

Nectar: Plant Interface for Complex Interaction with Biotic Environment

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ABSTRACT

Angiosperms' nectar, floral and extrafloral, is a valuable energetic alimentary resource for a large variety of animals from insects to small mammals, birds, marsupials and reptiles. It frequently mediates mutualistic relationships between the two partners. In recent years it was clearly demonstrated that this relationships actually often involves other partners: nectar dwelling micro-organisms. Yeasts and bacteria may alter considerably the nectar composition and can be the causal agents of some plant diseases. Nectar has biochemical defences to inhibit micro-organism proliferation, i.e., a heterogeneous arsenal of proteins with anti-fungal and anti-microbial activity that has just been discovered in the last years. Nonetheless yeasts are almost ubiquitous in nectar and their direct and indirect interactions with nectar foragers are almost unknown as well as the consequences for plant reproduction. A recent advance in nectar biology was the recognition of some secondary compounds, especially alkaloids and non-protein amino acids, in modulating the foraging behaviour of nectar feeders through several effects on insect neurophysiology. All these studies demonstrate that nectar has a wider range of more complex interactions than previously thought.

Keywords: nectar, defence, foragers behaviour, proteins, secondary compounds, non-protein amino acids

Introduction

Nectar is widespread amongst angiosperms and it is exploited as an alimentary resource by a great variety of animals (invertebrates and vertebrates) which may pollinate or defend the plant from herbivores (Nicolson 2007; Heil 2011). Until recently, most studies concentrated on the ability of nectar to attract foragers, largely due to its high concentrations of simple sugars, namely: sucrose, glucose and fructose, as well as its lower content of amino acids. Fundamentally an aqueous solution, nectar is easily ingested, digested and absorbed by the alimentary canal, and is thus a very cost-effective alimentary resource for a wide variety of animals (Nicolson 2007; González-Teuber and Heil 2009). This classical “alimentary” perspective of nectar has recently been challenged by other studies that have both detailed and shown the presence of compounds that are not directly related to the alimentary value of nectar. For example, it was shown that floral and extrafloral nectar may contain a large and heterogeneous assemblage of defence proteins that are active against micro-organisms (Carter and Thornburg 2000, 2004; Naqvi et al. 2005; Carter et al. 2007; Kram et al. 2008; González-Teuber et al. 2009, 2010; Hillwig et al. 2011; Nepi et al. 2011). Some of these micro-organisms are phytopathogens (Bubàn et al. 2003; Farkas et al. 2007). Others are not dangerous for the plant but may interfere with the pollinator’s alimentary choice. Yeasts are known to inhabit floral nectar, changing considerably the chemical composition of nectar and consequently affecting the relationships with nectar foragers (Herrera et al. 2008, 2009). It was hypothesized that nectar inhabiting micro-organisms may act as a third party in the mutualistic relationships linking plants and nectar foragers, an emerging tripartite relationship whose ecological and evolutionary significance is still far from being well established (Canto and Herrera 2012).

Moreover, data obtained from recent research that focused on secondary compounds in floral nectar contrasted markedly with the attractiveness of nectar to insects. Generally, nectary alkaloids and phenols are toxic to insects and deter insect foragers that are nectar-thieves, but not genuine pollinators, from visiting the flowers (González-Teuber and Heil 2009 and references therein). However, sometimes, these compounds deter both nectar-thieves and pollinators.

Within this complex context, one particular class of substances that potentially has an important role, namely, the non-protein amino-acids (NPAAs), has received almost no attention. Whereas only twenty amino acids are involved in building proteins, thousands of NPAAs have a myriad

of other functions. Of these amino acids, 250 are found in plants, and it is becoming increasingly clear that these play significant roles both in ecological and physiological processes (Vranova et al. 2011). The existence of non-protein amino acids in floral nectar was first reported in early surveys of nectar composition dating from the 1970s (Baker and Baker 1975; Baker 1977, 1978; Baker et al. 1978). Although several ecological and physiological functions have been attributed to a number of non-protein amino acids derived from both animals and plants, their role in nectar has still received little attention.

Based on the recent works mentioned above, it is evident that floral nectar exhibits an array of potential functions in its interaction with organisms that far exceeds that of a simple alimentary reward. Some of these functions are involved, directly or indirectly, in modulating the foraging behaviour of foragers. In this chapter we discuss the more significant recent advances in floral nectar biology and ecology namely the characterization of nectar proteome and nectar secondary compounds, as well as the interaction with yeasts. The purpose is to stimulate future research and scientific debate in the context of ecological functions of nectar in shaping the complex web of plant-animal interactions.

Nectar Proteins and Interaction with Micro-Organisms

Defence Against Pathogens

Because of its sugar composition, nectar is an excellent environment for the growth of airborne organisms or for those carried by pollinators (Fig. 1).

Some micro-organisms are phytopathogens and may penetrate plant tissues through nectarostomata such as *Erwinia amylovora* and *E. tracheifila* that are the causal pathogens of fire blight and bacterial wilt disease respectively (Bubán et al. 2003; Sasu et al. 2010). Thus the plants must defend their nectar from micro-organisms' proliferation by means of a heterogeneous array of anti-microbial substances. The anti-microbial activity of phenolic compounds, as well as alkaloids, substances that may be components of nectar (see next paragraph), is well documented (Adler 2000; González-Teuber and Heil 2009 and references therein). Nectar proteins are known to have a role in defence against fungi and bacteria as well. A new and exclusive metabolic pathway (the so-called Nectar Redox Cycle, NRC) that serves mainly to maintain high levels of hydrogen peroxide and involves 5 enzymes (nectarin I–V) has been found in the nectar of *Nicotiana langsdorffii* × *N. sanderae* (Carter and Thornburg 2000, 2004; Carter et al. 2007). In this way the nectar is maintained in a sterile state. The floral nectar of *Petunia hybrida* contains several RNases, a peroxidase and an endochitinase whose anti-microbial activity has recently been recognized (Hillwig et al.

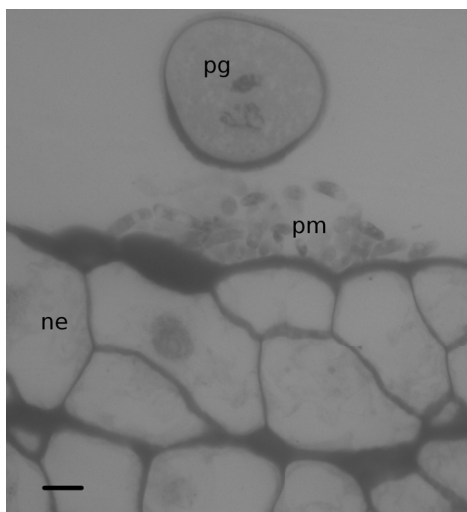


Figure 1. Pollen grain (pg) and proliferating micro-organisms (pm) on the floral nectary surface (ne = nectary epidermis) of *Helleborus foetidus* (Toluidine Blue O staining). Scale bar = 10 μ m.

2010, 2011). A lipase (JNP1) was reported for the floral nectar of *Jacaranda mimosifolia* but its anti-microbial activity has not yet been demonstrated (Kram et al. 2008). Four isoforms of β -xylosidases have recently been identified as main proteins in the male and female floral nectar of *C. pepo* and their putative antimicrobial activity has been hypothesized (Nepi et al. 2011). Extrafloral nectar also has its own enzymatic defence against invasion by micro-organisms, the predominant enzymes being chitinase, β -1, 3-glucanase and thaumatine-like proteins (González-Teuber et al. 2009, 2010). Defence proteins such as glucosidases, chitinases, hydrolases, and thaumatine-like proteins have also been detected in pollination drops (Wagner et al. 2007), secretions produced by the ovules of gymnosperms that possess a chemical composition similar to that of angiosperm nectar (Nepi et al. 2009; see also chapter by von Aderkas et al. in this book). It appears that pathogenesis-related proteins are abundant in all the environment-exposed sugary secretions of spermatophytes probably due to a convergent chemical evolution towards protection from invading micro-organisms. Apparently extra-floral nectar, being more exposed and less ephemeral, is characterised by a higher number of proteins than floral nectar (González-Teuber et al. 2009). In all these secretions defence proteins may explain their antibiotic activity in two ways: killing the micro-organisms, for example by degrading their cell wall through chitinases and glucanases, or by reducing their pathogenic potential. Defence proteins may limit the lytic activity of micro-organisms both directly, by inhibiting enzymes responsible of plant

cell wall degradation (Naqvi et al. 2005) and indirectly by reducing the quantity of elicitor molecules that stimulate the production of the degrading enzymes (Nepi et al. 2011).

Yeasts are Frequently Associated with Floral Nectar

Despite this heterogeneous defence “arsenal”, nectar is frequently contaminated by yeasts that deeply alter the chemical composition of nectar (Herrera et al. 2009). About half of the samples analyzed in tropical and temperate plant communities have tested positive for yeasts with the predominance of basidiomycetous and ascomycetous species from the genera *Cryptococcus*, *Metschnikowia* and *Candida* (Herrera et al. 2009; Canto and Herrera 2012). Persistence of microbial communities in nectar requires continuous cycles of immigration-multiplication-dispersal as new host flowers constantly appear and disappear in the landscape (Pozo et al. 2012). As nectar chemistry can vary strongly within and between different plant species (Baker and Baker 1975; Nicolson and Thornburg 2007) proliferation depends on the ability of the micro-organisms to cope with a broad range of nectar environments (comprising the heterogeneous presence of antimicrobial compounds) and thus requires the ability to rapidly adapt to different nectar conditions. Pollinators, most often insects, are likely the most important candidates for transferring microbes from one flower to another (Herrera et al. 2009). Recently it was established that ants also, although not generally involved in pollination but rather in plant defence while foraging for extra-floral nectar (Gonzalez-Teuber and Heil 2009), may participate in transferring micro-organisms into floral nectar (de Vega and Herrera 2012, 2013) and possibly they can contaminate extra-floral nectar when moving to extra-floral nectaries.

Immigration and dispersal therefore depend on patterns and frequency of forager visits and movements (Pozo et al. 2012). It was demonstrated that different nectar feeders have different probability to contaminate floral nectar with yeasts. In 22 plant species from Cazorla (southern Spain), yeast frequency and abundance were significantly related to differences in the relative importance of solitary bees vs. bumble-bees in the pollinator assemblage: yeast incidence was more marked in species pollinated mainly by bumblebees (Herrera et al. 2009). Identifications of yeast isolates suggest that the composition of nectar yeast assemblages varies among plant species and that that nectar yeasts impose a detectable imprint on community-wide variation in nectar sugar composition and concentration (Canto and Herrera 2012). This pattern is determined by several factors, including variation among plants species in concentration or effectiveness of antifungal defences in nectar, variability of composition of their associated nectar yeast communities, perhaps as a consequence of differences in pollinator type.

Yeasts are responsible of drastic changes in nectar attributes that may affect forager's behaviour mainly in three ways. First, the presence of yeast decrease the alimentary value of nectar because it causes a drastic changes in sugar and amino acids profiles. Through fermentation they cause a decrease in total sugar concentration and extensive reduction in sucrose percentage (Herrera et al. 2009; Canto et al. 2011; de Vega and Herrera 2013). Since sugars are the dominant chemical constituents of most nectar, providing the key energetic reward for several animals, their variation may influence choices during foraging bouts. Preferences for specific sugar profile are known for different classes of nectar feeding animals (Nicolson 2007). Yeasts cause also degradation of amino acids that they utilize as nitrogen source (Peay et al. 2012). As for sugars, pollinating animals and herbivore-defending ants have preferences for specific amino acid profiles. Thus drastic modifications of nectar composition induced by yeasts may significantly alter nectar attractiveness to specific foragers.

Second, nectar fermentation by yeasts produces ethanol that may rise to levels that are toxic to foragers. The fungus *Cladosporium* is responsible of ethanol production in the nectar of the orchid *Epipactis helleborine* (Ehlers and Olesen 1996). Wasps became very slow and "sluggish" when drinking this nectar and they groomed their body less for pollinia. This, lowering pollinia loss, may enhance pollinia transfer and thus orchid's reproduction (Ehlers and Olesen 1996).

Third, nectar fermentation is also responsible of the emission of volatile compounds that contribute to the scent of flower's headspace perceived by foraging insects (Raguso 2004 and references therein). It is well demonstrated that changes in flower odor may affect plant-animal interactions (Raguso 2009). The nectar of *Agave palmeri* contains sorbic acid that has antimicrobial activity. Sorbic acid is most probably transformed by yeasts into ethyl sorbate that confers to nectar a specific odor (Raguso 2004). While floral nectar scents have been considered in the interaction with pollinators, much less is known about odors in extra-floral nectar although it was recognized that they are likely to play a role in the attraction of beneficial insects to EFN (González-Teuber and Heil 2009).

It is clear that nectar dwelling yeasts may have several important effects in plant-animal interactions mediated by nectar. Very few studies deal with the extent to which these effects may interfere with the behaviour of foraging animals and, in turn, with plant reproduction. Kevan et al. (1988) failed to find evidence of yeasts influencing flower choice by honey bees. More recently Herrera et al. (2013) demonstrated that bumble bees can detect the presence of yeasts in artificial nectar and responded positively by paying proportionally more visits to yeast-containing flowers. This preference is detrimental for the reproduction of *Helleborus foetidus* probably because the longer visits by pollinators to yeast-containing flowers would enhance

autogamy with consequent reduction in number and size of seeds produced (Herrera et al. 2013). Unraveling details of these complex interactions will require further studies.

Nectar Secondary Compounds Interact with Foragers

Secondary metabolites, including tannins, phenols, alkaloids and terpenes, have been found in floral nectar in more than 21 angiosperm families (Adler 2000). These compounds have been known since the 70s and they were considered to be toxic deterrents against predators (Baker and Baker 1983). Recently, researchers have discovered that these compounds may play an important role in managing visitors' behaviour. Flowers face a multidimensional challenge: they need to attract visitors, to compel them to vector pollen with the least investment in rewards, and to repel nectar robbers at the same time. All of this is in the service of maximizing fitness. The bouquet of secondary compounds may serve a number of these objectives.

Alkaloids

Kessler et al. (2008) discovered that both the repellent nicotine and the attractant benzyl acetone were required to maximize capsule production and flower visitation by native pollinators, at the same time, nicotine reduced nectar robbing by non-pollinating animals. The presence of nicotine, a typical insect-repelling alkaloid, is necessary to optimize the number of flower visitors per aliquot volume of nectar produced by flowers of *Nicotiana attenuata*, thereby allowing plants to minimize nectar volumes, whilst maximizing transfer of pollen and seed production events (Kessler and Baldwin 2007).

According to Singaravelan et al. (2005) naturally occurring concentrations of secondary compounds such as caffeine, nicotine, anabasine, and amygdaline did not deter insects. Secondary compounds can be regarded as post-ingestion stimulants to pollinators. Low concentrations of psychoactive alkaloids, nicotine and caffeine, increased nectar feeding significantly. These compounds may have been part of the reward. The presence of psychoactive alkaloids, such as these, in nectar, may also impose a dependence or addiction on pollinators, as well as improving the short-term and early, long-term memory of honeybees (Singaravelan 2010). The latter effect was recently proved by Wright et al. (2013) demonstrating that honeybees rewarded with caffeine, which occurs naturally in nectar of *Coffea* and *Citrus* species, have a higher ability to remember a learned floral scent than honeybees rewarded with sucrose alone. Caffeine concentrations in nectar did not exceed the bees' bitter taste threshold, implying that

pollinators impose selection for nectar that is pharmacologically active but not repellent.

Secondary compounds in nectar are known to have antimicrobial properties (Adler 2000 and references therein). Compounds may not only provide a direct defence from microbial invasion, but can indirectly protect the consuming animal. Manson et al. (2010) demonstrated that consumption of the nectar alkaloid gelsemine found in *Gelsemium sempervirens* reduces pathogen loads in bumblebees. It also protects bees from infection, which, in the long run, improves their foraging efficiency.

Non-protein Amino Acids

Baker et al. (1978) reported the presence of NPAAAs in the floral nectar of 35% of 248 broadly distributed species of flowering plants. This percentage increased to 55% when these substances were searched for in the floral nectar of 69 species of tropical trees and lianes (Baker 1978). Guerrant and Fiedler (1981) reported the presence of NPAAAs in 13 out of 25 species (52%) growing in dry and wet forests of Costa Rica. Petanidou (2007) found NPAAAs in the nectar of 86% of 73 plant species of the phrygana community. While studying the nectar chemistry of the tribe Lithospermeae (Boraginaceae), we found NPAAAs in a total of 49 taxa (unpublished data). Surely we can state that NPAAAs are not so much uncommon, but rather that they are almost ubiquitous in floral nectar.

Unfortunately, most of the early surveys of nectar chemistry reported only the presence/absence of NPAAAs, without any specific determinations. In the years that followed, some NPAAAs were detected and determined in floral nectar. These included: β -alanine, γ -amino butyric acid (GABA), α -amino butyric acid (AABA), taurine, ornithine and citrulline, of which GABA and β -alanine appear to be the most common (Baker and Baker 1975; Baker 1977, 1978; Baker et al. 1978; Inouye and Inouye 1980; Guerrant and Fiedler 1981; Gardener and Gillman 2001; Kaczorowski et al. 2005; Petanidou et al. 2006; Nepi et al. 2012; Nocentini et al. 2012; Peay et al. 2012). GABA occurs at the highest concentration, ranging from 0.57 nmoles/ml in *Nicotiana glauca* (Kaczorowski et al. 2005) to about 750 nmoles/ml in *Agrostemma githago* (Gardener and Gillman 2001).

The ecological role of nectar NPAAAs

The early report of NPAA in floral nectar (Baker 1977) stated: "it is likely that at least some of them will prove to be toxic to certain kinds of flower-visitor". This hypothesis was later developed in the so-called pollinator fidelity theory applied to nectar secondary compounds. According to this latter theory, toxic compounds encourage foraging by specialist pollinators,

while deterring visits by erratic or undesirable (nectar-thieves) insects that either deliver less or no intraspecific pollen (Adler 2000). Furthermore, it assumes that specialist pollinators are more resistant to specific toxicants than generalists. Unfortunately, the toxicity of NPAAAs found in nectar to foraging insects is largely unknown. With regard to plant secondary compounds, the effect of NPAAAs has been mainly tested on herbivorous insects, not on nectar feeders. However, a direct toxic effect was reported in *Choristoneura rosaceana* (oblique-banded leaf roller, Lepidoptera) larvae fed on an artificial diet enriched with GABA (Bown et al. 2006). Toxic effects resulting in reduced growth and reduced survival rate were found for a concentration of 2.6 mM, i.e., about three-fold of the highest concentration found in nectar (see above).

The toxic or deterrent effect is dependent on the amino acid concentration of nectar, the rate of intake and the sensitivity of its consumers. It should be noted, however, that deterrent substances are not necessarily toxic, and that substances thought to be toxic are not necessarily deterrents (Singaravelan 2010). According to Inouye and Inouye (1980), the relatively high proportion of NPAAAs found in the extra-floral nectar of *Helianthella quinquenervis* suggests that these particular compounds are neither toxic to nor deter ants. Furthermore, they do not appear to have a deterrent effect on wasps, beetles, flies or any other insects that collect nectar in the absence of ants. Ants are also common floral nectar thieves, but NPAAAs present in the floral nectar rarely deter these insects from visiting flowers (Guerrant and Fiedler 1981).

Thus, from the scant literature available, it would appear that NPAAAs are not obviously involved in the exclusion of undesirable nectar foragers, rather, they seem to be more common in the floral nectar of species pollinated by specific pollinator guilds and in particular, those pollinated by Hymenoptera. Baker and Baker (1978) found that NPAAAs are more common in the floral nectar of tropical trees and lianes that are pollinated by Hymenoptera than in those that are visited by Lepidoptera, bats, or birds. Petanidou et al. (2006), who studied the nectar composition of the phrygana community species, presented similar data. These authors found that high levels of GABA could be correlated with long-tongued bees, anthophorid and andrenid bees, as well as anthomyiid and syrphid flies. Interestingly, bees and bumble bees are among the pollinators of those species that have higher GABA concentrations (Gardener and Gillman 2001; Kaczorowski et al. 2005; Petanidou et al. 2006; Nepi et al. 2012; Nocentini et al. 2012).

NPAAAs and defence against fungi and bacteria. Extracellular GABA was reported to be involved in plant communication with other organisms (Shelp et al. 2006) and accumulates in response to infection by fungi and bacteria (Chevrot et al. 2006; Oliver and Solomon 2004). It was also

demonstrated that accumulated, extracellular GABA reduced the virulence of *Agrobacterium tumefaciens* in tobacco leaves by inducing the synthesis of enzymes that modulate the infection process (Chevrot et al. 2006). Furthermore, GABA functions in communication between tomato plants and the fungus *Cladosporium fulvum* (Oliver and Solomon 2004). During infection, GABA concentration in the apoplast increases from about 0.8 mM to 2–3 mM, concentrations that resemble and are about three-fold greater, respectively, than the highest concentration found in floral nectar (Gardener and Gillmann 2001). No specific experiments have yet been undertaken to assess the eventual change in GABA concentration following infection by micro-organisms. The only report to date indicates that several species of yeast have little effect on GABA concentration in the floral nectar of *Mimulus aurantiacus*, whereas other amino acids (such as glutamic acid, aspartic acid and proline) were almost completely used as a nitrogen source by these fungi (Peay et al. 2012).

Moreover, β -aminobutyric acid (BABA), which is structurally related to GABA, but is much less common in nature, including nectar, seems to play a broad role in increasing plant defence against biotic stress, such as invasion by viral, bacterial and fungal pathogens, by priming plants to respond more rapidly and to a greater degree to future stress events (Huang et al. 2011).

This particular biological property of GABA and BABA may contribute to the function of nectar in protecting plants from invasion by pathogenic organisms that based on our present knowledge, it is mainly associated with nectar proteins (Nepi et al. 2011; Park and Thornburg 2009).

NPAAs may affect the foraging activity of nectar feeders. NPAAs may potentially affect the foraging behaviour of insects in three different ways: 1) by directly affecting the insect nervous system; 2) by contributing in regulating the feeding rate (phagostimulation); 3) by increasing the activity of flight muscles.

GABA, taurine and β -alanine are abundant in the nervous systems of insects (Bicker 1991; Gardener and Gillman 2001), where they function as inhibitory neurotransmitters. GABA acts in synergy with taurine, limiting excessive, potentially disruptive excitation states during stressful conditions, probably in antagonism with octopamine in arousal pathways (Stevenson 1999 and references therein). GABA is also the principal inhibitory neurotransmitter in the vertebrate brain and its levels are strictly regulated by transport across the blood–brain barrier. By contrast, GABA-receptors in invertebrates are located peripherally in muscle tissue and neuromuscular junctions, where they are bathed in haemolymph (Bown et al. 2006) and are thus more sensitive to changes in GABA eventually precipitated by GABA-rich nectar feeding. GABA is also important in the

development of the nervous system by acting as a cell-to-cell signal during embryonic and adult neurogenesis in animals, and changes to its synthesis or degradation can cause severe clinical disorders (Bouché et al. 2003) such as seizures, hypotonia, lethargy and severely retarded psychomotor development (Medina-Kauwe 1999). Recently, a colleague observed some bumble bees apparently in a semi-paralyzed condition after visiting the flowers and feeding on the nectar of *Gentiana lutea* (Marta Galloni, personal communication). Analysis of nectar amino acid profile revealed a very high concentration of β -alanine (2.3 mM) two-fold higher than that of proline, which is often the dominant amino acid in nectar. Despite the absence of unequivocal evidence, we suspect that insects accumulate sufficient neurotransmitter to induce lethargic behaviour.

Plants can regulate the feeding behaviour of animals via several metabolites—mainly sugars and amino acids (Shoonhoven et al. 2005). Unfortunately, once again, the feeding behaviour of herbivorous insects has been studied much more than that of nectarivorous species. In the case of locusts, beetles and caterpillars, sucrose and fructose are common and powerful feeding stimulants (Shoonhoven et al. 2005). The two protein amino acids proline and phenylalanine are amongst the most abundant nectar amino acids (Petanidou 2007; Nicolson and Thornburg 2007) and display strong phagostimulatory activity. Interestingly, another potential effect of a particular NPAA, namely, GABA, is the stimulation of chemoreceptors, and this results in the increased feeding behaviour of some caterpillars and adult beetles (Shoonhovet et al. 2005). Furthermore, co-administration of GABA can overcome the antifeedant activity of terpenoids (Passreiter and Isman 1997). Terpenoids can accumulate in the nectar of several species (Raguso 2004), and high levels of nectar GABA may contribute towards the maintenance of an adequate feeding rate (Nicolson and Thornburg 2007).

Several NPAAs appear to be involved in improving muscle performance. For example, β -alanine is a precursor of the dipeptide carnosine that is found in both vertebrate and non-vertebrate skeletal muscles, and acts as a limiting factor to its synthesis (Harris et al. 2006a). It has been demonstrated that carnosine can increase isometric endurance in humans, and β -alanine uptake can be enhanced by the assumption of simple sugars (Harris et al. 2006b; Sale et al. 2012). In fact, β -alanine, taurine and GABA are used by athletes to increase their performance and reduce fatigue (Hill et al. 2007; Zhang et al. 2004; Watanabe et al. 2002).

High concentrations of taurine have been found in several orders of insects, usually in the thoracic region of adults, where they are associated with fully functional flight muscles (Whitton et al. 1987).

The maintenance of good muscle performance by foraging insects is clearly advantageous to the plant as it ensures greater pollinator movements between flowers, plants and populations, thus favouring pollen and gene flow.

Concluding Remarks

Recent researches demonstrated that the relationships between nectar and organisms, from micro-organisms to large animals, are much more complex than we thought before (Fig. 2).

A recent fundamental step forward in this direction was provided by conclusive evidences that nectar sugar composition is not completely controlled by the plant and that this crucial food source for a variety

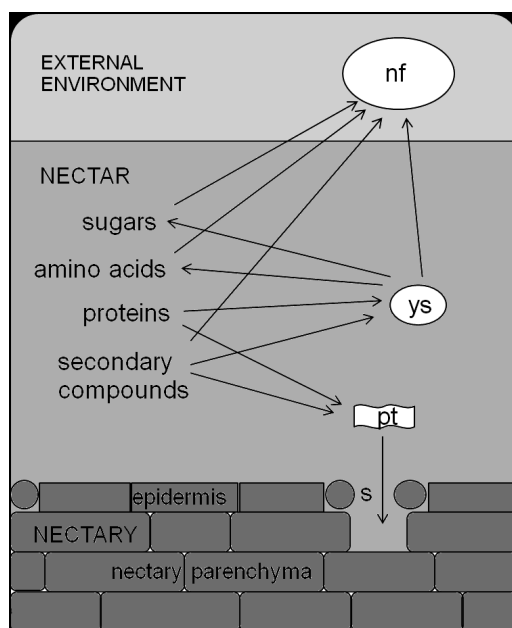


Figure 2. Web of interactions between nectar components and the biotic environment inside (yeasts and bacteria) and outside nectar (nectar feeders). The profile of the main nectar metabolites (sugars and amino acids) determine the alimentary choice of nectar feeders. Nectarins (nectar proteins) are a biochemical defence against micro-organisms (yeasts and bacteria). Secondary compounds (alkaloids and non protein amino acids) have multifunctional activity being involved in the interactions with micro-organisms and nectar feeders. Bacteria can be pathogens that invade the plant tissues penetrating through nectary stomata. Yeasts may affect feeder's behaviour directly, by producing alcohols or volatile compounds, or indirectly by changing considerably sugars and amino acids profile. Nectar feeders are responsible of micro-organism's transport from flower to flower. pt = pathogen bacterium; nf = nectar feeders; s = stoma; ys = yeast.

of organisms may be influenced by external biological factors such as contamination by micro-organisms, particularly yeasts (Canto and Herrera 2012; de Vega and Herrera 2013). Yeasts are also able to interfere with the behaviour of foragers and the functional links between nectar dwelling yeasts, nectar feeders and plant reproduction are going to be discovered (Herrera et al. 2013).

A further step that is awaited in the next future is a better integration between nectar chemistry and insect's physiology and neurobiology. Although specific studies are lacking for most insects, and there is currently little information available on the metabolism of non-protein amino acids and their fate following ingestion by insects, a complex, ecophysiological picture is beginning to emerge: proline powers the take-off of the insect from the flower (Carter et al. 2006), sugars propel its prolonged flight, and taurine, GABA and alanine promote the highly efficient functioning of flight muscles. Meanwhile, insects are forced to move from flower to flower by the combined phagostimulatory activity of proline, phenylalanine and GABA, which maintain a high feeding rate. Alkaloids, such as nicotine and caffeine, may serve to reinforce the fidelity of foragers by improving their memory ability and/or inducing dependence (Singaravelan et al. 2010; Wright et al. 2013). Excessive excitation due to hunger can be reduced by the intake of inhibitory neurotransmitters that keep the insect calm. All these are plausible hypotheses whose confirmation may open up new perspectives on plant-animal relationships.

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