrations in FNs[45,55] and seem to be generally important in the metabolism of insects. For example, phenylalanine is one of the ten essential amino acids for honeybees[56,57] and is phagostimulatory to insects, because it stimulates sugar cell receptors on the labellar hairs of flies,[58] whereas proline is preferentially utilized during highly energy-demanding processes such as the initial phases of insect flight.[59] For ants, comparable information is lacking and further physiological and ecological studies are needed to determine the significance of specific AAs for their metabolism. Some physiological factors involved may be associated with ant species-specificity in taste reception or digestive systems,[60,61] while ecological factors would be associated with differences in nutritional requirements or in preference responses to AAs according to their diet.[62]

Secondary Metabolites and VOCs (Volatile Organic Compounds)

Summarizing the above paragraphs, sugars and AAs shape the nectar visitor assemblage according to its specific composition. Thus, their function mainly appears related to the varying physiological suitability of consumers, and, consequently, to their attractiveness to the different visitor guilds. By contrast, secondary metabolites that are commonly associated with herbivore defence, such as alkaloids and phenols, have been also described as nectar components[63] and in FNs of at least 21 plant families.[64] These compounds are usually regarded as "toxic compounds"[64] that plants secrete into floral nectar in order to achieve a protection from nectar robbers. For example, FN of *Catalpa speciosa* contains iridoid glycosides that fended off nectar robbers but not legitimate pollinators.[65] Similarly, phenols in FN of *Aloe vryheidensis* lowered its palatability to generalist insects and, thus, the attraction of non-effective pollinators to the flowers of this species.[66] By contrast, Adler and Irwin[67] manipulated contents of gelsemine, which represents the principal FN alkaloid of *Gelsemium sempervirens*, and found that the supplementation of gelsemine to FN deterred not only nectar robbers but also effective plant pollinators, thus decreasing the number of flowers probed and the time spent per flower by both, pollinators and nectar robbers.

Why should plants present a nectar meal that fends off legitimate pollinators? Short visitation times by pollinators do not necessarily represent a fitness disadvantage for plants. For example, nicotine in FN of wild tobacco was found to decrease consumption rates by certain pollinators but consequently increased the number of individual visits and, thus, the number of successful pollen transfers.[7] In short, more studies based on experimental manipulations of nectar secondary metabolites are needed to understand the detailed functions of "toxic compounds" in repelling nectar robbers and achieving fitness benefits, which plants get from FN secretion. Besides behavioral assays, identifying the source of secondary metabolite production in plants seems to be crucial to understand their real ecological functions. Secondary metabolites might be synthesized in the nectaries themselves, a situation that would make an adaptive function highly likely, but they can also be derived directly from the phloem and xylem,[64] thus suggesting a function that is not necessarily related to their appearance in the nectar.

Putative further benefits associated with secondary metabolites in nectar could include the inhibition of microbial growth.[64] For example, yeasts are commonly present in nectar[15] and can produce fermentation products that repel pollinator visits or impair their effectiveness.[68] Thus, nectar should present some kind of defence against microbe infestation. Indeed, antimicrobial activity has been described for floral rewards, such as resins[69] containing polyisoprenylated benzophenones.[70]

Whereas the FN secondary metabolites discussed above have mainly been related to some kind of protective functions, volatile organic compounds (VOCs) were related to pollinator attraction. The attractive functions of odors that are released from the petals are known since many years, but nectar odors were only recently considered as a signal that facilitates the detection of flowers by pollinators. Butterflies and moths preferred artificial flowers containing scented nectar over others that contained pure sugar solutions.[71] Furthermore, flower mites, which can compete with pollinators for nectar secreted by the host, can also use nectar odors as a cue to distinguish between host and non host plants,[72] nectar odors can, thus, cause an ecological cost for plants.

Nevertheless, nectar scent could also serve a defensive function and protect floral nectar from nectar robbers such as, for example, ants.[73,74] Kessler and Baldwin[7] studied the origin of VOCs in floral nectar of *Nicotiana attenuata* (Solanaceae) and the preferences of pollinators (hawkmoths and hummingbirds) and nectar robbers (ants) to them. The origin of scent in nectar has been related to volatiles released by the floral tissue, which would be absorbed by nectar and then solubilized in it.[75] Nevertheless, a wide array of VOCs occurred in nectar of *Nicotiana attenuata* plants[7] and many of these compounds were not detected in other flower parts, suggesting that nectar emits its own scent. Benzyl acetone (BA) and nicotine were the most attractant and repellent, respectively, floral VOCs found in tobacco plants. Field experiments using genetically transformed plants of *N. attenuata* indicated that both compounds were necessary to increase pollinator visits and fitness in tobacco plants: BA increased of pollinator visits whereas nicotine modulated the "nectaring" time, that is, the time individual visitors spent consuming nectar from an individual flower.[74]

While even scents of FN have received scientific attention only recently, information on VOCs that are putatively released from EFN is lacking completely. Nevertheless, behavioral studies demonstrated that EFN releases some odors. Wasps located EFN from short distances by its odor alone and found it almost as fast as honey and much faster than odorless sucrose solution.[76] Furthermore, when we presented Eppendorf tubes containing EFN of *Acacia* myrmecophytes, sugar solution, or water, to ants without allowing direct physical contact, the ants still distinguished among these different types of liquids (Silva-Bueno JC, González-Teuber M and Heil M, unpublished data); thus, odors likely play a role in the attraction of beneficial insects to EFN as they do in the case of tobacco FN.

Proteins

The first reports on nectar proteins date back to the sixties of the last century but only reported the mere presence of proteins in nectar,[22,77] whereas first characterizations of nectar proteins were published in the eighties.[78,79] Interestingly, the majority of those nectar proteins for which detailed functional and/or biochemical information is available by now were found to serve the protection of nectars from exploiters. For example, Peumans et al.[78] identified a mannose-specific lectin, a 13 kDa protein that was very abundant in nectar of *Allium porrum* (Alliinase) and related to protection against sucking insects or nematodes.[80,81] Nectar proteins have been well characterized for the FN of ornamental tobacco (*Nicotiana langsdorffii x N. sanderae*) where a limited array of five proteins were identified, the so-called "nectarines",[79] which range in size from 29 kDa to 65 kDa. The biochemical functions of those five nectarines are well identified[82-85] as serve the protection from microbial infestation through a novel biochemical pathway called the *Nectar Redox Cycle*.[86] Mainly three of these five nectarines are involved in the *Nectar Redox Cycle*: NEC1, NEC3 and NEC5. NEC1 represents approx the 50% of the total nectar proteins in Nicotiana sp. and was characterized as a manganese superoxide dismutase,[82] whose enzymatic function is related to the removal of superoxide and the generation of hydrogen peroxide in nectar. In addition, NEC5 functions together with NEC1 in the production of high peroxide levels: nectar of ornamental tobacco can accumulate up to 4 mM of hydrogen peroxide,[82] concentrations that are clearly high enough to exhibit toxicity on microorganism. Thus, the production of hydrogen peroxide appears to maintain floral nectar free of microbes and also might protect the gynoecium from microbial attack.

Nevertheless, high levels of hydrogen peroxide in nectar could also become deleterious due to its instability in presence of metal ions and the consecutive generation of free hydroxyl radicals. Interestingly, NEC3 was found to detoxify those free radicals: in-gel enzyme assays demonstrated that NEC3 has both carbonic anhydrase and monodehydroascorbate reductase activity[83] and can, thus, stabilize nectar pH. Although NEC4 was found not to be involved in the *Nectar Redox Cycle* its function still could be associated with the defence of nectar, because NEC4 showed xyloendoglucanase inhibitory activity and thus serves to inhibit fungal endoglucanases, which otherwise could degrade the hemicelluloses in the nectary cell walls.[87]

While tobacco FN contained only 5 proteins, more than 50 proteins with sizes between 20 and 50 kDa were recently discovered in the EFN of *Acacia* myrmecophytes, plants that house entire ant colonies for their indirect defence.[14] Two-dimensional gel electrophoresis and subsequent analysis with nanoLC-MS/MS of proteins present in EFN of the myrmecophyte, *A. cornigera*, identified the majority of these proteins as pathogenesis-related (PR) proteins such as chitinases, glucanases and thaumatin-like and osmotin-like proteins. The first two groups contributed more than 50% to the overall protein content of this EFN. Interestingly, no fungal growth was detected in field-derived EFN samples of Acacia-myrmecophytes although fungi could be cultivated from samples that were derived from closely related, but non-myrmecophytic, species. Because EFNs of the first plant group contained much higher protein contents than EFNs of the latter group, these results demonstrate that proteins play a highly significant role in the protection of EFN from microbial infestation.[14]

Conclusions and Future Perspectives

Nectar plays a significant role in the functional ecology of plants. In the present review we tried to summarize the major knowledge on functions that certain nectar constituents play as signals of plants to mutualistic animals and as means of nectar protection from exploiters such as nectar robbers and nectar-infesting microorganisms. Although floral and extrafloral nectars play ecologically distinct roles and serve in pollination or in herbivore defence, respectively, the general functions of certain nectar constituents were found to be strikingly similar. Sugars and amino acids were usually related to the nutritional function of nectars and, thus, to the attraction of mutualists, although several compounds appear physiologically suitable only for certain groups of nectar consumers and tailor the plant-visitor network. Likewise, nectar odors play a role in the attraction of mutualists to both FN and EFN, but repellent functions have also been reported for odors released from certain FNs. By contrast, secondary compounds such as alkaloids and phenols usually protect nectar from unintended consumption. The same remains true for nectar proteins, whose function in keeping the nectar free of microbes has been reported for EFN and FN. Nectar chemistry serves both, the attraction of nectar visitors that exert a positive effect on plant fitness and the repellence or putative intoxification of exploiting organisms. However, we still lack crucial knowledge on the physiological and ecological functions of many nectar compounds and, particularly, on the regulation of nectar secretion and the sites of synthesis of most of its components. Future studies should figure out (1) the specific tissues in which nectar component production takes place and (2) how nectar secretion is controlled by plants, in order to completely elucidate the adaptive significance of nectar to plants.

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