Nectar Secretion: Its Ecological Context and Physiological Regulation

María Escalante-Pérez and Martin Heil

Abstract Plants secrete nectar to attract pollinators and indirect defenders. The chemical contents of both floral and extrafloral nectar appear adapted to attract and nourish these two classes of animal mutualists. Being rich in sugars and amino acids, however, nectar also requires protection from nectar robbers and infecting micro-organisms. This role is mainly fulfilled by nectar proteins (nectarins) and by secondary compounds such as alkaloids. Although much on the chemical ecology of nectar and the phenotypic patterns of its secretion is known, we have only limited knowledge on the molecular control of the synthesis of nectar components and of nectar secretion. Likewise, carbohydrates are uploaded from the phloem into the nectariferous tissue, where they might move via an apoplastic or a vesicle-bound, symplastic pathway. Cell wall invertases play a central role in creating the required source–sink relations and controlling the sucrose/hexose ratio of nectars. No information exits on the sites of synthesis of non-carbohydrate nectar components such as proteins and alkaloids, although it appears likely that at least the bulk of nectarins is synthesized in the nectariferous tissue itself. Most of the common model species do not depend on nectar secretion, and it might be this fact that has hindered nectar research over the last 50 years. We recommend the use of contemporary "omics" techniques in comparative approaches to understand how plants synthesize and secrete nectar.

1 Introduction

Nectar serves for the attraction and nutrition of animal partners that are engaged in two major types of plant–animal mutualisms, in which plants make use of the mobility of animals: pollination and indirect defence (Heil 2008, 2011;

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Brandenburg et al. 2009). Both types of mutualisms can also be enabled by other types of resources, but floral and extrafloral nectar appear by far the most important plant rewards in this context. By definition, floral nectar (FN) is secreted within the flower (Fig. 1) and serves pollination, whereas extrafloral nectar (EFN) is secreted commonly, but not necessarily, outside the inflorescences (Fig. 2) and serves the indirect defence of the plant by intensifying its tritrophic interactions with carnivores (Bentley 1977; Elias 1983).

Although its general importance is being widely appreciated, nectar remains a surprisingly understudied discipline within plant science (Sect. 6). For example, little is known about nectar components other than the sugars and amino acids (Nicolson et al. 2007; González-Teuber and Heil 2009a), and new classes of substances continue to be detected in nectar. Even less is known about the synthesis
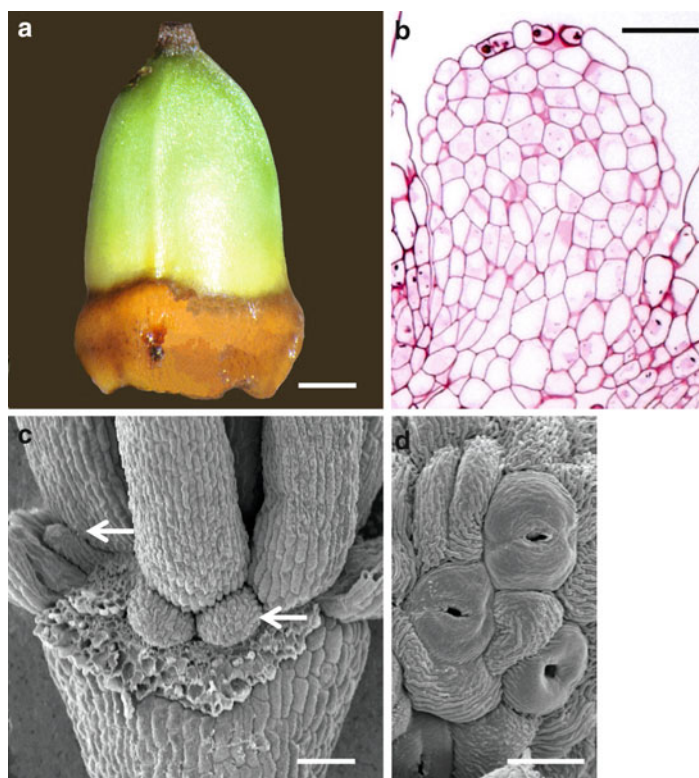

Fig. 1 Floral nectaries of *Arabidopsis thaliana* and ornamental tobacco. (**a**) Light microscopy of fresh, isolated S12 tobacco gynoecium showing the *light green* ovary and the *orange* nectary at its base, *bar* = 1 mm. (**b**) Light microscopy of 1-μm-thick sections of *Arabidopsis thaliana* floral nectaries stained showing two stomata, *bar* = 25 μm. (**c**) Scanning electron micrograph of dissected *Arabidopsis* flower showing two types of nectaries (*arrows*) located between petals (*removed*) and stamens, *bar* = 100 μm. (**d**) Enlargement of medial nectary (*right arrow* of **c**), *bar* = 25 μm. Reproduced from Ren et al. (2007b). Photographs courtesy of Harry T. Horner and Robert Thornburg (**a**) and Rosanne Healy (**b–d**)
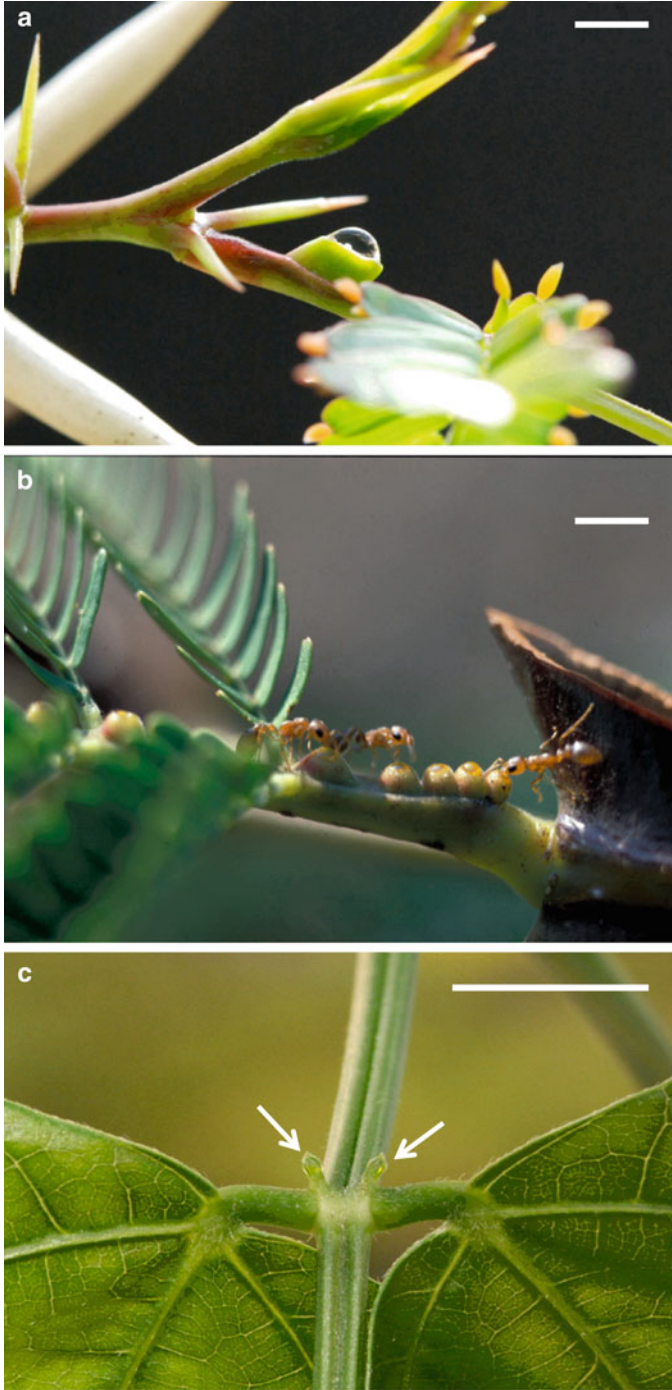
Fig. 2 Extrafloral nectary size depends on function. The size and anatomical complexity of extrafloral nectaries are often related to their function. Obligate myrmecophytes house and nourish

of nectar components and the regulation of their secretion. In this chapter, we review the general knowledge that is available on FN as well as EFN and focus particularly on those fields in which several exciting discoveries have been made over the last decade.

For example, jasmonic acid (JA) has been identified as a hormone that affects the secretion of both FN (Radhika et al. 2010) and EFN (Heil et al. 2001, 2004; Heil 2004), and a recently discovered gene that encodes an apoplastic invertase in *Arabidopsis* (*Arabidopsis thaliana*) represents the first gene whose function is required for FN secretion (Ruhlmann et al. 2010). Nectarins (nectar proteins) were identified in tobacco (*Nicotiana* spp.) FN, *Acacia* EFN and pollination droplets of gymnosperms. The function of these nectarins is likely to be the protection against microbial infection (Carter and Thornburg 2004; Carter et al. 2007; Wagner et al. 2007; Kram et al. 2008; González-Teuber et al. 2009, 2010). Other enzymes were found to play central roles in the post-secretory tailoring of nectar chemistry (Heil et al. 2005; Kram et al. 2008).

It is still not known, however, how nectar secretion takes place and how it is controlled at the physiological, cellular and genetic levels, how plants can adapt nectar secretion to consumption rates and/or consumer identity, and how nectar that has not been consumed can be re-absorbed, although all these phenomena are well described at the phenotypic level (Pacini et al. 2003; Pacini and Nepi 2007; Heil 2011). We therefore conclude our chapter with a short discussion on how contemporary methods could be used to "wake up the sleeping beauty" of nectar research. Ongoing research has produced many expressed sequence tags from nectaries or used gene chips to investigate large-scale transcriptional changes during nectar secretion (Kram et al. 2009; Hampton et al. 2010), and the first proteomes have been obtained from nectars of various species (Peumans et al. 1997; González-Teuber et al. 2009; Park and Thornburg 2009; Hillwig et al. 2010). The field is ready for breakthroughs with regard to the (bio)chemistry of nectar and the physiological and genetic regulations of its synthesis and secretion.

2 The Role of Nectar in Plant Reproduction and Defence

How important are nectars in the above-mentioned mutualisms? Pollinators of higher plants are most commonly insects and birds. They are attracted to flowers by pollination syndromes combining flower shape, colour and odour with nectar and pollen at different quantities and qualities. Pollination by insects is generally

Fig. 2 (continued) specific ant colonies and are characterized by large nectaries that secrete a chemically highly complex EFN. (**a**) Extrafloral nectary of *Acacia cornigera*; (**b**) *Pseudomyrmex peperi* ants feeding on extrafloral nectaries of *A. collinsii*. By contrast, the extrafloral nectaries of lima bean, *Phaseolus lunatus* (**c**: *white arrows*), serve only in the facultative attraction of defenders, are localized on stipules, much smaller, and secrete a chemically more simple EFN. *Bars*: 1 cm. Photographs taken by Antonio Cisneros (**a**), Martin Heil (**b**) and Christian Kost (**c**)

assumed to increase the specificity of the pollen transport among conspecific flowers and thereby guarantee adequate fertilization and outcrossing (de la Barrera and Nobel 2004; Brandenburg et al. 2009). In spite of its apparent frequency and presumed importance, however, little experimental evidence exists for the positive effect of plant nectar on pollination success and, ultimately, plant fitness. Among the Orchidaceae, for example, nectariferous species are more successful at setting fruits than nectariless species (Neiland and Wilcock 1998). However, we are not aware of similar correlational evidence from other study systems.

Although we can assume that the mere presence of nectar clearly benefits plants by increasing pollination rates (Neiland and Wilcock 1998), we must ask: does more nectar always mean more service, and what can plants do to minimize the resulting costs? Apparently, only two studies have investigated the correlation among FN consumption rates and pollinator efficiency, and both have found contrasting patterns. In wild tobacco (*Nicotiana attenuata*), pollinators are attracted from distance via the nectar-released odour, in which particularly benzyl acetone played a crucial role in increasing the number of pollinator visits. Surprisingly, the nicotine that is present in tobacco FN reduced the nectar uptake during single visits. The combination of these compounds ultimately increased the number of floral visits, particularly by hummingbirds (Kessler and Baldwin 2007; Kessler et al. 2008). By combining a long-distance attractant with a presumably bitter taste of the nectar, wild tobacco appears to have developed a strategy to maximize outcrossing with minimum investment in FN amounts. Can all plants minimize their energy investment in nectar and still maintain a high number of pollinator visits? Flowers of *Petunia* plants that were bred to produce reduced FN amounts with unchanged morphological traits of the flower paid for this attempt to "cheat" their pollinators with reduced visitation frequency by *Manduca sexta* moths and a concomitant reduction in seed production (A. Brandenburg, personal communication, and Brandenburg 2009). Do plants gain or lose fitness when reducing their FN secretion? Generalizations are impossible based on these two studies. However, it seems likely that the *Petunia* system represents the more usual scenario because *Nicotiana attenuata*, owing to its special ecology, does usually not compete for pollinators with other species.

The importance of FN in pollination has apparently never been questioned and it might be this fact that prevented quantitative experimental studies and detailed cost–benefit analyses from being planned, conducted, and published. By contrast, the ecological role of EFN has been controversially discussed (Sect. 6). Has this situation led to a better empirical investigation? In fact, the answer is "yes". During the sixties and seventies of the last century, literally hundreds of ant exclusion experiments demonstrated that the presence of ants can benefit plants by reducing overall herbivore pressure (Bentley 1977; Davidson and McKey 1993; Heil and McKey 2003). Inducing plants to produce more EFN has positive effects on the number of ant workers foraging on plants (Heil et al. 2001; Kost and Heil 2005). Several studies in different ecosystem have demonstrated that higher EFN availability can increase the survival rates of ant workers (Lach et al. 2009) and other predators (Limburg and Rosenheim 2001) and also ant activity and aggressiveness

(Sobrinho et al. 2002; Ness 2006; Heil et al. 2009). Studies using wild cotton (*Gossypium thurberi*) demonstrated that fewer ants visited plants with experimentally reduced numbers of extrafloral nectaries; leaf damage on these plants was higher and seed number was lower compared with plants with natural levels of EFN, indicating that EFN mediates the benefits of ants (Rudgers 2004). Indeed, indirect defence via ants represents one of the few anti-herbivore defence strategies for which a clear effect on net herbivory rates and plant fitness has been shown for different species (Chamberlain and Holland 2009). In summary, a positive correlation of investment with benefit for the plant has been shown for EFN, but the generality of this assumption has yet to be proved for FN.

3 Nectar Chemistry and Function

Plants secrete nectar to attract pollinators and defenders. Therefore, nectar needs to be chemically attractive to these insects. Nectars in general contain sugars and amino acids in an aqueous solution, which can be supplemented by proteins, lipids, and even attractive volatiles (Heil 2011). The content of essential amino acids is often enriched, and consumers are known to respond positively to this enrichment (see below). In short, nectar is chemically designed to present an appetizing meal to a large group of mutualistic animals. However, every reward that partners exchange in a mutualistic interaction is prone to being abused by exploiters: non-mutualistic species that do not render the respective benefit to the reward-producing partner (Bronstein 2001). How can plants protect their nectar from these "nectar thieves"? In the following chapter we discuss how the chemical properties of nectar create its "Janus-like" face: nectar presents a nice, attractive face to its legitimate consumers but might appear very different from the perspective of putative exploiters (Fig. 3).

3.1 The Attractive Components of Nectar

Carbohydrates and free amino acids in the nectar are most important for the function of attraction. Because this aspect of the chemical composition of nectar has been reviewed repeatedly (Nicolson et al. 2007; González-Teuber and Heil 2009a), we provide only a short overview here. Most authors assume nectar to be adapted in its composition and concentration to the nutritional preferences of the consumers (Baker and Baker 1982, 1983; Pacini et al. 2003; Johnson and Nicolson 2008; Kromer et al. 2008). Even nectar viscosity has been reported to determine the spectrum of pollinators, because certain animals might not be able to consume too viscous nectars (Kohler et al. 2010). Besides this physical trait, the composition of the nectar determines, at least in part, the spectrum of nectar consumers, because animals differ in their nutritive requirements. For example, hummingbirds, butterflies, moths and long-tongued bees usually prefer sucrose-rich floral nectars, as do most ant species

that feed on EFN, whereas short-tongued bees and flies prefer FN rich in hexoses (Blüthgen and Fiedler 2004; Nepi and Stpiczyńska 2008; González-Teuber and Heil 2009a; Nepi et al. 2009). However, some nectarivorous birds and ants lack the sucrose-cleaving enzyme invertase and are thus not able to assimilate sucrose, and hence prefer sucrose-free nectars (Martínez del Rio 1990; Heil et al. 2005).

Sugars represent the dominant compound class in nectar and are usually about 100–1,000 times more concentrated than amino acids. However, amino acids can significantly affect the attractiveness of nectar. Birds and bats can also gain nitrogen from other sources, whereas many adult insects feed only on liquids. We can therefore assume that insect-pollinated flowers should possess more amino acids
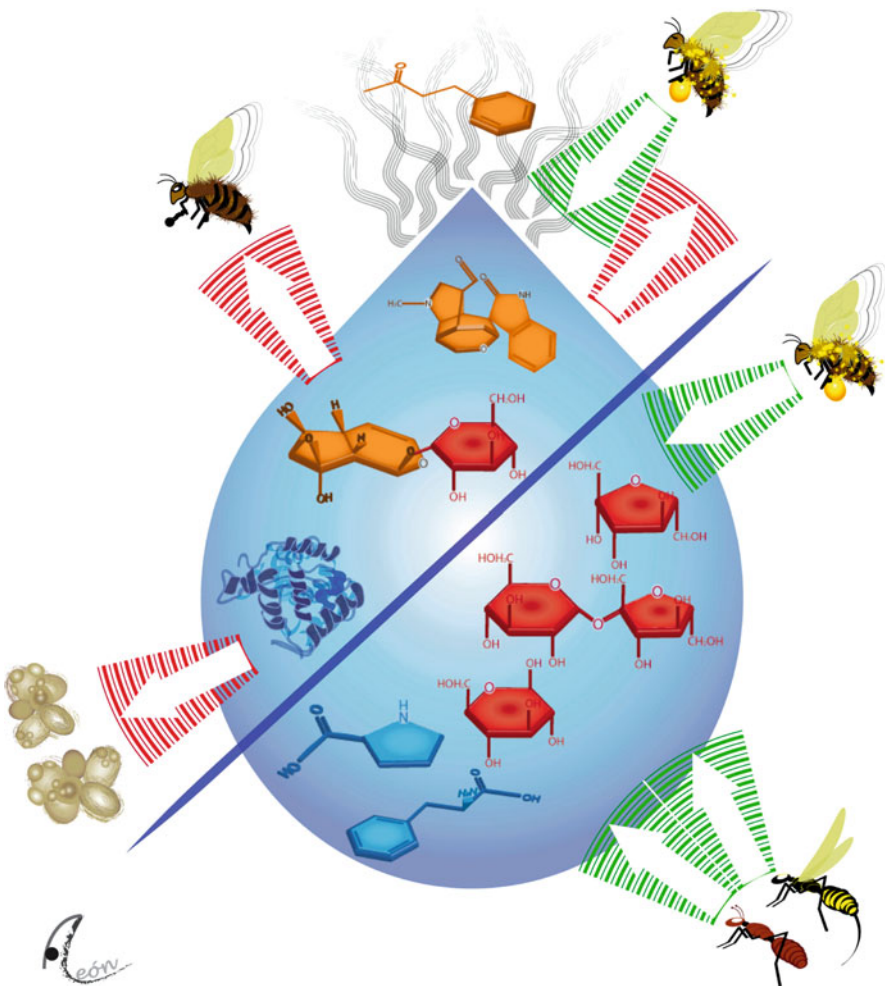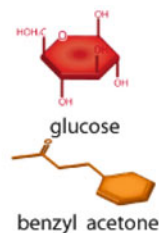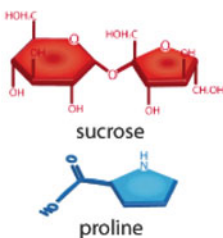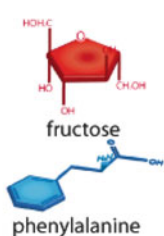

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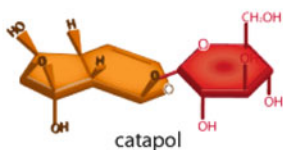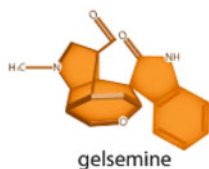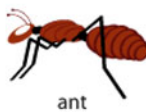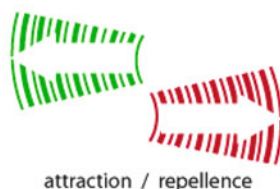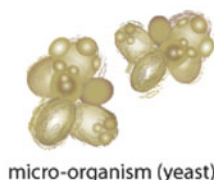
Fig. 3 The Janus-faced nature of nectar. Nectar chemistry serves both the attraction of mutualists such as pollinators and defenders and the protection from nectar robbers and nectar-infecting microorganisms. The most important attractive classes of compounds are mono- and disaccharides, amino acids and volatile components such as benzyl acetone. Repellent effects are exhibited by secondary compounds such as gelsemine and iridoid glycosides. Interestingly, gelsemine has also repels legitimate pollinators. Nectar proteins (nectarins) mainly serve its protection from microbial infections

in their nectar than vertebrate-pollinated flowers, although high amino acid concentrations in FNs of bird-pollinated flowers have been reported in specific cases (Nicolson 2007). High amino acid concentrations have indeed been reported

for FNs from flowers that are adapted to butterflies (Baker and Baker 1982), flies (Potter and Bertin 1988), or bees (Petanidou et al. 2006). Similarly, ants prefer nectars rich in amino acids, and ants as well as many insect pollinators can show strong preferences for specific, usually essential amino acids (Blüthgen and Fiedler 2004; Carter et al. 2006; González-Teuber and Heil 2009b).

Which other compound classes are involved in the attractive function of nectar? That flowers attract pollinators via attractive odours is known since centuries and readily used by the perfume industry. In fact, flower petals are still the most important source of natural odours. By contrast, nectar odours were only recently considered as a relevant signal for pollinators (Raguso 2004). Butterflies and moths preferred artificial flowers containing scented nectar over those that contained pure sugar solutions (Weiss 2001), and parasitoid wasps localized cotton (*Gossypium hirsutum*) EFN using only its odours (Röse et al. 2006). Even spiders can use odours to localize nectar (Patt and Pfannenstiel 2008) and flower mites use nectar odours to distinguish between host and non-host plants (Heyneman et al. 1991). The origin of FN scent has been linked to volatiles released by the petals that are absorbed and re-released by the nectar (Raguso 2004). However, a wide array of VOCs occurred in the nectar of wild tobacco (*Nicotiana attenuata*), and many of these compounds have not been detected in other flower parts, suggesting that in certain species, nectar emits its own scent (Kessler and Baldwin 2007).

3.2 Protection from Nectar Thieves

Nectar carbohydrates, amino acids and volatiles are apparently composed to provide an appetizing meal to legitimate nectar consumers and/or to signal the presence of nectar to these mutualists from a distance. Being a nutritionally valuable reward, however, nectar must also be protected from illegitimate consumers, which can be animals ("nectar thieves") but also microorganisms, such as bacteria, fungi and yeasts, which might use nectar as a suitable growing medium (Herrera et al. 2008, 2009; González-Teuber and Heil 2009a). The most well-known protection from non-legitimate nectar consumers in the case of FN is achieved by anatomical rather than chemical adaptations. Many flowers have evolved particular structures and shapes that allow access only to a few – or even one – coevolved species. This strategy is known in particular from bird- and butterfly-pollinated flowers, which produce their FN in nectar spurs: long, usually tube-shaped structures to which only their long-tongued specific pollinators have access. Most plant species, however, interact with a large number of different pollinators, and EFN in particular is usually openly presented. Moreover, long tubes cannot protect nectar from being infected by micro-organisms. Nectar, thus, requires also some kind of chemical protection. Mainly three different compounds classes are known to be involved in this protective function: secondary compounds, non-proteinogenic amino acids and proteins.

Surprisingly, nectar proteins appear to be one of the most important compound classes in the protective context. Nectar proteins were discovered more than

80 years ago (Buxbaum 1927; Lüttge 1961). One could suppose that these proteins supply nectar consumers with organic nitrogen, as described above for free amino acids. However, although a nutritive function of nectar proteins cannot be excluded at present, their main function appears to be protection. For example, the nectarins in the FN of ornamental tobacco (*Nicotiana langsdorffii* × *Nicotiana sanderae*) have been biochemically characterized and are likely to protect FN from microbial infestation through the Nectar Redox Cycle (Carter et al. 1999, 2006, 2007; Carter and Thornburg 2004; Park and Thornburg 2009). Floral nectar proteomes appear to be small: for example, five proteins have been found in the FN of ornamental tobacco, eight proteins have been detected in the FN of *Jacaranda mimosifolia* (Kram et al. 2008) and ten in the FN of *Rhododendron irroratum* (H.-G. Zha, personal communication). By contrast, more than 50 proteins are present in the EFN of *Acacia* myrmecophytes, which house ant colonies for their indirect defence (González-Teuber et al. 2009). The majority of these nectarins was identified as pathogenesis-related (PR) proteins, such as chitinases, glucanases and thaumatin-like proteins. The first two groups contributed more than 50% to the overall protein content, and similar numbers were then also found in nectars of other *Acacia* myrmecophytes (González-Teuber et al. 2010). Chitinases were also found in the FN of *Rhododendron irroratum* (H.-G. Zha, personal communication) and, thus, might be another common class of nectarins.

A role in antimicrobial defence has even been suggested for the GDSL lipase in the FN of blue jacaranda (*Jacaranda mimosifolia*) (Kram et al. 2008). Most nectarins, thus, appear to be involved in protecting nectar against microorganisms. The biological function of this effect is likely to be twofold. First, nectars are commonly infested by microorganisms, particularly yeasts, whose metabolic activities can dramatically change nectar chemistry (Herrera et al. 2008, 2009). The chemistry of nectar is closely linked to its function (see above). Therefore, although the presence of some nectar-infecting microorganisms or nectar robbers might have beneficial effects on the plant (Lara and Ornelas 2002), we can assume that most plants gain a benefit from keeping the nectar as sterile as possible, in order to maintain control over its chemical composition (Herrera et al. 2008). Nectaries might, however, also serve as an entrance for phytopathogens (Ivanoff and Keitt 1941; Keitt and Ivanoff 1941). Therefore, antimicrobial nectarins should serve to protect the nectary tissue from infection. More empirical evidence will be required to decide whether anti-microbial nectarins play an important role in reducing infections of tissues other than the nectary.

Protection of the nectar against non-mutualistic insects is often assumed to be the function of non-proteinogenic amino acids or of secondary metabolites (Baker 1977; Baker et al. 1978; Adler 2000). Several studies failed to demonstrate a negative effect of nectar robbers on plant fitness (Maloof and Inouye 2000; Lara and Ornelas 2002), but most authors assume that nectar consumption by non-mutualists represents a loss of a valuable resource. In fact, floral nectars of numerous plant families are "toxic". For example, FN of *Catalpa speciosa* contains iridoid glycosides that fend off nectar robbers but not legitimate pollinators (Stephenson 1982). However, toxic nectar components appear to function as

a double-edged sword. For example, Adler and Irwin (2005) experimentally manipulated the concentration of gelsemine, the principal FN alkaloid of *Gelsemium sempervirens*. Gelsemine also deterred effective plant pollinators, thus decreasing the number of flowers probed and the time spent per flower by both pollinators and nectar robbers. Why should FN deter legitimate pollinators? Several scenarios are possible (Adler 2000; Adler and Irwin 2005). First, short visitation times by pollinators do not necessarily represent a fitness disadvantage for plants (Kessler et al. 2008). Second, preventing the loss of nectar to robbers might even be worth some reduction in pollinator visits. Third, gelsemine lowers the rate of selfing in *G. sempervirens* by reducing the proportion of pollen that is transferred from the same individual (Irwin and Adler 2008). Finally, secondary metabolites in nectar could inhibit microbial growth (Adler 2000) and, thus, complement the function of nectarins. However, following Adler and Irwin (2005), no clear fitness benefits for plants with toxic nectars have been demonstrated to date. An alternative explanation might be that secondary compounds, which are transported through the vascular system in a functional context of systemically induced defence, passively "leak" into the nectar (Adler 2000; Adler et al. 2006). Identifying the site of synthesis of toxic nectar components and elucidating their secretion mechanism would be the first steps towards an explanation of their function.

4 Nectary Structure and Evolution

Nectaries are structures, or simply areas on the plant surface, where nectar is being secreted. While floral nectaries are located within the floral organs (ovary, filaments or petals), extrafloral nectaries are found on all vegetative organs besides roots, commonly even within the inflorescences (outside sepals) (Elias 1983; Heil 2007, 2011). They may be located at the surface level, forming an outgrowth on the organ, or be sunken (Fahn and Rachmilevitz 1979). In some cases nectaries are engulfed very deeply, as in the case of gynopleural nectaries, which represent the most common type of floral nectaries in monocotyledons although they are almost absent from the dicotyledons (Smets et al. 2000).

4.1 Nectary Anatomy and Secretion Sites

Nectaries can be extremely diverse with respect to their localization, their structure and even the secretion mechanism (Elias 1983; Pate et al. 1985; Fahn 1988) (Sect. 5). For example, the anatomically most simple nectaries are "gestaltless" (Frey-Wyssling and Häusermann 1960) (i.e., without any externally visible structure), and in this case can only be identified as areas where nectar appears on the plant surface. Gestaltless nectaries appear to be more frequent among the extrafloral

nectaries (Kirchoff and Kennedy 1985), whereas floral nectaries are normally well defined (Bernadello 2007). Because of their nature, however, such non-structural nectaries are difficult to identify when no nectar is observed, and gestaltless nectaries may indeed be strongly under-reported. Other nectaries form anatomically distinct and sometimes highly conspicuous structures with a complex ultrastructure (Schmid 1988), defined nectaries as a localized, multicellular glandular structure, although this definition does not cover nectaries that are formed by unicellular hairs.

Interestingly, many nectaries are characterized by a continuous cuticle present on the surface of the nectar epidermis (Gaffal et al. 1998), although the permeability for aqueous solutions of the cuticle is extremely low. Several different strategies have been evolved to reach high permeability during the active secretion. In some cases, the cuticle might simply break at the onset of the secretion process, and nectar is released through these ruptures (Durkee 1982). In other cases, specialized pores have been described, where the cuticle is forming "secretory pits" through which nectar can be exuded (Kronestedt-Robards et al. 1986; Arumugasamy et al. 1990; Stpiczyńska 2003b). Finally, the cuticle may have microchannels, narrow tubular interruptions of the cuticle (Stpiczyńska et al. 2005). In most cases, however, the nectar exits through modified stomata that remain permanently open, or through specialized trichomes (Figs. 1 b, d and 4) (Fahn 1988; Wist and Davis 2006; Gaffal et al. 2007; Vassilyev 2010).

4.2   Fine Structure and Vascularization

In general, the nectariferous tissue consists of two main components: an epidermis, with or without stomata or trichomes through which nectar is released to the exterior and a specialized parenchyma that produces and/or stores the pre-nectar (Fig. 4) (Fahn 1979). A third component can sometimes be distinguished by its more loosely packed cells as subnectary parenchyma (Stpiczyńska 2003a; Kaczorowski et al. 2008). The components of this general anatomical structure, however, are not necessarily recognizable in all nectaries. For example, gestaltless nectaries often do no exhibit a modified secretory tissue that can be clearly differentiated from the surrounding tissue (Zimmermann 1932; Elias 1983).

Nectaries can be connected to the phloem, the xylem, both or have no direct vascular connection (Fahn 1988; Wist and Davis 2006). Vascular bundles can be found in the nectariferous or the subnectariferous parenchyma. In most species studied, floral nectaries are vascularized by phloem only, or are not directly vascularized at all. For example, Wist and Davis (2006) reported that 50% of the Asteraceae lack direct vascular connections in their floral nectaries and also a taxonomically broader review found that nectaries of more than one-third of all plant species lack any direct vascularization (Fahn 1988). Only a minority of nectaries known receive direct vascular supply comprising xylem and phloem and, even in those ones, the last branches reaching the parenchyma or the epidermis are usually from the phloem (Elias et al. 1975; Davis et al. 1988). That nectar represents
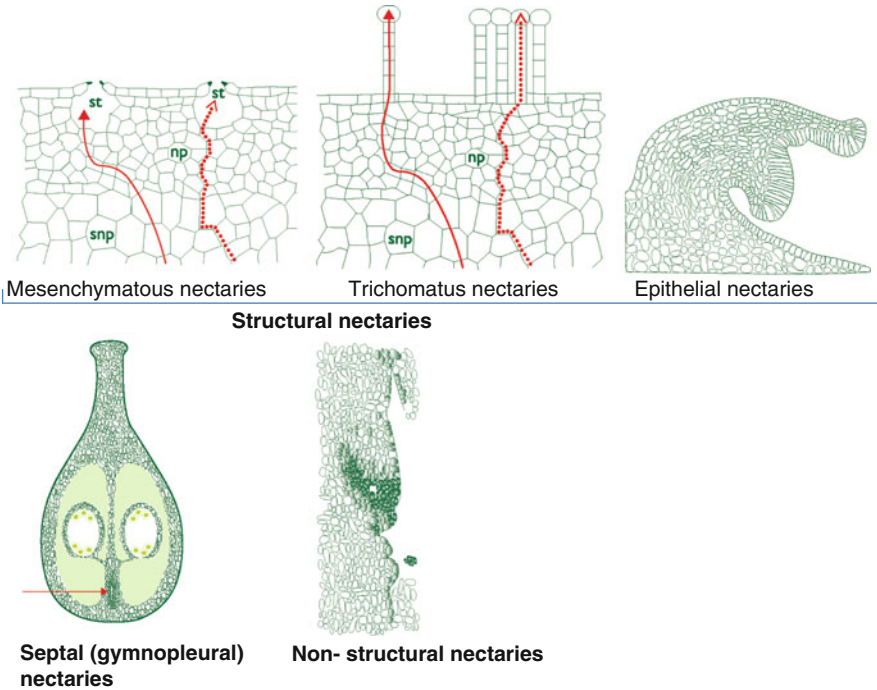
Fig. 4 Ultrastructure of nectaries. Nectaries can have different ultrastructural properties but most are characterized by a nectary parenchyma (np) and a sub-nectary parenchyma (snp). Nectar can be secreted to the outside via modified stomata (st), as illustrated in the case od mesenchymatous nectaries, via trichomes (tricomatous nectaries) or via the epidermis (epithelial nectaries). See text for further details

"secreted phloem sap" for most species (Agthe 1951; Frey-Wyssling et al. 1954; Zimmermann 1954; Lüttge 1961; Fahn 1988; de la Barrera and Nobel 2004) is further supported by an interesting characteristic of the companion cells of the nectary phloem. These companion cells commonly possess well developed wall ingrowths, which increase the surface area, thereby facilitating the uploading of pre-nectar component into the adjacent nectar parenchyma (Davis et al. 1988; Wist and Davis 2006).

4.3 Ultra Structure of Nectaries

Nectaries are structurally highly diverse and no "typical" or "representative" type of nectary can be defined. In the following, we shortly describe what appear to be the most widely distributed structural properties of nectariferous tissues (Fig. 4). Floral and extrafloral nectaries are not principally different, although several characteristics might be more common in one than in the other functional group.

Epidermal cells in the area of a nectary are in general small and polyhedric and have an anticlinal orientation. The storage of starch in the epidermis has been reported in some cases where nectaries are characterized by a particularly high energy requirement (Durkee et al. 1981; Nepi et al. 1996; Razem and Davis 1999), but does not appear to be a common phenomenon. As described above, the epidermis itself may represent the secretory complex, but more commonly the epidermis includes secretory structures, such as trichomes or modified stomata. Vogel (1997, 1998) defined four basic nectary types based on epidermal characteristics: mesenchymatous nectaries, epithelial nectaries, tricomatous nectaries and nectarioles. (1) Mesenchymatous nectaries form a very common type and are composed of glandular and storage tissues that usually secrete nectar via modified stomata (Davis 2003; Bernadello 2007; Ren et al. 2007b). The stomata can be localized on the surface of the nectary or in deep depressions and their regulation differs from that of leaf stomata: for instance, nectar stomata lack turgor- and ion-mediated movements (Davis and Gunning 1992, 1993). (2) Epithelial nectaries are basically formed by a glandular epidermis, whereas (3) tricomatus nectaries secrete via specialized trichomes. These trichomes show a variety of distributions and patterns (Fahn and Rachmilevitz 1970) and can be both of the uni- or the multicellular type. Most of the multicellular trichomes comprise a basal part at the level of epidermal cells, a stalk, and tip cells that possess all the characteristic traits of secretory cells such as an elaborate ER, dictyosomes, a high number of mitochondria and a high number of vesicles (Kronestedt-Robards et al. 1986; Sawidis et al. 1989). (4) Finally, an anatomically heterogenous group of small, discontinuous nectar-secreting structures, which are composed only of few cells, has been termed nectarioles (Vogel 1997, 1998).

Below the epidermis, a specialized parenchyma can be found in all cases of morphologically specialized nectaries. The nectary parenchyma is generally composed of several layers of small isodiametric cells with thin walls, dense granular cytoplasm, small vacuoles and relatively large nuclei (Heil 2011). These cells represent typical secretory cells and share their characteristics (D'Amato 1984) such as, for example, being rich in ribosomes and mitochondria and possessing an elaborate ER, many dictyosomes and – usually – a high number of vesicles. Another well-defined characteristic of this tissue is the high number of plasmodesmata, a trait that is supposed to ensure rapid trafficking of metabolites among the cells (Fahn 1979). Prior to and during secretion, the nectary parenchyma is subject to significant ultrastructural changes. For example, parenchyma cells frequently undergo continued cell division during secretion (Gaffal et al. 1998). While vacuoles in the pre-secretory phase are usually small, their volumes increase at the time of secretion. During this phase, a boost in the energy requirements leads to a more active endoplasmic reticulum and an accumulation of mitochondria and ribosomes in the cytoplasm of parenchymatous cells, while dictyosome numbers are reduced (Zhu and Hu 2002).

Below the nectary parenchyma, some nectaries present a subnectary parenchyma that can be differentiated by its large cells with bigger vacuoles, less dense cytoplasm and larger intracellular spaces (Durkee 1982; Cawoy et al. 2008). In contrast

to the nectary parenchyma, minor or no changes take place in the subnectary parenchyma during secretion. Its main function appears the communication with the vascular system and perhaps some contribution to the synthesis of nectar sugars. When the nectary is vascularized, phloem and xylem bundles are always present in subnectary parenchyma. The xylem vessels in general terminate in this tissue, whereas phloem strands branch into the nectary parenchyma. The subnectary parenchyma is generally rich in chloroplasts and thereby might contribute to the production of nectar sugars, although it is commonly believed not to be directly involved in nectar production (Nepi et al. 2007).

4.4 Evolution of Nectaries

In summary, nectaries are structurally and functionally highly diverse, and they appear difficult, if not impossible, to define the "most typical" nectary. These great variations in nectary morphology and structure occur even at the intraspecific level: floral nectary structure might differ within individual flowers, among the flowers of the same plant, and among plants of a population. As an example for variability within individual flowers, Arabidopsis flowers contain two types of nectaries, lateral and meridian, which are characterized by significant morphological and structural differences. Lateral nectaries are more pronounced and more heavily innervated by sieve elements and realize the largest part of overall FN secretion (Davis et al. 1998). Variability has also been observed among extrafloral nectaries, where even central morphological traits might differ among nectaries of the same plant, only depending on their localization. For example, stipular extrafloral nectaries of cowpea (*Vigna unguiculata*) form an area of widely spaced secretory trichomes that lacks any direct connection to the vascular system, whereas extrafloral nectaries on the inflorescence stalk consist of a region with secretory, cone-shaped tissues that are connected to the phloem and release EFN through permanently open stomata (Kuo and Pate 1985). Extrafloral nectaries appear particularly variable, at least within the *Fabaceae*, where several species within the same plant posses both vascularized and non-vascuralized extrafloral nectaries of several different morphological types (Díaz-Castelazo et al. 2005).

Unfortunately, little is known on the evolution of nectaries, and their origin is still a matter of discussion (de la Barrera and Nobel 2004). Although floral nectaries are likely to have evolved from extrafloral nectaries (Matile 1965; Heil 2007), the high variability in contemporary nectary function and morphology might be related to a low evolutionary stability. It is likely that nectaries can be easily lost and gained during evolution (de la Barrera and Nobel 2004; Sugiura et al. 2006; Wooley et al. 2007). Moreover, extrafloral nectaries, and possibly floral nectaries, are phenotypically highly plastic (Mondor et al. 2006; Doak et al. 2007). For example, the number of extrafloral nectaries increased when plants of aspen (*Populus tremuloides*) or broad bean (*Vicia faba*) were damaged by herbivores or defoliated experimentally (Mondor and Addicott 2003; Wooley et al. 2007). The other way

round, African *Acacia* myrmecophytes reduced the number of extrafloral nectaries when being released from herbivore pressure by large mammals over several years (Huntzinger et al. 2004; Palmer et al. 2008). *Malotus japonicus* plants cultivated under high light conditions produced larger extrafloral nectaries (Yamawo and Hada 2010), a trait that is likely related to higher EFN secretion rates (Petanidou et al. 2000; Yamawo and Hada 2010). Morphological traits also can vary with seasonal changes; Petanidou et al. (2000) compared various species of the Lamiaceae with different flowering times and observed lower floral nectary sizes, FN secretion rates and stomatal openings during Mediterranean summer. Although likely to minimize nectar flow when water supply is limited, it cannot be decided whether these differences were indicative of interspecific (adaptive) variability or of phenotypic plasticity.

It is likely that the ease with which nectaries are gained and lost, together with the different selective pressures on their functionality, provides explanations for their structural and mechanistic diversity. However, much more data will be required until we understand the evolution of nectaries. Few studies have so far focused on the degree of heritability in the morphological attributes of nectaries. Rudgers (2004) used sire-offspring regression analyses of *Gossypium thurberi* and reported that both the proportion of leaves with extrafloral nectaries and the size of these nectaries exhibit heritable variation. This observation is likely to represent a common case, because Mitchell (2004) reported high heritability values of nectary traits in a survey of various species. However, the high phenotypic plasticity of many – even morphological – traits of nectaries might cause that "even substantial amounts of genetic variation may be swamped out in the field (Mitchell 2004). Hints towards a possible selection by environmental parameters can also be seen in the observation that species located in hot and arid climates usually present stomatal secretion in their floral nectaries (Petanidou 2007), although it is again not possible to decide whether this pattern indicates differences among species or rather the effects of a high phenotypic plasticity. In summary, nectaries are phenotypically highly plastic in spite of the fact that central structural and morphological traits are clearly heritable, and many more studies will be required to understand their micro- and macro-evolution.

5 Secretion Mechanisms

The morphological diversity that we have described above is also resembled by a multitude of secretion mechanisms (Kram and Carter 2009). Scientists have discussed since more than a century whether or not the accumulation of starch represents a prerequisite of a functioning nectar secretion, and also the questions "holocrine or merocrine secretion?" and "apoplastic or symplastic transport?" date back to the thirties of the last century (Sect. 6). In fact, it is most likely that plants have evolved several solutions for the same problem and secrete nectar using various different mechanisms. In the following, we describe what appear to be

the most common types of secretion mechanisms. In general, we can distinguish three different phases: (1) carbohydrate uploading, (2) processing of pre-nectar and synthesis of non-carbohydrate components and (3) nectar secretion (Heil 2011, see video on a putative mechanism of nectar secretion: http://www.youtube.com/watch?v=Nd8ryN_7BP8).

5.1   Uploading of Carbohydrates

Most authors agree that nectar basically represents "secreted phloem sap" for most species (Agthe 1951; Frey-Wyssling et al. 1954; Zimmermann 1954; Lüttge 1961; Fahn 1988; de la Barrera and Nobel 2004). However, nectar and phloem sap clearly differ in their chemical composition. The processing from phloem sap to pre-nectar and to the finally secreted, mature nectar certainly comprises more than a simple transport of phloem sap through the nectariferous tissue. In the most likely scenario, photosynthates are uploaded as sucrose from the phloem, either directly into the nectariferous tissue in the case of vascularized nectaries (about 60%), or into neighbouring parenchyma cells in non-vascularized nectaries, from where they might diffuse further into the secretory cells of the nectary (Vassilyev 2010). There are two alternative routes for the movement of the pre-nectar from the phloem into the nectary cells: a symplastic and an apoplastic route.

Although both pathways might exist and in fact could be active even within the same nectary, the symplastic component appears to be more common (Heil 2011). Fahn (1988) postulated that the pre-nectar is stored in vesicles and moves through the nectariferous tissue to the secretory cell via numerous plasmodesmata. During this process, pre-nectar is modified into mature nectar. This hypothesis is confirmed by the great number of plasmodesmata present between the phloem parenchyma and the nectar parenchyma cells (Sawidis et al. 1987) and by the high number of vesicles that can usually be observed in nectariferous tissues (Heil 2011). Furthermore, nectar secretion via trichomes excludes an apoplastic transport for the respective species, owing to apoplastic barriers in the external cell walls in the stalk and intermediate cells of the trichomes (Kuo and Pate 1985; Fahn 1988). For the symplastic pathway, the transport of sugars and amino acids is likely to be driven passively by the osmotically generated hydrostatic pressure differences between source and sink tissues (Lalonde et al. 2003).

The second possibility, an apoplasmic pathway, comprises a transport via intercellular spaces and cell walls to the secretory cells (Vassilyev 2010) where sucrose transporters are conceived to function as membrane complexes that respond to alterations in the source/sink balance. Effluxers that release sucrose and amino acids to the surrounding apoplasm during uploading from the phloem remain to be discovered. However, recent evidence indicates that cell wall invertases might play an important role in the uploading of sucrose from the phloem. An apoplastic invertase (AtCWINV4) has been discovered in *Arabidopsis thaliana* that is required to create the sink status for active nectar secretion (Ruhlmann et al. 2010). A mutant

line, which lacked this activity, showed reduced levels of starch accumulation within the nectary (Kram and Carter 2009; Ruhlmann et al. 2010). However, at least nectar proteins are most likely transported in vesicles and their transport depends, thus, on the symplastic pathway.

A generally driving factor in the unloading of starch from the phloem is represented by the large transmembrane differences in sucrose concentration (Lalonde et al. 2003). Moreover, many floral nectaries (e.g., of tobacco and *Arabidopsis*) accumulate starch in the pre-flowering phase and thereby continuously remove sucrose from the cell plasma, thereby avoiding an equilibrium between sucrose concentrations in the phloem and the nectariferous tissue (Horner et al. 2007; Ren et al. 2007b). Thus, source–sink relations appear a major driving force during the uploading of carbohydrates to the nectariferous tissue.

5.2 Processing of Pre-nectar and Synthesis of Non-carbohydrate Components

As mentioned above, floral nectaries might rely on the accumulation of starch in order to reach peak nectar secretion rates. Besides Arabidopsis and tobacco, an accumulation and consecutive breakdown of starch have also been described for further, taxonomically unrelated species such as soya bean (*Glycine* max) and common foxglove (*Digitalis purpurea*) (Horner et al. 2003; Pacini et al. 2003; Gaffal et al. 2007). Many species possess amyloplasts in their nectary tissue (Pacini et al. 2003) that can become directly connected to the vacuole and consecutively emptied during the phase of most active FN secretion (Gaffal et al. 2007). All these processes appear linked to the mobilization of nectar carbohydrates. However, the accumulation of starch has not been reported for extrafloral nectaries so far and does therefore not represent an absolute requirement for a functioning secretion. In fact, the direct secretion of products of the current assimilation process has been shown repeatedly for FN, using girdling of flower shoots as well as darkening and defoliation experiments (von Czarnowski 1952; Gaffal et al. 2007). Experiments with ^{13}C-labelled CO_2 demonstrated that also EFN contains sugars that have been assimilated during the last hours before secretion (Radhika et al. 2008).

In summary, carbohydrates are uploaded as sucrose from the phloem to the secretory tissue where they are stored and/or processed (Wenzler et al. 2008; Kram and Carter 2009). During active secretion, sucrose is metabolized by cell wall invertases, which serve to produce hexose-rich nectars and to create the required source–sink relationships that prevent reloading of sucrose into the phloem (Agthe 1951; Zimmermann 1953; Frey-Wyssling et al. 1954; Peng et al. 2004). The abovementioned apoplastic invertase, AtCWINV4, also plays a role in this context, as demonstrated by the absence of nectar in "lack of function" mutants (Ruhlmann et al. 2010). In fact, genes coding for the complete sucrose biosynthesis are upregulated in *A. thaliana* nectaries (Kram et al. 2009) and expression patterns of

genes involved in starch metabolism allow a clear separation of an anabolic phase before anthesis and a catabolic phase during secretion in nectaries of ornamental tobacco (Ren et al. 2007a). A more primary role of extracellular invertases may be to double the osmotic contribution of uploaded sucrose that affects turgor and hence rates of different photoassimilate import (Roitsch 1999). However, secreted nectars are characterized by a wide range of concentrations and sucrose:hexose ratios (Baker and Baker 1975, 1982) and, therefore, cannot be produced only by a passive flow or transport mechanisms exclusively driven by sucrose-cleaving enzymes.

Unfortunately, research into the mechanisms of nectar production (de la Barrera and Nobel 2004; Gaffal et al. 2007; Wenzler et al. 2008; Kram and Carter 2009; Vassilyev 2010) has focused exclusively on FN carbohydrates. It remains completely unknown where non-carbohydrate nectar constituents are produced, where and how they are added to the pre-nectar and how they are secreted. Considering the abundance and chemical diversity of nectar proteins and the lack of reports of many of these nectarins from other tissues, synthesis in the nectary tissue appears likely. In fact, secretory cells of *Vigna unguiculata* extrafloral nectaries contain protein-rich inclusions (Kuo and Pate 1985) and all *NECTARIN* genes that correspond to nectar proteins in the FN of ornamental tobacco are expressed in the nectary tissue (Carter and Thornburg 2004; Thornburg 2007), where some of them are under the control of an MYB305 transcription factor (Liu et al. 2009). Furthermore, tobacco nectarins contain signal peptides for secretion and can therefore only be secreted by the fusion of vesicles with the plasma membrane. A similar evidence for other systems is lacking and is urgently needed to understand how plants produce nectar compounds and secrete them into the nectar. It appears likely, however, that pre-nectar is processed within vesicles and that nectar proteins are synthesized in the nectary parenchyma itself and then added to the pre-nectar during this process (Heil 2011).

5.3   Secretion

At the initiation of secretion, the vacuoles increase their volume, the number of dictyosomes is reduced, the endoplasmic reticulum becomes more active and the number of mitochondria increases (Zhu and Hu 2002). Together with this increase in the number of metabolically active organelles, the starch grains reduce their size or completely disappear (Zhu and Hu 2002; Horner et al. 2007; Ren et al. 2007b).

In principle, two different mechanisms of nectar secretion have been described so far: holocrine and merocrine. Holocrine secretion involves programmed cell death at the moment of secretion (Horner et al. 2003; Pacini et al. 2003; Gaffal et al. 2007). In this case, the nectar is produced within the cell and released by the rupture of the plasma membrane (Horner et al. 2003; Vesprini et al. 2008). The merocrine secretion allows the secreting cells to survive and to continue with their secretory activity (Nepi and Stpiczyńska 2007). Most extrafloral nectaries that have been investigated so far release EFN in response to herbivory or mechanical damage and

can do so repeatedly (Heil et al. 2000, 2001; Heil 2009). By contrast, FN is usually secreted in an ontogenetically programmed manner. Therefore, holocrine secretion might be a common case in floral nectaries but we do not regard it as likely that this type of secretion has frequently evolved for EFN (Heil 2011).

The merocrine-type secretion of nectar usually occurs as a granulocrine secretion, which is characterized by the presence of vesicles that stem from the endoplasmic reticulum or the dictyosomes and that contain the pre-nectar. These vesicles move through plasmodesmata towards the epidermal cells and finally fuse with the plasma membrane to release their content into the extracellular space. Apparently, this secretion mechanism represents the most common and/or most accepted one (Jürgens and Geldner 2002; Heil 2011). Several aspects support this model. First, vesicles are common in nectariferous tissues (Benner and Schnepf 1975; Kuo and Pate 1985; Fahn 1988) and the number of dictyosomes in secreting and non-secreting nectary cells of *Billbergia nutans* (Bromeliaceae) differed in coincidence with secretory activity (Benner and Schnepf 1975). Second, non-carbohydrates such as lipids and proteins (Carter and Thornburg 2004; Kram et al. 2008) are likely to be synthesized in the nectariferous tissue and then must enter the pre-nectar before its secretion.

Although the secreted nectar is likely to be chemically mature in most cases, further changes to the biochemistry of nectar can occur in the post-secretory phase. For example, sucrose can also be eliminated from nectar by post-secretory hydrolysis, which is mediated by invertases that are secreted into the nectar itself (Zimmermann 1953; Heil et al. 2005). Second, nectars are commonly infested by microorganisms, particularly yeasts, whose metabolic activities can dramatically change nectar chemistry (Herrera et al. 2008, 2009). It has been assumed that the presence of some nectar-infecting microorganisms or nectar robbers might have beneficial effects on the plant (Lara and Ornelas 2002).

5.4 Regulation and Re-absorption

Although nectar sugars in some species represent less than 2% of the current net photosynthates (Pate et al. 1985), nectar production by *Asclepias syriaca* can consume up to 37% of daily assimilated carbon during blossoming (Southwick 1984). Nectar secretion undoubtedly can be costly, although many more studies on different plant groups will be required to obtain a realistic estimate of the "usual" costs of nectar production. In order not to waste energy, floral and extrafloral nectaries are commonly able to adjust the net secretion rates to the consumption rates and/or to re-absorb nectar that has not been consumed. An adjustment of FN net production to consumption rates has been demonstrated in various species (Corbet and Delfosse 1984; Gill 1988; Pyke 1991). *Macaranga tanarius* reduced EFN secretion in the absence of consumers and increased it immediately after consumers were allowed to feed for 1 day on the nectaries (Heil et al. 2000). However, quantitative adjustments of the apparent net production could result

from either an inhibited de novo secretion or the re-absorption of accumulated nectar. Although it is not known whether plants really can adjust the de novo secretion, a re-absorption of FN has been shown unambiguously with different methods (Nepi and Stpiczyńska 2008), including labelling studies that demonstrated the uptake and allocation to other plant organs of externally applied ^{14}C-labelled sucrose or glutamine (Pederson et al. 1958; Ziegler and Lüttge 1959). Re-absorption of non-consumed FN appears common (see examples in Stpiczyńska 2003b), although this phenomenon has yet to be demonstrated for EFN (Nepi and Stpiczyńska 2008).

Nectar resorption has been hypothesized to be mediated by vesicles or by programmed cell death in the nectary tissue in combination with a phloem that remains active and the resulting changes in source–sink relationships (Kuo and Pate 1985; Gaffal et al. 2007). Under this scenario, extrafloral nectaries are likely to lack the capacity to re-absorb nectar because the regulation of EFN secretion is not dependent on ontogenetically programmed patterns. Numerous vesicles became associated with the plasma membrane at the moment of resorption in the nectar spurs of *Platanthera chlorantha*, and cell death was apparently not involved in this process (Stpiczyńska 2003b). Similarly, the observation of numerous mitochondria surrounding the plasmalemma may indicate active transport (Zhu and Hu 2002). It appears likely, thus, that the resorption of non-consumed nectar represents an active process in most species. Although most studies concern sugar resorption, other nectar components such as amino acids or proteins may also undergo resorption (Nepi et al. 1996). For example, water has been demonstrated to be reabsorbed as well (Nepi et al. 2001), a phenomenon that could be explained by cell turgor modifications and rapid changes in the osmolarity as a passive process (Castellanos et al. 2002). Many more studies will however be required until we truly understand the biochemical and physiological mechanisms that underlie the re-absorption of non-consumed nectar.

Another raising question is how plants sense the unconsumed nectar. The most accepted hypothesis is "sugar sensing"; sugars are able to act as central signalling molecules, interacting with hormones, light and stress signals (Rolland et al. 2006). Membrane-bound receptors are involved in sugar sensing and transduction (Loreti et al. 2001). It appears possible that the same mechanism is acting in nectaries. Alternatively, the re-absorption might be driven by changing source–sink relations; unconsumed nectar accumulates, becomes more concentrated due to evaporation and therefore the concentrations outside the nectary might become higher than inside. It will be a future goal to demonstrate whether similar or other mechanisms allow nectaries to control nectar homeostasis.

5.5   Regulation by Internal and External Factors

Obligate myrmecophytes among Central American *Acacia* species can adjust their EFN secretion to the identity of the inhabiting ants and provide high levels of the

resource only to defending mutualists, but much lower amounts to non-defending parasites (Heil et al. 2009). Unfortunately, the cues that enable the defending partner to be identified are not yet known. In general, the regulation of nectar secretion seems to be complex and dependent on internal and external factors such as, for example, light availability, time of the day, nutrient availability and the presence of consumers or, in the case of EFN, herbivores.

Many nectaries secrete their nectar in a diurnal rhythm that appears to be adapted to the activity patterns of the respective consumers (Tilman 1978; Heil et al. 2000). On the other hand, EFN secretion with few exceptions represents an inducible defence mechanism that is activated by mechanical damage and/or herbivory. An induction of EFN secretion by herbivory has been demonstrated for 11 genera from six families (*Prunus* [Rosaceae], *Acacia, Phaseolus, Prosopis, Vicia* and *Leucaena* [Fabaceae], *Macaranga* and *Sapium* [Euphorbiaceae], *Populus* [Salicaceae], *Gossypium* [Malvaceae] and *Paulownia* [Scrophulariaceae]) (Wäckers and Wunderlin 1999; Heil et al. 2001, 2004; Mondor and Addicott 2003; Rogers et al. 2003; Wooley et al. 2007; Kobayashi et al. 2008; Pulice and Packer 2008) and, thus, appears to be very common.

Interestingly, FN and EFN secretion is, at least in part, under the control of the same hormone – jasmonic acid. Mechanical damage and herbivory increased endogenous jasmonic acid levels, and this change was correlated with an increase in EFN secretion, moreover, external application of jasmonic acid also increased EFN secretion rates by lima bean, *Macaranga tanarius* and several *Acacia* species (Heil et al. 2001, 2004; Heil 2004). Recently, it has been demonstrated that FN secretion by *Brassica napus* is influenced by the same hormone (Radhika et al. 2010). Interestingly, FN secretion by *Brassica* was not affected by folivory or JA application to the leaves, an observation that points to a functional separation of the hormonal controls of FN and EFN secretion (Radhika et al. 2010). In short, nectar secretion responds to multiple endogenous and exogenous triggers and appears generally adapted to ensure that plants obtain maximum mutualistic benefits from a minimum of investment in energy and other limiting resources. However, the physiological and genetic mechanisms by which this likely adaptive regulation is achieved remain to be investigated in most cases.

6   Nectar Research: Past and Future

Nectar serves in the interactions of plants with mutualistic animals and its presence and attractive function can be observed easily. It is likely for these reasons that research into nectar has a long history. However, the topic has turned into the "sleeping beauty" of plant sciences over the last 50 years and many recent observations represent in fact re-discoveries of formerly well-known phenomena (Heil 2011). In this last chapter, we present a short insight into the history of nectar research in order to acknowledge some of those brilliant scientists of the nineteenth and the first half of the twentieth century whose results still represent a considerable

part of our current knowledge on nectar biology and physiology. We then discuss how contemporary "omics" techniques could be used to wake up the sleeping beauty and convert nectar research back into an active and lively component of the contemporary plant sciences.

6.1 A Short Historical Overview

The involvement of FN in the attraction of bees and other insects to flowers is likely known since thousands of years. Although being comparatively young, even the suggestion that EFN might be involved in plant defence has a history of more than a century, since it has been suggested by Delpino and Belt in 1874 (Belt 1874; Delpino 1874, 1886). Just like many other provocative ideas, this hypothesis has had a very hard life until being accepted: It was questioned by Darwin 2 years after its first publication (Darwin 1876) and was regarded as "finally rejected" by Nieuwenhuis von Uexküll-Güldenband in 1906 (Nieuwenhuis-von Uexküll-Güldenband 1906; Zimmermann 1953). By that time, most botanists agreed that EFN serves the excretion of "excess carboydrates" and it was not until the seventies of the last century that literally hundreds of ecological field studies were presented to support the defensive role of extrafloral nectar (Bentley 1977; Elias 1983; Koptur and Lawton 1988).

Do further recent reports also represent confirmatory work rather than truly novel discoveries? The answer is "yes". Matile (1965) suggested that floral nectaries are evolutionarily derived from extrafloral ones. Nectar proteins were discovered by Buxbaum (1927) and its invertase activity in the 1950s (Zimmermann 1953). Because the original publications were in German, they remained widely unknown, and the presence of invertase in the secreted nectar was published erroneously as a new discovery again more than 50 years later (Heil et al. 2005). Nectar re-absorption was suggested originally by Bonnier (1878) and already in experimentally demonstrated using radioactively marked amino acids and disaccharides (Pederson et al. 1958; Ziegler and Lüttge 1959). Another controversial discussion that dates back to the nineteenth century concerns the mechanisms of nectar secretion. Behrens related starch degradation to nectar secretion in 1879 (cited after Gaffal et al. 2007) whereas a direct involvement of the phloem was suggested by von Wettstein (1889). In summary, the current discoveries in nectar research represent a renaissance of a previously fashionable field, rather than a truly novel development.

6.2 Steps into the Future

Research into the biology and physiology of nectar was an important topic in plant research during the late nineteenth and the first half of the twentieth century.

However, little progress has been made since then, although central questions remained unresolved. Which factors have inhibited nectar research over the last 50 years? The field has probably from of the focus of the plant sciences on model plants that either do not produce nectar (i.e. wind-pollinated crops such as maize, rice and wheat), or do not rely on nectar because they are highly selfing (Arabidopsis). However, "omics" techniques have enabled non-model plants to be studied, and the first nectar-producing species (in particular, cotton, poplar, tobacco and *Petunia* species) have now reached the status of genetically tractable model systems.

Despite the number of publications related to nectaries and nectar, only few studies have treated the topic at a molecular level by now. Remarkably, the molecular events involved in the synthesis, regulation and secretion of nectar, as well as in the development of the nectary, remain poorly understood. Little information exists regarding genes that are involved in nectar synthesis and secretion and these mostly stem from *Arabidopsis thaliana* (Bowman and Smyth 1999; McKim et al. 2008; Kram et al. 2009; Ruhlmann et al. 2010). Three genes encoding for putative transcriptions factors have been established to be involved in nectary development: CRABS CLAW (CRC) and BLADE-ON-PETIOLE 1 and 2 (Bowman and Smyth 1999; McKim et al. 2008), whereas one gene coding for an invertase, CELL WALL INVERTASE 4, has been demonstrated to be essential for nectar secretion (Ruhlmann et al. 2010). As to our knowledge, these four genes are the only ones for which a function in the development of floral nectaries or in FN secretion has been demonstrated, and nothing appears to be known regarding genes involved EFN secretion. More research is needed to understand how plants produce nectar: the most important mediator of their interactions with mutualistic animals (Heil 2011).

Where is the future of nectar research, and which are the plant species and techniques to chose? Kram and Carter (2009) emphasized the genomic tools that are available for *Arabidopsis thaliana* and have therefore suggested Arabidopsis as a "model for functional nectary analysis". However, Arabidopsis is characterized by small flowers with tiny nectaries that release minute levels of nectar. Besides these technical problems, Arabidopsis is a highly selfing plant and does not rely on FN for its pollination, and it does not possess extrafloral nectaries. Thus, neither FN nor EFN play an important role in the life of *Arabidopsis* and many traits that characterize more important nectars might not be expressed in this species. In fact, nectar secretion schemes and nectar biochemistry can depend strongly on the biological importance of the nectar in the particular life history of a species and therefore can significantly vary even among closely related species (Heil et al. 2004, 2005). *Arabidopsis* is therefore not likely to possess all genes and mechanisms that are required for the full functioning of biologically important nectaries.

It is likely that specialized structural and functional properties of nectaries as well as nectar chemistry are based on the expression and regulation of a nectar-specific set of genes. Economically important plants such as orchard trees (apple, *Malus domestica*; pear, *Pyrus communis*; cherry, *Prunus avium*, etc.); crops (cotton, *Gossypium* sp.; soya, *Glycine max*; beans, *Vicia faba* and *Phaseolus* sp.; zucchini,

Cucurbita pepo, etc.) and timber trees (aspen, *Populus* sp.; cedar, *Cedrus* sp., ect.) depend on their nectaries for pollination or defence and should therefore express all genes that are required for a complete nectary development, a functioning nectar secretion and a complete and complex nectar biochemistry. New model plants such as poplar trees (which bear extrafloral nectaries: Wooley et al. 2007) or *Brassica rapa* (with its large floral nectaries: Hampton et al. 2010) combine benefits such as the availability of genomic and bioinformatic tools with relatively large nectaries and high nectar flow rates, which facilitates metabolomic studies. *B. rapa* can be a useful alternative to *Arabidopsis thaliana* not only for the size of its nectaries and the volumes of nectar secreted, but also because it is highly dependent on pollinators. First efforts to discover the genes involved in nectar development on this plant have already been made (Hampton et al. 2010). Regarding extrafloral nectaries, a member of the poplar genus, *Populus trichocarpa*, has been fully sequenced (Tuskan et al. 2006) and several tools are available (e.g., commercial microarrays, bioinformatic tools, mapman). Some characteristics making it useful for nectary/nectar research are its rapid growth, an easy vegetative propagation and tractability to *Agrobacterium*-mediated transformation.

Several research groups have initiated genomic projects over the recent years, projects that could help elucidate the molecular mechanisms involve in nectar biology (Table 1). The so-called next-generation sequencing methods (454 pyrosequencing, Ilumina Solexa, SOLiD) will provide a faster and more accurate option for genomic analyses. The possibility of affordable genomic studies and the advances in bioinformatics will promote solutions to address this gap in knowledge surrounding nectar specific genes. The "omics" approach has potential to provide answers to as yet unresolved questions: How is the phloem sap uploaded to the nectariferous tissue and how is the unidirectional transport ensured? How can this direction be reversed when nectaries start to re-absorb nectar? How is the pre-nectar modified to produce mature nectar, and where are the non-carbohydrate components of nectar synthesized? How is the "mature" nectar release on the apical side, and which transport processes are involved?

Future research should realize comparative biochemical analyses of nectar and phloem sap in order to identify nectar compounds that stem exclusively from the nectary itself. In general, the suggested use of large-scale untargeted attempts such as transcriptomics, proteomics and metabolomics will be most fruitful when

Table 1 Potential candidate species for genomic studies on nectaries

Name	Nectary	Scope	Taxon	Status	PI
Glycine max	Floral	Standard draft	Whole genome	Complete	Schutz J
Brassica rapa	Floral	Resequencing	Whole genome	Awaiting release	Rokhsar D
Salix purpurea	Floral	Standard draft	Whole genome	Assembly	Tuskan G
Eucalyptus sp.	Floral	Resequencing	Whole genome	Complete	Myburg A
Gossypium sp.	Extrafloral	Standard draft	Whole genome	Production	Paterson A
Phaseolus sp.	Extrafloral	Resequencing	Whole genome	Awaiting release	McClean P
Cedrus sp.	Extrafloral	EST	Transcriptome	Complete	Dean J

Source: JGI, DOE joint genome institute (http:\crwww.jgi.doe.gov)

following comparative strategies, using functionally similar nectaries of taxonomically unrelated species and functionally different nectaries within the same species. This strategy should enable an understanding of the general principles that underlie the synthesis of nectar components and the regulation of nectar secretion.

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