

Nectaries and Nectar

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Editors

Springer

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Cover illustrations from left to right:

Left: Cross section through the base of an ornamental tobacco (*Nicotiana langsdorfii* x *Nicotiana sanderae* Hort var Sutton's Scarlett Line LxS8) flower showing the large, bright-orange floral nectary located at the base of the ovary (picture by Robert Thornburg).

Middle: Flower in an inflorescence of *Fatsia japonica* with large nectar droplets on the surface of the yellow nectary (picture by Massimo Nepi).

Right: *Lycus fernandezi* (Lycidae) drinking nectar of *Aloysia wrightii* (Verbenaceae), New Mexico (picture by Bob Barber).

Background: Scanning electron micrograph of the nectary surface of *Cyclanthera pedata*. Nectar droplets are secreted by multicellular capitate trichomes (picture by Fabrizio Ciampolini).

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Preface

"Nectar is the drink of the gods"… since the time of Homer (the *Iliad*, 800 BC), nectar has been known as a unique biological fluid with mystical properties; yet it is only now that the true chemistry of nectar is being defined. Nectar is a complex biochemical milieu offering much more than sugars to visiting pollinators. Its consumption is central to one of two types of plant–animal interaction that have contributed so much to global biodiversity: herbivory and pollination. All types of plants, regardless of their position on the evolutionary scale, are eaten by herbivorous animals. Nectar, however, is the product of a mutualism in which animals consume nectar and are involuntarily responsible for the transport of pollen or, in some cases, for plant defence. The presence of nectaries, in either reproductive or vegetative parts of a plant, symbolizes that plant's benevolent relationship with animals.

Nectaries are interesting not only for our knowledge of plant biology, but also because they are involved in the pollination of many edible and rare plants, thus having huge economic and ecological importance. About a third of our food may be derived from bee-pollinated crops. In addition, nectar is the raw material of honey. Other than bees, nectar is food for an enormous variety of insects, a tenth of all bird species, and some mammals; when nectar is not an animal's main food, it often provides an energy drink. Nectar biology has many overlapping facets, evident in the chapters that follow: botany, chemistry, zoology, and ecology.

The stimulus for this volume was the meeting of a group of nectar biologists in Italy, at the first international conference dedicated exclusively to nectar and nectaries. The meeting was held in Montalcino, Tuscany, in May

2002, and the proceedings were published as a special volume of *Plant Systematics and Evolution* (238, issues 1–4, 2003). The topics ranged from the molecular biology of tobacco nectar to the potential effects of global climate change on floral nectar production, and we decided it was the right time for a new book on nectar. The cooperation of the three co-editors was also assisted by an award from the Joint Italy/South Africa Science and Technology Agreement (2002–2003).

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Chapter 1

INTRODUCTION

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1 EVOLUTIONARY ORIGINS

The evolutionary origins of nectaries and nectar are relatively obscure, but several researchers, working on a broad scale on the evolution of angiosperm families, have provided overviews of nectary incidence, diversity, origin, and function. Two contrasting examples below show how concepts regarding the origin of nectaries and nectar have been modified in the light of new information. Firstly, in his outline of the classification of the angiosperms, Armen Takhtajan (1980) gives a simple, concise statement on the purpose and origin of nectaries:

> The original pollinators were most probably beetles …. The original attractant in insect pollination was the pollen …. But the necessity for pollen economy leads to a course of evolution in which the flower starts producing a cheaper foodstuff, nectar, as its alternative. For the production of nectar special structures are formed as nectaries. They originated independently in the most diverse lines of angiosperm evolution and on a most widely varying morphological basis. With the emergence of nectaries the plant gets an opportunity for producing pollen in more limited quantities and using it only for transport to other flowers.

Later, based on new data from paleobotany and molecular systematics, Peter Endress (1994a) discusses recent concepts of the evolution of angiosperm flowers and states:

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> The reward(s) to pollinating insects in early angiosperm flower evolution were floral secretions, and not pollen, in contrast to earlier hypotheses. The pollination drop on the ovular micropyle and later the stigmatic secretion may have served as nectar reward for pollinators...

The first two lines of Tahktajan's statement were disputed in the light of new evidence from different sources, but Endress also presents the concept that nectaries may have multiple evolutionary origins and can be induced in a wide range of positions and tissues in the flower (Simpson & Neff, 1983).

The history of ideas about nectaries and nectar shows that it was a long time before the role of nectar in insect pollination was recognized: earlier it was assumed that nectaries originated as excretory organs to rid the flower of superfluous liquid (Lorch, 1978). This physiological explanation for nectar-secreting structures was recently revived by de la Barrera and Nobel (2004), in the context of the carbon and water relations of flowers. According to their "leaky phloem" hypothesis, nectar secretion could result from high hydrostatic pressure in the phloem and the structural weakness of developing phloem tissue. Their complementary "sugar excretion" hypothesis is based on sugar accumulation due to rapid growth and associated high transpiration rates of floral structures. However, we consider the primary function of nectaries to be ecological rather than physiological, as sites where liquid substances involved in interactions with animals are produced and offered in exchange for benefits to the plant. Animals that are attracted by nectar rewards not only involuntarily disperse pollen in the environment, thus enabling plants to avoid self-fertilization and competition with parents and siblings, but may also help protect plants from herbivores.

The most ancient extant plant with nectaries is the bracken fern *Pteridium aquilinum*, which has extrafloral nectaries on its fronds (Heads and Lawton, 1985). The phylogeny of extant seed plants shows three separate origins of animal pollination: in cycads, gnetaleans, and angiosperms (Pellmyr, 2002). In gymnosperms, secretions resembling nectar occur in Gnetales and are involved in pollination (Bino et al., 1984; Wetschnig & Depisch, 1999). Nectaries are far more common in angiosperms, dating back to the late Cretaceous. Early-branching lineages of the angiosperms (the ANITA grade, based on molecular studies) are characterized by tiny flowers with wet stigmas, the stigmatic secretions being a potential reward, included among plesiomorphic traits in angiosperms (Endress, 1994a, 2001). Most angiosperms are pollinated by insects, which are rewarded with nectar during visits to flowers with floral nectaries, whereas extrafloral nectaries reward a more limited set of animals, mainly ants, that keep herbivores away.

Nectaries are specialized tissues that secrete a sugary solution involved in interactions with animals. The term does not indicate a uniform or well-defined anatomical structure, however (Fahn, 1979; Pacini et al., 2003). There are various types of nectary, situated anywhere in the flower and in widely different parts of plants, with different origins and types of organization. The diversity of nectaries is evident in Vogel's exhaustive description of the types and structures of nectaries in many angiosperm families (Vogel, 1997, 1998a,b,c). In general terms, nectaries consist of three components (Fahn, 2000; Pacini et al., 2003):

- An epidermis, with or without stomata and trichomes, where nectar is released to the exterior.
- Specialized parenchyma that produces or stores nectar solutes.
- The vascular bundle that conveys water and nutrients to the parenchyma.

The conventional view that nectar originates from phloem sap but may be modified by the nectary parenchyma is perhaps oversimplified. The sugar component of nectar is derived from photosynthesis by the nectary itself, or probably more commonly by photosynthesis in other parts of the plant, with or without starch as an intermediary storage product. At least some extrafloral nectaries, which secrete for prolonged periods, photosynthesize. The breakdown of stored starch makes high rates of nectar production possible, at any time of the day (Pacini et al., 2003).

2 SECRETIONS ANALOGOUS TO NECTAR

The floral secretions mentioned by Endress (1994a) as potential early rewards to pollinating insects were pollination drops and stigmatic secretions. The exposed ovules of gymnosperms secrete a sugary fluid at the micropylar end, and this so-called pollination drop acts as a nectar reward for insects in *Gnetum*, *Ephedra,* and *Welwitschia* (Owens et al., 1998; Gelbart & von Aderkas, 2002). Strong evolutionary, cytological, and chemical similarities exist between the pollination drop and nectar. Both are liquids containing carbohydrates and proteins. The function of the pollination drop is to rehydrate pollen and to serve as germination medium, and rehydration of pollen grains is only possible if the sugar concentration of the drop is relatively low: more concentrated solutions would tend to dehydrate it. Retraction of the pollination drop draws pollen into the ovule, whereas nectar is consumed by animals visiting the flower and may be reabsorbed if not collected by visitors. Pollination drops always retract to draw pollen into the micropyle, whereas reabsorption of unconsumed nectar only occurs in some species,

mainly those investing heavily in nectar production and having many ovules per ovary, such as *Cucurbita pepo*, *Linaria vulgaris*, and certain orchids (Pacini et al., 2003).

The stigma of many plant species exudes liquids consisting mainly of lipids that facilitate pollen adhesion, but in some monocots (e.g., certain Araceae) the stigmatic secretion is a clear sugary fluid containing few or no lipids (Heslop-Harrison & Shivanna, 1977). The watery exudate forming drops on the spadix of *Anthurium hookerianum* contains 7% sugar (Vogel, 1983). In *Asclepias syriaca* the exudate produced by the stigmatic chamber moves through a capillary system to nectar reservoirs, which are visited by insects. The nectar thus functions as both germination medium for pollen and reward for pollinators. Its concentration may increase through evaporation, but rates of pollen germination are highest in sucrose solutions of 11–15% w/w (Kevan et al., 1989). This shows a clear link between the funtions of stigmatic secretions and nectar.

In the vegetative and reproductive organs of angiosperms there are other types of cells and tissues secreting liquids with different ecological functions, not always related to plant reproduction and dispersal. Analogies between nectary and other secreting tissues are more evident when the secretion is liquid and "exported" outside the organ. In certain flowers elaiophores may produce a reward rich in lipids (Vogel, 1988), and osmophores produce a fragrance attractive to animals (Effmert et al., 2005). Vegetative parts, mainly leaves, of plants living in wet environments may have hydathodes, structures that passively secrete water and excess mineral ions from xylem vessels by a process known as guttation (Feild et al., 2005). According to Feild et al. (2005), this process may be defensive in that it prevents flooding of the mesophyll. Carnivorous plants have modified leaves covered with various glands which function in attracting and digesting the prey (Joel, 1986). Pitcher plants (*Sarracenia* and *Nepenthes*) have large extrafloral nectaries above the pitcher (Dress et al., 1997; Owen & Lennon, 1999). All these types of secretory cells have been considered and analysed from an anatomical point of view (reviewed by Fahn, 2000). The structural similarity between nectaries, hydathodes, and elaiophores has often been noted (Schmid, 1988; Vogel, 1997).

Sugary secretions are also produced by fungi and insects. Fungal infection of the ovary of *Secale cereale* (Poaceae) attacked by *Claviceps purpurea* (Ascomycetes) elicits production of a sugary fluid that the parasite exploits to disperse its conidia (Alexopoulos et al., 1996). The comparison with nectaries is valid from an ecological point of view, because the pathogenically induced

exudate attracts insects that disperse the spores. The cost of fungal spore dispersal is, however, at the expense of the reproductive function of the plant. Wäckers (2002) gives other examples, such as rust fungi that produce, near their spores, sugar droplets consumed by dispersing insects.

Honeydew is the greatest non-floral source of sugar, and was probably a flight fuel for insects before the appearance of flowering plants. It is the excretory product of homopteran insects, such as aphids, whiteflies and scale insects, which must feed more or less continuously on phloem sap in order to obtain sufficient nitrogen. The excess sugar and water in their diet is excreted as honeydew, which differs from nectar in containing oligosaccharides synthesized by the insects from the dietary sugars. The sugar composition of honeydew depends on both the sap-sucking homopteran and its host plant and, in addition to sugars, amino acids from the phloem are also excreted to some extent (Byrne & Miller, 1990; Völkl et al., 1999). It was suggested by Downes and Dahlem (1987) that honeydew use may have preceded nectar feeding in early Diptera, which appeared long before the angiosperms: the pseudotracheate labellum of flies would have been ideal for dissolving and then imbibing dried films of honeydew on leaves. These sponging mouthparts are likewise suited for drinking stigmatic secretions which, like honeydew, are shiny fluids which would be visually attractive to flies. The fossil history of surface fluid feeding involves a wide range of imbibed fluids, not necessarily involved in pollination (Labandeira, 2002).

Many small insects such as flies, ants, and parasitoid wasps meet their carbohydrate requirements from a mixture of floral nectar, extrafloral nectar, and homopteran honeydew, although in laboratory experiments it has been found that the oligosaccharides in honeydew are less likely to elicit feeding responses and are of less value nutritionally (Wäckers, 2000, 2001). Ants in Australian rainforests obtain sugar and amino acids from many different nectar and honeydew sources (Blüthgen et al., 2004). Ants frequently tend phloem-feeding homopterans, protecting them from natural enemies in exchange for carbohydrate-rich fluids (Völkl et al., 1999). Honeydew is also a valuable sugar source for honeybees, particularly in forests when nectar is in short supply, and honeydew and other sugary fluids may substitute for nectar in the diets of nectarivorous birds (Paton, 1980; Gaze & Clout, 1983). Finally, the manna mentioned in the Biblical chapter Exodus was apparently honeydew produced by a scale insect (*Trabutina mannipara*) associated with tamarisk; it accumulates when attending ants are absent (Bodenheimer, 1947; Ben-Dov, 1988).

3 FLORAL AND EXTRAFLORAL NECTARIES

Two types of nectaries, floral and extrafloral, were recognized by Bonnier (1879). They may differ considerably in anatomical structure, source of nectar components, and mode of presentation (Davis et al., 1988; Pacini et al., 2003). Nevertheless, both have the same function: to reward animals that provide the mobility which plants lack—vectors for pollen dispersal and ants for physical defence—and their exudates are chemically similar. Floral nectaries, however, are better known than extrafloral ones and receive more attention in this volume. The reason for this "asymmetrical knowledge" is that floral structure and the different reproductive strategies of plants have long intrigued biologists and have resulted in comparatively more attention being directed to floral nectaries. These nectaries are also important sources of food for honeybees and are involved in the reproduction of many plants of economic significance and in the production of many fruit and seed crops. Extrafloral nectaries, which occur mainly in tropical plants, are noteworthy from an ecological point of view, but have limited economic applications, at least at present. Some of the differences between floral and extrafloral nectaries are summarized in Table 1. Koptur (1992) provides a detailed review of the interactions between insects and plants mediated by extrafloral nectaries.

Extrafloral and floral nectaries may be found in the same plant species with their secretion being collected by different kinds of animals. The structure, composition, and ecology of extrafloral and floral nectaries in the same species have been compared in various papers, e.g., *Croton sarcopetalus*, Euphorbiaceae (Freitas et al., 2001); *Tabebuia serratifolia*, Bignoniaceae (Thomas & Dave, 1992); *Thryptomene calycina*, Myrtaceae (Beardsell et al., 1989); *Turnera ulmifolia*, Passifloraceae (Elias et al., 1975).

The distinction between floral and extrafloral is topographical, but this separation is artificial. The distinction is certainly not clear in the genus *Euphorbia*, where the extrafloral cyathial nectaries are very close to the flower and are involved in pollination even if this is not clearly stated in the literature (Proctor et al., 1996, Fig. 2.16). Floral nectaries of *Ruellia radicans* (Acanthaceae) produce dilute nectar collected by hummingbirds. The nectary does not cease its secretory activity after the corolla has fallen, but continues producìng nectar with a higher sugar concentration, collected by ants (Gracie, 1991). This example is important because it demonstrates that nectary cells may produce nectar with different concentrations according to developmental stage and ecological necessities. The higher concentration of nectar

Table 1. Summary of the main differences between floral and extrafloral nectaries.

	Floral nectaries	**Extrafloral nectaries**
Function	Reward animals transporting pollen	Reward animals defending plant from herbivores
Position	In different parts of flower: ovary, stamen, calyx, corolla, receptacle	Common in leaves: petiole, stipule, blade Less often in developing inflorescence, e.g., *Euphorbia*, on floral parts (e.g., calyx or corolla) and developing or mature fruit, e.g., certain Bignoniaceae (Thomas & Dave, 1992)
Nectar consumers	Insects: especially Hymenoptera, Diptera, Lepidoptera Birds: e.g., hummingbirds, sunbirds Mammals: e.g., bats, small marsupials	Mainly ants
Duration of secretion	Few hours to several days, rarely exceeding a week as in *Helleborus* (Vesprini et al., 1999)	Few days in "tender" young growth, few weeks (when in fruit) to months (nectaries last as long as leaves)
Amount of nectar produced	Less than 1 µl to few ml: proportional to the nectary parenchyma volume	Generally few µl per day
Variability of nectar quality	Chemical and physical features (viscosity) vary widely in relation to different nectar consumers	Nectar physicochemical features vary less because ants are main consumers

during the extrafloral phase may be related to greater exposure of these photosynthesizing nectaries to light once the corolla falls.

Benefits to plants from the associations between ants and extrafloral nectaries are not always obvious. Extrafloral nectaries of ferns are best studied in the cosmopolitan bracken *P. aquilinum*, and are hypothesized to provide rewards for ants that defend the plants from herbivores. Experiments with British populations of bracken have, however, seldom provided any evidence that ants visiting foliar nectaries influence levels of herbivory (Heads and Lawton, 1985; Heads, 1986). In South African populations these extrafloral nectaries confer protection only when ant densities are high and homopterans

producing honeydew are also present (Rashbrook et al., 1992). In these experiments the ants strongly preferred honeydew to foliar nectar.

4 NECTAR COMPONENTS

Nectar composition varies widely, quantitatively more than qualitatively, presumably because it is produced to reward different kinds of animals (Faegri & van der Pijl, 1979; Cruden et al., 1983). Not surprisingly, nectar consumers and pollen vectors are primarily taxa that have evolved the ability to fly—insects, birds, and bats (Pellmyr, 2002). However, nectar rewards also attract many non-pollinators. Dissolved substances in nectar have multiple functions: in addition to rewarding animals with water, ions, carbohydrates, amino acids and low molecular weight proteins, nectar contains scented compounds to attract consumers (Raguso, 2004), and enzymes and antioxidants to maintain homeostasis of nectar composition (Carter & Thornburg, 2004). It may also contain toxic compounds to discourage unwanted consumers (Adler, 2000). For solutes other than sugars and amino acids, there is generally much more information available for floral nectars than for extrafloral nectars. Although many constituents of nectar originate in phloem sap, the latter fluid is more difficult to sample than nectar and studies comparing the composition of both fluids in the same plant are rare (for studies comparing phloem sap and extrafloral nectar see Baker et al., 1978 for *Ricinus communis*; Pate et al., 1985 for *Vigna unguiculata*).

The major constituents of nectar (see Nicolson & Thornburg, 2007, Chapter 5 in this volume) are given below, with a brief indication of their origins and their importance for animal consumers. Only for the carbohydrate component of nectar are the origins well understood. The early emphasis was on the energetics of the relationship between flower and pollinator, based on considering nectar as predominantly a sugar solution and also on the high energy demands of many pollinators. More attention is now being paid to the non-sugar components of nectar (it was Herbert and Irene Baker who first drew attention to these; Kevan, 2003) and to their role in pollinator attraction and nutrition. This is by no means an exhaustive list: see Jakubska et al. (2005) for an example of the chemical complexity that becomes evident when nectar is subjected to suitable analytical techniques.

Water. Depending on nectary structure, water may be derived from both xylem and phloem or phloem alone, with a lower water content being expected as the proportion of phloem in the vasculature increases. Nectar water content depends on floral microclimate, and may be greatly affected by

evaporation in exposed flowers. The nectar concentration determines its viscosity and hence influences the feeding responses of animals; water in nectar may also be an important reward for pollinators in dry conditions.

Carbohydrates. The main nectar solutes are the sugars sucrose, glucose and fructose, and their total concentration ranges from 7% to 70% w/w. Invertase activity in the nectary determines the proportion of sucrose to hexoses. Considerable attention has been paid to the question of whether the relative proportions of these three sugars in nectars are a result of adaptation to pollinators (Baker & Baker, 1983; 1990) or phylogenetic history (e.g., Nicolson & van Wyk, 1998; Galetto & Bernardello, 2003). Other monosaccharides and disaccharides may be present in minor amounts, as well as oligosaccharides such as stachyose, and sugar alcohols such as sorbitol. However, oligosaccharides are much less abundant in nectar than in honeydew. Sometimes polysaccharides may be responsible for a jelly-like consistency of nectar (Sazima et al., 2001). The sources of nectar carbohydrates are phloem sap (in which case nectary parenchyma is reduced or absent); photosynthesizing nectary parenchyma, starch stored in parenchyma and derived from photosynthesis in that tissue or other floral parts, or the degeneration of certain nectary parts (Pacini et al., 2003). Sugars in nectar are usually the primary energy source for consumers, and the study of plant–pollinator relationships has long been based on energetics, with clear correlations between the sugar content of flowers and the energy requirements of the animals pollinating them (Heinrich, 1975).

Amino acids and proteins. Amino acids are the most abundant nectar solutes after sugars, and include a wide array of both essential and non-essential amino acids, as well as some non-protein amino acids (e.g., Petanidou et al., 2006). Proteins occurring in nectar include enzymes and preservatives (Carter & Thornburg, 2004). These nitrogenous components are derived from one or more of the following sources: phloem sap, protein bodies in the nectary parenchyma, cytological activity or degeneration of certain parts of the nectary, or the epidermis of the nectary parenchyma. Nectar amino acids may play a role in taste preferences of insects (Gardener & Gillman, 2002) and in their nutrition, depending on other food sources (Mevi-Schütz & Erhardt, 2005). Proteins appear to have various homeostatic and regulatory roles.

Ions. These are derived from xylem and/or phloem sap, although information on ion concentrations in floral nectars is scarce. Again, the nutritional benefits to pollinators will depend on other food sources. High K^+

concentrations in the nectar of onion flowers have a deterrent effect on honeybees (Waller et al., 1972).

Antioxidants such as ascorbate are involved in nectar homeostasis (Carter & Thornburg, 2004).

Lipids are a high energy source but usually occur only in trace amounts in nectar. In some flowers, oils secreted by elaiophores or glandular trichomes are offered as rewards instead of nectar.

Terpenoids. Volatile terpenoids are important components of floral scents (Raguso, 2004) and may accumulate in nectar.

Secondary compounds associated with resistance to herbivory have often been documented in floral nectar (Adler, 2000). Toxic compounds such as phenols and alkaloids may have a selective effect on pollinators, deterring some and attracting others.

Cytoplasmic remnants result mainly from holocrine secretion where the secretory cells break down in the process, e.g., *Strelitzia reginae* (Kronestedt-Robards et al., 1989), *Glycine max* (Horner et al., 2003).

Spores of fungi and bacteria dispersed in the air may fall into nectar, especially if it is exposed, and grow. Thus nectar may be a portal for plant pathogen infections. However, antimicrobial substances with a homeostatic function may prevent the spread of harmful organisms (reviewed by Carter & Thornburg, 2004), because examples of infection are rare. In only a few cases have these invasions been demonstrated to occur via the nectar. Spores of the mould *Aureobasidium pullulans* and *Cladosporium herbarum* enter the nectary via the nectar and destroy extrafloral nectaries in the leaves of *Ailanthus altissima* (Clair-Maczulajtys & Bory, 1982). The pathogen bacterium *Erwinia amylovora*, the agent of fire blight also enters flowers via nectar (Bubán et al., 2003).

Nectar should be seen as a complex and dynamic fluid. Pollinators reduce the volume, sometimes stimulating further secretion in the process, and contaminate it with microbes. Changes in nectar sugar are caused by activity of the nectary (secretion or reabsorption) as well as removal by foragers, which may stimulate further secretion. Nectar water content depends on activity of the nectary, removal by foragers, and is additionally affected by equilibration with ambient humidity (Corbet, 2003). This is particularly noticeable in the more exposed extrafloral nectaries. The resulting spatial and temporal variation is a frequent theme in subsequent chapters.

5 ORGANIZATION OF THIS VOLUME

Two major volumes on nectar and nectary biology were published in 1983 and are long out of print: *The biology of nectaries* (Bentley & Elias, 1983) and *Handbook of experimental pollination biology* (Jones & Little, 1983). The publication of *Nectary biology* (Bahadur, 1998) was delayed and the volume is not widely available. Endress' (1994b) book, *Diversity and evolutionary biology of tropical flowers*, first considers the nectary per se, from a morphological point of view, and subsequently from a systematic point of view in families where it is present. It also provides some historical background to the study of flowers and nectaries. *The natural history of pollination* by Proctor et al. (1996) is an update of an earlier edition and a detailed account of pollination biology. The publication of three books dealing with the practical aspects of pollination biology (Dafni, 1992; Dafni et al., 2005; Kearns & Inouye, 1993) indicates strong interest in this field in recent years. The topical theme of specialization versus generalization in pollination systems has led to a new multi-author volume (Waser & Ollerton, 2006). Volume 238 (2003) of *Plant Systematics and Evolution*, entitled *Nectary and nectar: from biology to biotechnology* and edited by A.R. Davis, M. Hesse, M. Nepi and E. Pacini, is devoted to papers presented at a meeting held in Montalcino in Tuscany, Italy, in 2002. In the journal *Ecology* 2004, vol. 85 there is a special section devoted to papers on *Community and Evolutionary Ecology of Nectar*, with contributions on both floral and extrafloral nectaries from different ecological points of view.

The present book, *Nectaries and nectar*, emphasizes both the plant side of the interaction (nectary structure and function) and the animal viewpoint (nectar composition and consumption). The remaining seven chapters are organized into four conceptual areas, which are discussed in more detail below.

Nectary systematics (Chapter 2)

This chapter reviews the distribution of floral nectaries throughout the angiosperms in a systematic context. Nectar-secreting tissues show great variety in their location and histological structure, previously surveyed in dicots and monocots respectively by Smets (1986) and Smets et al. (2000). Plant diversity is often linked to adaptive radiation of pollination systems, and the variety of nectar-secreting tissues is to some extent associated with the varying morphology and behaviour of pollinators. However, there is also a strong relationship with specific plant phylogenetic lineages, and hence to plant

systematics. In some plant families the nectaries differ greatly in position, morphology, and nectar composition, while others are relatively homogeneous. Species of either small or large families may resemble each other in nectary organization but others, such as Ranunculaceae, show wide variation.

Nectary structure and nectar production (Chapters 3 and 4)

Nectary structure may vary with nectary position in the flower. Though nectary histological components have long been recognized, ultrastructural studies of secretory cells are revealing new details of organelles during nectary development and secretion. Independently from the anatomical organization, the extent of nectary parenchyma determines the quantity of nectar produced and hence the type of pollinator. The anatomical diversity of nectaries may be matched by a similar diversity in the mechanisms of nectar secretion and presentation. The concept of secondary nectar presentation, namely when nectar is not exposed close to the nectary but elsewhere in the flower, as in spurs, was recently developed.

Nectar components may be produced and elaborated in different parts of the nectary tissue. It is generally assumed that nectar carbohydrates are derived from phloem sap, but photosynthesis in the nectary parenchyma may be an important supplementary source of carbohydrates. The storage of starch in non-photosynthesizing nectaries is an advantage when rapid production of nectar is required. We discuss the dynamics of nectar production, including reabsorption of unconsumed nectar. The interaction between the dynamics of nectar production by the plants and nectar feeders defines the nectar standing crop. Animal–plant interactions also affect the site and manner of nectar presentation. Variability in nectar quantity and quality is apparent at many different spatial and temporal levels and is strongly affected by environmental parameters.

Nectar chemistry and molecular biology (Chapters 5 and 6)

The chemical complexity of nectar has been apparent since the prolific work of Herbert and Irene Baker, but analytical methods have naturally improved and a new look at nectar chemistry is appropriate. Nectar sugar composition has been extensively studied, particularly the relative proportions of sucrose, glucose and fructose, and the data have pointed to convergence in nectar characteristics of unrelated plant species and pollinator type (Baker & Baker, 1983); however, the fact that there are phylogenetic constraints on the adaptation of nectar to pollinators has become more apparent in recent years. Nectar is also much more than a dilute sugar solution, and there is renewed

interest in its non-sugar components, such as proteins which inhibit microbial growth, amino acids which contribute to taste and the nitrogen balance of pollinators, and alkaloids and phenols which deter certain pollinators but not others. The water component in nectar, greatly affected by microclimatic conditions, is an important factor in pollinator drinking rates and water balance.

The molecular biology of nectar is a relatively new area of research. Chapter 6 describes the developmental processes that change the *Nicotiana* floral nectary from a non-secretory organ into a secretory one. There is a dramatic decline in levels of starch in the nectary to produce sugar for nectar production. A general analysis of gene expression in nectaries is included, with special reference to proteins with a defence function against microbial attack.

Nectar consumption and ecology (Chapters 7 and 8)

Flowers differ in size by orders of magnitude, and so do their nectaries and the volumes of nectar produced for nectar-consuming animals, which range from 10 mg flies to 30 g bats. Nectar, especially in more open and accessible flowers, is also consumed by nectar robbers, which provide no benefit to the plant. The most numerous nectar consumers are found in three of the four largest insect orders (Diptera, Lepidoptera, and Hymenoptera), and nectarivorous birds and bats provide reliable pollination services in warmer parts of the world. Nectar feeding has physiological implications for all these animals in terms of water, energy, and nitrogen balance, but many are able to utilize nectars of varying composition and concentration. As stressed by Galetto and Bernardello (2003), "success in attracting pollinators is a relative matter", depending on alternative nectar sources available, so animal visitors should not be too particular. The ability of flower-visiting animals to deal with all kinds of nectar seems appropriate in view of the broad generalization apparent in many plant–pollinator relationships.

Most of the individual studies on nectaries, nectar, and nectar consumers included in this book concern a few plant species (either sympatric or related) and a few animal species that visit them. The final chapter takes a much broader approach, examining nectar resources at the community level in Mediterranean habitats. The information is derived from a unique data set including extensive analyses of nectar sugars and amino acids, combined with a complete survey of insect visitors. It has enabled consideration of several hypotheses about the evolutionary ecology of nectar production in Mediterranean environments, where summer drought is common, flowers

tend to produce small volumes of concentrated nectar, and bees are the dominant pollinators. The role of pollination mutualisms in structuring communities is a rich and rewarding field of study.

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Chapter 2

A SYSTEMATIC SURVEY OF FLORAL NECTARIES

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1 INTRODUCTION

The construction of classifications, as well as the understanding of biological diversity, depends upon a careful comparison of attributes of the organisms studied (Stuessy, 1990). It is widely known that data from diverse sources showing differences from taxon to taxon are of systematic significance. During the 20th century, systematists have emphasized that their discipline involves a synthesis of all knowledge (Stevens, 1994) or, in other words, the variation of as many relevant characters as possible should be incorporated into the natural system to be constructed. The extent to which particular characters are constant or labile will determine their usefulness to systematics. In general, more conservative characters will be valuable in defining families and orders, whereas more labile characters may be useful at the generic and specific levels (Webb, 1984). There is no doubt that floral characters are among the most used in the classification of flowering plants. At the same time, they constitute essential features in diagnostic keys to taxa in both taxonomic treatments and Floras (Cronquist, 1981, 1988).

The diversification of many plant families has been attributed to adaptive radiation of pollination and mating systems accompanying changes in ecology and life history (e.g., Grant & Grant, 1965; Dressler, 1990; Barrett et al., 1996). Reproductive traits in seed plants provide a potentially rich source of diversity for comparative and phylogenetic studies. When considering the evolution of the breeding or pollination systems of particular species or genera,

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it is important to appreciate the constraints that operate on reproductive characters in that family. Although adaptation to pollinators has played a major role in floral evolution, sexual selection associated with pollen competition may have generated conflicting evolutionary pressures acting in opposition to selection generated by pollinators (Armbruster, 1996).

As nectar is, by far, the most important reward for animal-pollinated flowering plants (Simpson & Neff, 1983; Proctor et al., 1996), the occurrence, position, and characteristics of nectaries in flowers are helpful comparative data for assessing relationships. Owing to the diversity in nectary positions and structures, floral nectaries are thought to have independently evolved several times after the angiosperms were already highly diversified, with a great potential to contribute to the diversification of both plants and animals (Fahn, 1979; Cronquist, 1988; Lee et al., 2005b). Effectively, fossil records of insects and angiosperms suggest that the timing of the radiations of some groups were coincident (Meeuse, 1978; Crepet & Friis, 1987; Crepet et al., 1991; Pellmyr, 1992).

Despite their biological significance, floral nectaries have been neglected in plant systematic studies in particular (Chesselet et al., 2002). At the same time, morphologists have generally not considered nectaries as special structures and have not paid adequate attention to them (Lorch, 1978). Brown (1938) attributed the apparent lack of interest in the systematic significance of nectaries to the fact that as taxonomic studies normally use herbarium specimens they are not always evident in dried plants, as they are small, soft-tissued structures. Isolated attempts were made at the end of the 19th and the first half of the 20th centuries (Lorch (1978) and Schmid (1988) give a thorough historical account) to investigate nectaries with a systematic background, e.g., Bonnier (1879), Knuth (1906–1909), Brown (1938), Norris (1941), Fahn (1953), Brown (1961), and Kartashova (1965), among the most relevant. In later years, Cronquist (1981, 1988) has to be commended for his great interest in nectaries and the careful effort put into his taxonomical descriptions regarding the presence of nectaries, as well as his attempts to define trends within families and higher order groups. In parallel, there has been a tremendous interest in reproductive biology, pollination, and breeding system research, which indirectly includes nectaries. However, in spite of a century of recognition, and more than two decades of intensive study by systematists and ecologists, the impact and meaning of reproductive characters as a whole on systematic treatments is still overlooked or undervalued (Anderson, 1995). The synthesis of both types of data is sorely needed, because understanding plant reproductive biology helps to clarify the potential use and value of these

characters in systematic treatments and sound phylogenies can clarify the origin of reproductive characters or syndromes (Anderson et al., 2002).

Fortunately, several authors have demonstrated the taxonomic and evolutionary value of floral nectaries in understanding the classification of many angiosperm groups. Examples of the groups that have been analysed with interesting and challenging results are: Apocynaceae–Asclepiadoideae (Christ & Schnepf, 1988), Bignoniaceae (Galetto, 1995a; Rivera, 2000a), Bromeliaceae (Böhme, 1988; Sajo et al., 2004), Costaceae (Newman & Kirchoff, 1992), Crossosomatales (Matthews & Endress, 2005a), Dipsacales (Wagenitz & Laing, 1984), Haemodoraceae (Simpson, 1993), Iridaceae (Rudall et al., 2003a), Lamiaceae (Dafni et al., 1988; Petanidou et al., 2000), Liliaceae (Khaniki & Persson, 1997), Malvales (Judd & Manchester, 1997; Vogel, 2000), Melastomataceae (Stein & Tobe, 1989), Melianthaceae (Decraene & Smets, 1999a; Decraene et al., 2001), Aizoaceae–Mesembryanthemoideae (Chesselet et al., 2002), Polygonaceae (Decraene & Smets, 1991a), Rhamnaceae (Medan & Aagesen, 1995), Solanaceae (Bernardello, 1987; Cocucci & Galetto, 1992), among others. More importantly, the survey publications on dicot groups by Smets (1986, 1988) and Smets and Cressens (1988) and on monocot groups by Daumann (1970), Vogel (1981a), Endress (1995), and Smets et al. (2000) were fundamental to appreciation of the significant role of nectaries in systematics and evolution. Nevertheless, their potential impact is still underexploited. Taking this background into account, I here summarize the biodiversity of floral nectaries and their general position and distribution in gymnosperms and angiosperms, with emphasis on their use in systematic classifications and phylogenies.

2 NECTARIES IN GYMNOSPERMS

Although most gymnosperms are anemophilous, entomophily has evolved in Cycadales and Gnetales, albeit differently in each group. In many cycads, thermogenic cones characterize pollination: here beetles and thrips find rewards in the form of food, mating sites and brood sites for larvae, and in return act as pollen vectors (e.g., Tang, 1987; Donaldson, 1997; Terry et al., 2004).

The three genera of Gnetales—*Ephedra* (Bino & Meeuse, 1981; Bino et al., 1984a, b; Meeuse et al., 1990), *Gnetum* (Kato et al., 1995), and *Welwitschia* (Wetschnig & Depisch, 1999)—differ in that their ovules secrete a sugary droplet at the micropylar end (Fig. 1), i.e., the nucellar apex of the ovule (Martens & Waterkeyn, 1974; Moussel, 1980; Carafa et al., 1992),

a circumstance reported long ago (e.g., Pearson, 1909; Porsch, 1910; van der Pijl, 1953). Several authors have considered this exudate to be nectar (e.g., Jaeger, 1957; Martens, 1971; Singh, 1978; Endress, 1996), but the producing structure has been named a nectary only by Jaeger (1957). Nevertheless, Schmid (1988, p. 208) pointed out that this term is improper, as the pollination drop of gymnosperms and the nectar of angiosperms are not homologous. He indicated that the use of the terms nectar and nectary for gymnosperms "applies only in a loose, ecological sense of the words since the primary role of the pollination drop relates to manipulation of pollen rather than reward to insects". In my opinion, the droplets in Gnetales may be regarded as a nectar reward for pollinators produced by ovular nectaries, according to the broad topographical classification of nectaries of Schmid (1988). The fact that these micropylar secretions primarily serve to draw pollen into the inner regions of the ovule where pollen germination takes place, as happens in most gymnosperms (e.g., Gelbart & von Aderkas, 2002), does not prevent insects that visit the plants being attracted by the exudate and pollinating them, as proved in several instances. As this micropylar nectar secretion can take place either nocturnally or diurnally, the type of insect visitors varies accordingly: moths, flies, bees, and wasps are the most relevant groups recorded (Meeuse et al., 1990; Kato et al., 1995; Wetschnig & Depisch, 1999). More importantly, the droplet of these species has a higher sugar concentration (10–80%; Bino et al., 1984b; Kato et al., 1995; Wetschnig & Depisch, 1999) than the droplets of the strictly anemophilous conifers (~1%; McWilliam, 1958), and thus, can be considered equivalent to angiosperm nectar.

From the anatomical point of view, the ovular nectaries of *Ephedra*, *Gnetum*, and *Welwitschia* can be regarded as non-structural nectaries. Effectively, they are not histologically or cytologically recognizable (cf. Martens, 1971; Fahn, 1979), but they regularly secrete a solution that contains sugars serving as reward for pollinators. The secretion takes place at the nucellar apex (Martens, 1971; Martens & Waterkeyn, 1974; Moussel, 1980; Carafa et al., 1992). The inner epidermis of the micropylar tube and the integument may take part in the secretion as well, but this fact is not clear (Martens, 1971; Endress, 1996).

In addition to these ovular nectaries, in some *Ephedra* species nectaries have been found on the bracts of male and female plants enveloping the reproductive units and on the integuments of the female plants (Bino & Meeuse, 1981; Bino et al., 1984a, b). In a *Gnetum* species (Kato et al., 1995), in which sterile female flowers are lacking in male inflorescences, nectar is secreted between and on the collars. In these taxa both types of nectaries

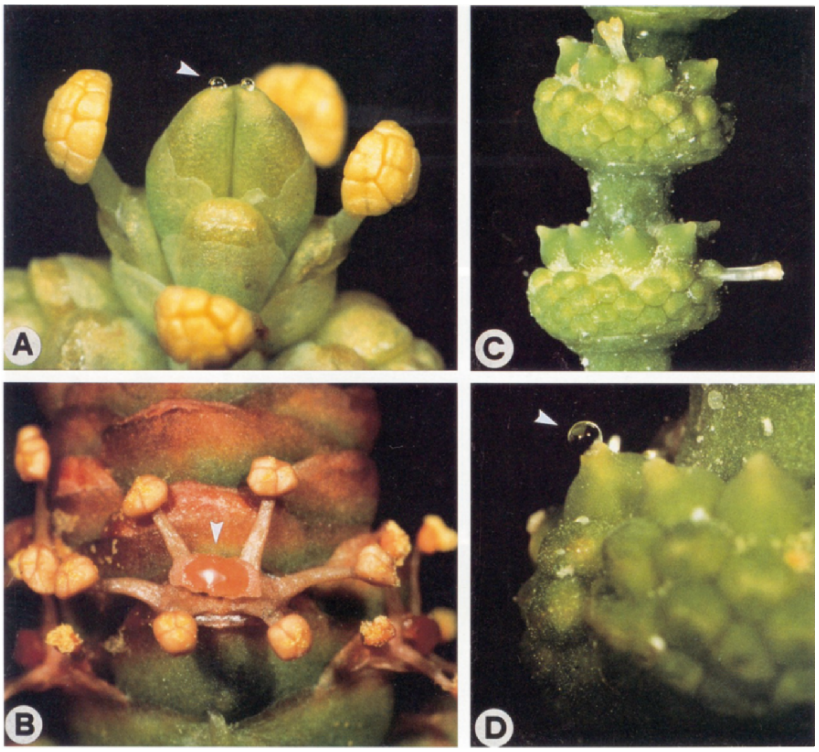

Figure 1. Nectaries in gymnosperms. Structurally bisexual organ complexes showing male organs and sterile female organs with secretion drop at the micropylar orifice (*arrowheads*). A, *Ephedra fragilis*, inflorescence with two upper female flowers, stamens of male flowers still closed, ×9. B, *Welwitschia mirabilis*, flower with ovule in the centre, stamens still closed, those of flower on the right side open, ×7.5. C, D, *Gnetum gnemon*. C, Two whorl complexes, two male flowers open, each showing a stamen, other male flowers still in bud stage, ×5. D, part of a whorl complex, a sterile female flower with secretion drop, ×12. (From Endress, 1996, p. S119, Fig. 7; reprinted with permission of the University of Chicago Press, Chicago.)

are located close to each other; they are visited at the same time and are related to the pollination of the plants bearing them.

In addition to insect pollination, wind pollination has been reported in several *Ephedra* species (e.g., Bino & Meeuse, 1981; Niklas & Buchman, 1987), but it is now considered either impossible or negligible for *Gnetum* and *Welwitschia* (Kato et al., 1995; Wetschnig & Depisch, 1999), although earlier articles suggested it.

The prevalence of entomophily in the extant Gnetales and its monophyly as implied by recent molecular analyses (Rydin et al., 2002; Soltis et al.,

2002; Ickert-Bond & Wojciechowski, 2004) suggests that entomophily, accompanied by nectar secretion, may be considered a synapomorphy for this order and that it has been important in its evolution.

3 NECTARIES IN ANGIOSPERMS

3.1 Diversity

Floral nectaries are comparatively simple structures of different origins that are involved in the pollination process. They can occur in virtually all parts of the flower and produce a variety of sugary exudates. Even though for more than a century the shape, structure, and position of these nectaries has been used for taxonomic and phylogenetic considerations (e.g., Behrens, 1879; Bonnier, 1879; Knuth, 1906–1909; Brown, 1938, 1961; Norris, 1941; Fahn, 1953; Kartashova, 1965), only relatively recently has the systematic value of nectaries been acknowledged (e.g., Daumann, 1970; Zandonella, 1977; Fahn, 1979; Cronquist, 1981, 1988; Vogel, 1981a; Smets, 1986, 1988; Smets & Cressens, 1988; Smets et al., 2000, 2003). As has happened with many other reproductive characters, nectaries have, to some extent, been overlooked by systematists. These specialized structures can, however, show evolutionary trends in plant groups because they are either reasonably homogeneous, or because they have dramatically changed within groups. In addition, they may be easily lost or acquired within a lineage, helping to understand the evolution of the group and of its mating systems.

In angiosperms, the diversity of nectaries may be related to several causes involving three basic aspects of nectary biology: nectar presentation, nectary structure, and nectary fate. As a whole, the extent to which these variations are correlated with the delimitation and history of coherent plant groups is largely unexplored. Nevertheless, all these aspects may reveal significant trends and should be taken into account when trying to elucidate them. In addition, symmetry, number, and colour of nectaries are of interest in evaluating their diversity.

3.1.1 Nectar presentation

Floral nectaries may either be at the same level as the surface of the organ that bears them, form a protrusion, or be deeply embedded (Fahn, 1979, 1988). In addition, how nectar is presented within the flower—i.e., its degree of exposure—is highly significant from the functional point of view. Thus, the more exposed the nectar, the easier it is to reach and remove for different

kinds of animals. The relative shape, size, behaviour, and mouthparts of visitors compared to the size and shape of the flowers are essential in determining access of animals to the nectar and their efficiency as pollinators. In addition to and as a consequence of exposure, nectar concentration may be affected directly by corolla depth, since the concentration in flowers with long corolla tubes is lower than in flowers with short or no tubes, from which water evaporates readily (Corbet, 1978; Plowright, 1987).

As flowers can be regarded as pollination units, their functional structure is closely related to their pollination mechanisms. If the well-known structural blossom classes of Faegri and van der Pijl (1979) are taken into account and are correlated to nectariferous flowers, several possibilities arise regarding how nectar is offered and can be foraged by visitors within the group of conspicuous and advertising flowers. This system may be equally applied to whole inflorescences that actually operate as single floral units, e.g., a capitulum. Generally, nectar exposure is more intense when flowers are chasmogamous, opening when shedding pollen. There are several degrees of exposure, here listed from most to least accessible, according to the different flower classes (Fig. 2):

- Dish- or bowl-shaped flowers (e.g., in some Apiaceae, Asteraceae, Ranunculaceae, Rosaceae)
- Head- or brush-shaped (e.g., in some Caryophyllaceae, Combretaceae, Myrtaceae, Proteaceae, Fabaceae–Mimosoideae, Salicaceae)
- Bell- or funnel-shaped (e.g., in some Alliaceae, Campanulaceae, Gentianaceae, Rubiaceae)

Alternatively, flower shape may ensure that nectar is comparatively hidden and more difficult to forage, as in the next flower classes to an increasing degree:

- Gullet-shaped flowers (e.g., in some Acanthaceae, Convolvulaceae, Lamiaceae, Scrophulariaceae)
- Flag-shaped flowers (e.g., in some Asparagaceae, Fabaceae–Papilionoideae, Papaveraceae–Fumarioideae)
- Tube-shaped flowers (e.g., in some Apocynaceae–Apocynoideae, Caprifoliaceae, Polemoniaceae, Solanaceae)
- Flowers that have to be opened by visitors forcing their way in (e.g., in some Fabaceae–Papilionoideae, Polygalaceae, Scrophulariaceae)

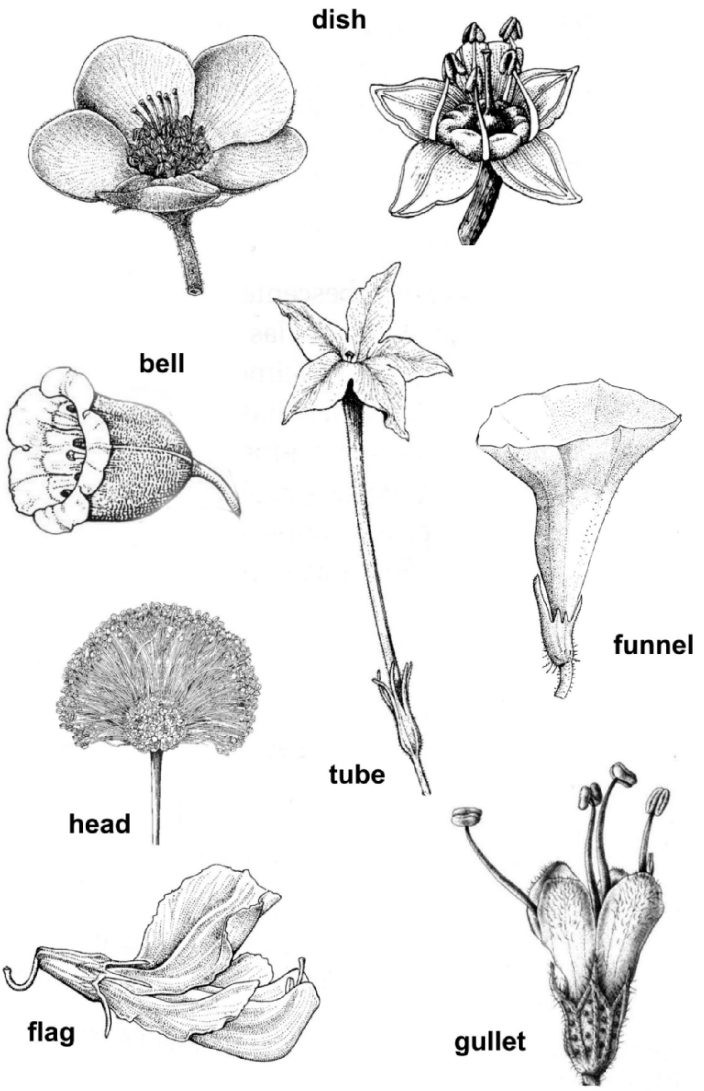

Figure 2. Examples of some of the structural blossom classes of Faegri and van der Pijl (1979) in relation to nectar presentation. Dish-shaped flowers: *Malvella leprosa* (Malvaceae) and *Condalia microphylla* (Rhamnaceae), respectively. Bell-shaped flower: *Anisodus tanguticus* (Solanaceae). Tube-shaped flower: *Nicotiana longiflora* (Solanaceae). Funnel-shaped flower: *Sclerophylax caducifructus* (Solanaceae). Head-shaped flower: *Anadenanthera colubrina* var. *cebil* (Fabaceae). Flag-shaped flower: *Galega officinalis* (Fabaceae). Gullet-shaped flower: *Mentha* × *rotundifolia* (Lamiaceae).

Let us now consider how nectaries release their exudate, a feature also related to nectar presentation. Commonly, nectar is released where it is produced, called primary presentation by Pacini et al. (2003), i.e., nectar is exposed to pollinators on the surface of capitate nectaries or on continuous surfaces. Sometimes, however, the nectar is conducted to other floral parts and is then termed secondary presentation (Pacini et al., 2003). This is usually accomplished through nectar ducts—auxiliary structures whose function is to conduct nectar from its production source to a comparatively distant site of presentation (cf. Vogel, 1998c). Generally, nectar flows along these ducts by capillarity, secretion pressure, or gravity. The variability of nectar ducts within a certain plant group may also be systematically important. Some examples of secondary nectar presentation are:

- Several Alliaceae and Xanthorrhoeaceae, whose papillate septal nectaries principally bear their three primary outlets at the top of the superior ovary, but only rarely exude nectar there; they have several possibilities, such as ducts along the external carpellary sutures towards the base of the ovary, towards the base of the style, or along an ovarial stipe (Daumann, 1970; Vogel, 1998b; Nepi et al., 2006).
- In some Haemodoraceae with half-inferior ovaries, septal nectaries are drained by lateral channels conducting the nectar to spur-like auricles of the tepals (Simpson, 1993; Vogel, 1998b).
- In some Caryophyllaceae, nectar collects at the bottom of a tubular calyx and flows from the disc through ten channels along a columnar stipe (Zandonella, 1977; Vogel, 1998b).
- In some Solanaceae (*Capsicum*), nectar is produced by a typical ovarian nectary from where it flows upwards through ducts from the corolla tube towards apertures located in the staminal fascicle (Huber, 1980; Vogel, 1998b).

The nectariferous spurs of some Scrophulariaceae and Violaceae use different strategies to supply spurs with the nectar produced by relatively distant nectaries (Vogel, 1998b; Nepi et al., 2003). However, in other nectariferous spurs, nectar may be produced and accumulated directly in the spurs.

3.1.2 Structure

Nectaries can be divided into two groupings: **structural** and **non-structural** nectaries. Structural nectaries are sharply localized, histologically differentiated areas where nectar is regularly exuded, whereas non-structural nectaries are non-differentiated areas that are able to secrete nectar sporadically (Zimmermann, 1932; Daumann, 1970; Fahn, 1979). From this perspective,

any aerial plant part could act as a nectar-secreting tissue. As the origin of the secreted nectar is the phloem sap (Fahn, 1979, 1988), the pre-nectar can move from the sieve elements to the cells of the neighbouring tissue (either specialized nectariferous or ordinary parenchymatous tissue) and then to the plant surface.

Because of their nature, non-structural nectaries are hard to distinguish if the actual exudate is not observed. The main issue is that when visitors actively patrol and frequently forage the nectar, no droplets or nectar accumulation can be seen; therefore, the secretion is overlooked. This may be the reason why non-structural nectaries are apparently rare in angiosperms. So far, they have been reported in a few families, such as Bromeliaceae (Galetto & Bernardello, 1992), Cactaceae (Elias, 1983), Costaceae (Zimmermann, 1932; Elias, 1983), Fabaceae (Vogel, 1997), Melastomataceae (Stein & Tobe, 1989; Vogel, 1997), Orchidaceae (Frey-Wyssling & Häusermann, 1960; Galetto et al., 1997), Paeoniaceae (Frey-Wyssling & Häusermann, 1960; Hiepko, 1966), and Ranunculaceae (Kartashova, 1965). Non-structural nectaries may indeed be overlooked and under-reported among the vascular plants, and warrant careful observation.

When the nectary is structural, its three main histological components (epidermis, specialized parenchyma, and vascular system; see Nepi, 2007, Chapter 3 in this volume) for structural and ultrastructural features) may differ and, in addition to their functional impact, this variation may be of systematic value. Let us consider these aspects separately:

Epidermis. Except for the septal nectaries, which are deeply sunken in inner floral tissues (Daumann, 1970; van Heel, 1988; Rudall, 2002), floral nectaries are related to the surface of the different floral whorls. The epidermis may be standard, wholly secretory, or include some secretory structures that are responsible for producing nectar, which can be referred to as **epidermal nectaries** in general.

Epidermal nectaries. Nectar-secreting structures located in the epidermis are relatively common in flowering plants. Although they can consist of trichomes, papillae, idioblasts, or glands, they have a common origin.

In 1977, Vogel recognized three basic nectary types: mesenchymatous (composed of glandular and storage tissues), epithelial (basically a glandular epidermis), and trichomatous (secreting trichomes). Recently, Vogel (1998a) described **nectarioles** as small, few-celled nectaries that are glandular modules or idioblasts, which can occur singly or in clusters and are anatomically

heterogeneous. He lists four kinds of nectarioles: glands, idioblasts, clusters of mesenchymatic cells, as well as trichomes. Accordingly, there is some overlap among nectarioles, trichomatous nectaries, and epithelial nectaries. Considering the great variability of these few-celled epidermal structures, it seems easier to refer to them plainly as epidermal nectaries, even though they may contain a few subepidermal cells. Larger, conspicuous, more complex, frequently vascularized secreting structures will be referred to as nectaries of a floral whorl (e.g., receptacular, sepal, petal, or staminal nectaries), and correspond to the mesenchymatous type of Vogel (1977).

Trichomes are by far the most common epidermal nectaries. They may be unicellular (e.g., Dipsacales; Wagenitz & Laing, 1984; Orchidaceae; Stpiczyńska et al., 2005; Tropaeolaceae; Rachmilevits & Fahn, 1975), or multicellular (Adoxaceae; Erbar, 1994; Wagenitz & Laing, 1984; Anacardiaceae; Wunnachit et al., 1992, Malvaceae *s.l.*; Vogel, 2000; Leitao et al., 2005). Multicellular trichomes can be either uni- or multiseriate, although both types are usually present in the same plant group. Nectariferous trichomes may be scantily dispersed (Sterculioideae; Vogel, 2000) or form compact, sharply circumscribed cushions or carpets whose shapes may be taxonomically important in defining related plant groups (e.g., Bombacoideae, Malvoideae; Vogel, 2000).

The following list includes the families (all dicots) known to have trichomes, at least in some genera, where the trichomes are located on the floral structure, as well as a reference:

- Anacardiaceae: corolla (Wunnachit et al., 1992)
- Aristolochiaceae: calyx (Daumann, 1959; Sakai, 2002)
- Bignoniaceae: corolla tube (Lopes et al., 2002)
- Capparaceae: receptacle (Schmid et al., 1984)
- Convolvulaceae: sepal (Keeler & Kaul, 1984)
- Cucurbitaceae: petal or hypanthium (Vogel, 1997)
- Dipsacales: corolla tube (Fahn, 1979; Wagenitz & Laing, 1984; Smets, 1986; Erbar, 1994; Davis, 2003)
- Lentibulariaceae: petal spur (Vogel, 1998a)
- Malvaceae *s.l.* (Bombacoideae, Byttnerioideae, Dombeyoideae, Grewioideae, Helicterioideae, Malvoideae, Sterculioideae, and Tilioideae): petal, sepal, or androgynophore (Vogel, 2000; Leitao et al., 2005)
- Myrsinaceae, Primulaceae, Theophrastaceae: generally corolla, rarely ovary surface (Vogel, 1986, 1997)
- Ranunculaceae: ovary surface (Peterson et al., 1979; Smets & Cresens, 1988)

- Tropaeolaceae: sepal spur (Rachmilevits & Fahn, 1975)
- Verbenaceae: corolla tube (Smets, 1986)

Within the monocots, trichomes in tepal nectaries have been observed occasionally, e.g., in Orchidaceae (Galetto et al., 1997; Stpiczyńska et al., 2005) and Liliaceae (Kanikhi & Persson, 1997; Rudall et al., 2000). Smets et al. (2000) mention trichomes in *Sisyrinchium* (Iridaceae), although uncertain if they are nectariferous or not.

Other epidermal nectaries are small secreting glands or nectarioles (Vogel, 1998a), e.g., in the tepals of some Aristolochiaceae, Calycanthaceae, and Cabombaceae, the sepals of one species of Gentianaceae, the perianth of Nepenthaceae and Lentibulariaceae, the perianth and ovary wall of Sarraceniaceae, and the bracts and tepals of Cephalotaceae.

In some groups, trichomes and papillae are related to mesenchymatic nectaries and are located in the epidermis of the nectary, e.g., in Aizoaceae (Ihlenfeldt, 1960), Caryophyllaceae (Zandonella, 1977), and Polygonaceae (Decraene & Smets, 1991a).

Nectar exudation. There are several ways in which nectar can be exuded though epidermal cells (Fahn, 1979, 1988; Pacini et al., 2003):

- Crossing the plasmatic membrane and the cell wall, accumulating between the cell wall and the cuticle, which breaks under the nectar pressure (Wunnachit et al., 1992).
- The cell walls may have ingrowths like transfer cells that facilitate secretion (Kronestedt et al., 1986).
- Through the cuticle, either with rupture of its outer layer (Figueiredo & Pais, 1992; Stpiczyńska, 2003), or through microchannels or micropores (Robards & Stalk, 1988; Vassilyev & Koteyeva, 2005; Wist & Davis, 2006).
- The epidermal cells gradually die, producing nectar for a given period (Vesprini et al., 1999).
- Through orifices or small pores (Vogel, 1997).
- Through modified stomata which have lost the capacity to open and close (Fahn, 1988; Davis & Gunning, 1992; Gaffal et al., 1998); they are named sap-holes (Vogel, 1997), nectarostomata (Smets, 1988), or nectarthodes (Schmid, 1988).

Modified stomata are the most frequent way of nectar exudation and have been extensively reported for many dicots: Apocynaceae (Galetto, 1997),

Asteraceae (Wist & Davis, 2006), Bignoniaceae (Galetto, 1995a), Boraginaceae (Weryszko-Chmielewska, 2003), Brassicaceae (Davis et al., 1998), Campanulaceae (Galetto et al., 1993), Caryophyllales (Zandonella, 1977), Convolvulaceae (Galetto & Bernardello, 2004), Crassulaceae (Said, 1982), Cucurbitaceae (Nepi et al., 1996), Ericaceae (Palser et al., 1991), Euphorbiaceae (Freitas et al., 2001), Fabaceae (Davis & Gunning, 1991), Gesneriaceae (Maldonado & Otegui, 1997), Hydroleaceae (Di Fulvio, 1997), Lamiaceae (Dafni et al., 1988), Loranthaceae (Galetto et al., 1990), Melianthaceae (Decraene & Smets, 1999a; Decraene et al., 2001), Myrtaceae (Davis, 1997), Plantaginaceae (Nepi et al., 2003), Rosaceae (Radice & Galati, 2003), Rubiaceae (Galetto, 1998), Solanaceae (Rodriguez, 2000), and Tropaeolaceae (Fabbri & Valla, 1998), among many other families.

According to Endress (1995), modified stomata are apparently absent from monocotyledons. In these plants, nectar is secreted by trichomes or by diffusion through the epidermis. Alternatively, nectar is mainly collected in inner cavities of septal nectaries and is released to the outside through special slits. Recently, Davies et al. (2005) reported the existence of stomata in the labellum of an orchid, a finding that may imply that stomata may be present in other orchids as well, but data are needed to support this assertion.

Both the number and location of stomata on nectaries may differ among closely related species. Although available data are scarce, this feature may have taxonomic importance and should be considered more carefully (e.g., Palser et al., 1991; Galetto, 1995a, 1997, 1998; Petanidou et al., 2000; Rodriguez, 2000; Weryszko-Chmielewska et al., 2003; Galetto & Bernardello, 2004). In addition, occasionally the distribution of stomata may be restricted to specific parts of the nectary, forming a single group or multiple fields of stomata (e.g., Davis & Gunning, 1992; Galetto, 1995b; Vogel, 1998c). The significance of these features in plant systematics is mostly unexplored, although the diversity of stoma fields was regarded by Vogel (1998c) as a promising criterion.

In relation to the epidermis, the position of stomata may be sunken, isobathic, or elevated. Data on several Lamiaceae species showed that this position was affected by growing conditions (Petanidou et al., 2000); therefore, the alignment of stomata on the nectary surface cannot be considered a reliable taxonomic character, at least according to this evidence.

Parenchyma. Turning to the modifications of the specialized parenchyma of the nectary, the main differences include being either uni- or multilayered, and presenting chloroplasts (Vassilyev & Koteyeva, 2004a), amyloplasts (Nepi et al., 1996), or both (Maldonado & Otegui, 1997). The presence and

abundance of starch in the nectary parenchyma may vary as well (see Pacini et al., 2003), but its systematic value is uncertain until more data are gathered.

Vascular system. The type of vascular bundle which supplies the nectariferous parenchyma may, more commonly, consist of phloem only, or of both phloem and xylem (e.g., Fahn, 1979, 2000; Mani & Saravanan, 1999; Galetto & Bernardello, 2004; Vassilyev & Koteyeva, 2004b; Leitao et al., 2005; Wist & Davis, 2006). On the other hand, no special bundles may irrigate the nectaries, these being supplied by the vasculature of the organs located near them (e.g., Fahn, 1979, 1988, 2000; Said, 1982; Galetto, 1995b; Ma et al., 2002). It should be mentioned that within a family the type of floral vasculature may differ among genera and species (e.g., Frei, 1955; Kartashova, 1965; Fahn, 1979). Its systematic importance is therefore relative, although trends might be defined. At the same time, a correlation between the type of vascularization and the level of nectar concentration and type of sugars present in the nectar does not always exist (e.g., Kartashova, 1965; Fahn, 1979; Dafni et al., 1988).

Unfortunately, the majority of the available articles that deal with nectaries frequently do not report information on these basic anatomical and cytological aspects. Furthermore, most studies have been done in either single species or in a reduced number of related taxa; it is therefore hard to fully appreciate the systematic value of many of these traits. Further data on these aspects for large numbers of taxa, as well as on patterns of infraspecific variability, are required.

3.1.3 Fate

The fate of the organs where the secretory tissue is located may be an indication of the nectary fate. This fact was taken into account by Smets (1986, 1988) who broadly divided nectaries into **persistent** (associated with non-falling floral parts) and **caducous** (associated with falling floral parts). This, however, does not mean that persistent nectaries will always continue to secrete nectar for long periods after anthesis, or during fruit development. Such long-standing secretion occurs exclusively in **post-floral** nectaries (or post-floral secretion, as preferred by Schmid, 1988).

The function of post-floral nectaries was interpreted as promotion of seed dispersal (Faegri & van der Pijl, 1979) or attraction of insects, mainly ants, for defence against herbivores, thus preventing fruit predation (e.g., Keeler,

1981; Falcao et al., 2003). Three types of post-floral nectaries can be differentiated, according to Schmid (1988):

- Non-ovarian, persistent nectaries that continue to secrete nectar during fruit development, i.e. they are indirectly related to the fruit.
- Ovarian nectaries that directly continue to secrete nectar as ovaries develop into fruits.
- Fruit nectaries that are newly developed after pollination in growing fruits.

Post-floral nectaries seem to be comparatively less frequent than the remaining nectary types, but may have been overlooked. As an illustration, in *Croton sarcopetalus* (Euphorbiaceae; Freitas et al., 2001), only one of the two nectary types present in female flowers functions as post-floral nectary.

More attention should be paid to taxa with persistent nectaries in fieldwork (Smets, 1986), because more species may continue to secrete nectar during stages of fruit development, and trends may be observed in some families. The same consideration applies to families with extrafloral nectaries located on persistent sepals, because they may continue to secrete nectar throughout fruit development (e.g., Convolvulaceae; Keeler, 1980), as well as extrafloral nectaries on the pedicels of buds and fruits (e.g., Bixaceae; Bentley, 1977a). In some publications, the fruits are described as bearing secreting structures, but as these are not specified as post-floral nectaries and are not included in keywords, they are difficult to find.

According to the available literature, post-floral nectaries have been reported in a small number of species from the following families:

- Acanthaceae (Gracie, 1991)
- Anacardiaceae (Wunnachit et al., 1992)
- Annonaceae (Koptur, 1992)
- Apiaceae (Szujko-Lacza, 1975)
- Apocynaceae (Koptur, 1992)
- Asparagaceae (Keeler, 1979)
- Bignoniaceae (Elias & Gelband, 1975; Elias & Prance, 1978; Thomas & Dave, 1992)
- Brassicaceae (Delpino, 1898, after Schmid, 1988)
- Buxaceae (Daumann, 1974)
- Cactaceae (Davidson, 1988)
- Convolvulaceae (Keeler, 1980)
- Euphorbiaceae (Freitas et al., 2001)
- Gesneriaceae (Davidson, 1988)

- Loasaceae (Keeler, 1981)
- Meliaceae (Morellato & Oliveira, 1994)
- Orchidaceae (Jaffe et al., 1989; Rico-Gray et al., 1989)
- Oleaceae (Daumann, 1932)
- Onagraceae (Keeler, 1979)
- Rubiaceae (Bentley, 1977b; Faegri & van der Pijl, 1979)
- Solanaceae (Davidson, 1988; Falcao et al., 2003)
- Vitaceae (Sernander, 1906, after Schmid, 1988)

3.1.4 Symmetry

Structural nectaries are predominantly symmetrical structures. Effectively, they show typically continuous arrangements, largely annular, which are radially symmetrical (e.g., ovarian nectaries in many Lamiales and Solanales, petal nectaries in Malvales; Fig. 3A). They may also be composed of a number of independent glands that are evenly distributed in the flowers and show radial symmetry as well (e.g., receptacular nectaries in many Malpighiales and Geraniales).

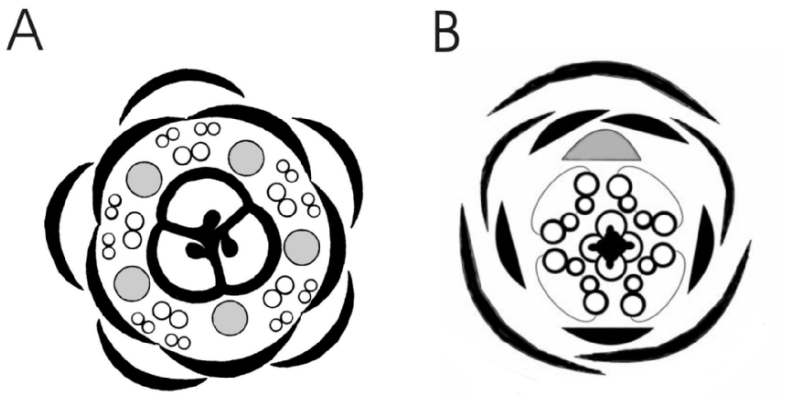

Figure 3. Nectary symmetry in floral diagrams. A, radial symmetry in *Nitraria retusa* (Nitrariaceae), modified from Decraene et al. (1996). B, bilateral symmetry in *Melianthus major* (Melianthaceae), modified from Decraene et al. (2001). Symbols: *black* = sepal (*outer*) and petal (*inner*), *gray* = nectary, *white circle* = stamen, *central structure* = gynoecium (showing number of carpels and ovules).

On the other hand, nectaries may have a different organization: either unilaterally located in the flower, having an irregular shape (usually with a part larger than the rest), and being a component of a spur (e.g., in Balsaminaceae, Lentibulariaceae, and Orchidaceae). Therefore, they have only one plane of symmetry and are thus bilaterally symmetrical, although in the literature they have been referred to, inappropriately, as asymmetrical (e.g., Zer & Fahn, 1992; Galetto, 1995b; Petanidou et al., 2000). Concerning the unilaterally organized nectaries (Fig. 3B), the receptacular nectaries in some species of Lythraceae, Melianthaceae, and Sapindaceae, and the ovarian nectaries in a number of Goodeniaceae, Lamiaceae, Gesneriaceae, Scrophulariaceae, Pedaliaceae, Phrymaceae, and Plantaginaceae (mainly the former Globulariaceae) can be mentioned (e.g., Cronquist, 1981; Dafni et al., 1988; Leins & Erbar, 1989; Zer & Fahn, 1992; Galetto, 1995b; Petanidou et al., 2000; Decraene et al., 2001; Nepi et al., 2003; Ilhenfeld, 2004b). In some cases, e.g., in Lamiaceae and Scrophulariaceae, radially and bilaterally symmetrical nectaries have been found in different genera of the family, but more data at the specific and generic levels are needed to assess their systematic significance.

3.1.5 Number

Within a flower, nectaries can be differentiated as a single structure of nectariferous tissue (e.g., annular rings on the receptacle or at the base of the ovary), or as a number of individual glands (e.g., cushions on each petal base or several glands on the receptacle), whose shape and size may be equivalent or dissimilar. All these traits may have taxonomic value and have to be considered when studying a particular plant group. For instance, in Brassicaceae (Norris, 1941; Deng & Hu, 1995; Davis et al., 1996, 1998, 1999), nectaries can be annular, or composed of a number of individual glands (two, four, or eight), depending on the genus. On the other hand, SanMartin-Gajardo and Sazima (2005) reported that in Gesneriaceae the number of nectaries per flower not only shows interspecific variation, but also intraspecific variation (e.g., mostly five glands or two fused plus three in *Vanhouttea*, or one, two, or four glands in *Sinningia*).

In 1998, Davis et al. proposed the resurrection of the term **nectarium** (coined by Linnaeus, 1735) to represent collectively the multiple nectaries that can be found in individual flowers. This proposal for the Brassicaceae, a family with a ring of nectariferous tissue, or two, four, or eight individual nectaries that may be interconnected (Davis et al., 1996, 1998), may well be applicable to any flowering plant. Thus, nectarium would represent all separated nectaries of a flower abstractly (whether the mentioned connections

exist or not), whereas nectary or nectary gland represents a tangible entity having distinct boundaries (Davis et al., 1998, p. 317).

3.1.6 Colour

Nectaries normally have the colour of the organ where they are located, a state of affairs that makes it difficult to find them. For instance, in species of Solanaceae with nectaries on the ovary base, the nectaries are indistinguishable from the gynoecium tissue when the nectariferous tissue is green like the ovary. However, in several taxa the nectariferous tissue can become coloured and can therefore be recognized macroscopically, e.g., in the tribe Lycieae (Bernardello, 1986, 1987) in *Nicotiana* (Cocucci & Galetto, 1992). This feature has been used to recognize taxonomic groups.

Convolvulaceae species (*Ipomoea*) have either yellow or cream-coloured nectaries surrounding the ovary base (Collins, 1992). When a mutant with a green nectary was discovered, it was demonstrated that this colour is inherited as a monogenic dominant trait over the yellow nectary. The colour in these cases is produced by the presence of carotenoids in the nectary tissue (Bernardello, 1986; Mann et al., 2000).

On the other hand, environmental variations were reported to be responsible for nectary colour change in *Hedera helix* (Araliaceae: Vezza et al., 2006). In this species, the nectary is situated above the inferior ovary and its colour varied from green to brown during the flowering period, a change resulting from the accumulation of anthocyanins in subepidermal layers of the parenchyma; the authors suggested that with this change the nectary may protect the inferior ovary from sunlight (Vezza et al., 2006).

3.2 Factors influencing nectary diversity

Floral nectaries are comparatively simple structures that are variable in their morphology, anatomy, and location (e.g., Brown, 1938; Daumann, 1970; Fahn, 1979, 1988; Cronquist, 1981; Smets, 1986, 1988; Schmid, 1988; Smets et al., 2000, 2003). Both phylogenetic and ecological constraints have been reported to influence nectary traits. These constraints will principally depend on the plant group, a circumstance that prevents wide generalizations. Let us examine some particular cases.

In Lamiaceae, nectary structure is shaped largely by both phylogenetic (nectar volume depends positively on the volume of nectariferous tissue; Fahn, 1949; Dafni et al., 1988; Petanidou et al., 2000) and climatic constraints

(some ecological parameters interfere on nectar secretion such as time within the season, life history, and light requirements of the plants; Petanidou et al., 2000).

Galetto and Bernardello (2004), in several *Ipomoea* species (Convolvulaceae), pointed out that flower length was correlated with nectary size and the total volume of nectar secreted, suggesting that structural constraints play a major role in the determination of nectar traits of these species.

Galetto (1995a) demonstrated in Bignoniaceae that the longer the flower, the more voluminous the nectary and the higher the stomata number, but these traits were not related to nectar secretion. On the other hand, Petanidou et al. (2000) showed in Lamiaceae that the number of stomata does not appear to influence nectar secretion, in agreement with findings by Teuber et al. (1980), and Davis and Gunning (1991) in Fabaceae species.

In *Campanula* species (Campanulaceae), reproductive attributes are far more conservative than flower phenology and pollination-related features (Blionis & Vokou, 2005); the patterns of change of reproductive attributes indicate, therefore, prevalence of phylogenetic over environmental constraints.

Other studies suggest that flower morphology is evolutionarily more labile and that corolla traits can frequently change (e.g., Cubas et al., 1999; Harrison et al., 1999) in comparison to changes in nectar features (e.g., Asteraceae; Torres & Galetto, 2002; Fabaceae; van Wyk, 1993; Gesneriaceae: Perret et al., 2001; Solanaceae; Galetto et al., 1998). In some taxa nectar composition seems to be a more conservative trait than flower morphology. The same argument was used to explain the absence of convergence in sugar composition between plants growing in two different South American biogeographical regions (Chaco and Patagonia) that share the same animal visitor guilds (Galetto & Bernardello, 2003).

It has to be kept in mind that flowers are the most complicated parts of plants, as they are composed of a number of organs that form an ordered pattern (Endress, 2001a). For instance, in families with sympetalous flowers, the evolutionary flexibility of floral length and shape provides an excellent means for isolating mechanisms in pollination and they have a similarly wide spectrum of pollinators (e.g., Acanthaceae, Bignoniaceae, Gentianaceae, Gesneriaceae, Polemoniaceae, Solanaceae; Grant & Grant, 1965; Vogel, 1991; Endress, 1994). Even though the key function of nectar is to attract pollinators, new evidence suggests that selection on flower shape and

size is a complex process, involving not only the well-accepted interactions with pollinators, but also interactions with herbivores, nectar robbers, seed predators, and seed dispersers that may influence different reproductive traits in plants (e.g., Armbruster, 1997; Galen, 1999; Aizen, 2003; Irwin et al., 2004).

3.3 Basic types of floral nectaries

There have been numerous attempts to classify nectaries from different perspectives. No single classification system can take into account the remarkable diversity of locations, shapes, histology, and functions of nectaries. Different classification systems may therefore have to be applied depending on the purpose of our research; the classification used must be specified so that readers understand our point of view. The simpler the classification system, the better—most likely the tremendous variety of nectaries could be fitted into an uncomplicated system with less difficulty.

The essential topographical nectary distinction was first proposed by Caspary (1848): **floral** (on the flowers) and **extrafloral** (on vegetative organs). But in recent times, as first recommended by Elias and Gelband (1975), these terms have been extensively used as Delpino's (1868–1875) functional classification into **nuptial** (related to the pollination process) and **extranuptial** (not related to pollination) nectaries, respectively. This situation creates a terminological inconsistency because some "extrafloral" nectaries are located in the "flower", e.g., nectaries located abaxially on sepals and petals. To avoid this problem, the more recent classification proposed by Schmid (1988) favours the use of **reproductive** (on any reproductive structure from inflorescences, bracts, pedicels, to flowers and fruits) and **extrareproductive** (on strictly vegetative organs) nectaries. Despite this, the traditional use of the terms floral and extrafloral nectaries as suggested by Elias and Gelband (1975) is still standard in botanical papers published all over the world.

There are a few general nectary classifications that are helpful for floral (and extrafloral) nectaries:

- Zimmermann (1932) distinguished between (a) **structural** and (b) **non-structural** nectaries, based on the basic structure of the nectaries and the possibility of recognizing them macroscopically and/or microscopically, as previously mentioned (sharply localized, histologically differentiated areas where nectar is regularly exuded, and non-differentiated areas that sporadically are able to secrete nectar, respectively).

- Vogel (1977) suggested (a) **mesenchymatous** nectaries (consisting of glandular and storage tissues that usually secrete nectar into interstitial spaces or the cell wall apoplast and then exude it via modified stomata), which certainly is the most widespread nectary type (Davis, 2003), (b) **epithelial** nectaries (consisting of a permeable, glandular epidermis often with an underlying glandular tissue), and (c) **trichomatous** nectaries (consisting of secreting glandular trichomes often aggregated into cushions). Vogel supported this categorization according to histological structure and the way of secreting the nectar to the plant surface.
- Fahn (1979) identified nectaries exuding nectar (a) from **morphologically unchanged epidermal cells or trichomes** (unicellular or multicellular), (b) from **stomata**, (c) from **septal nectaries**, or (d) from **lysigenous cavities**, based on the anatomy and ultrastructure of the nectaries and their mode of secretion.
- Smets (1986, 1988) and Smets and Cresens (1988) proposed (a) **nectaria caduca** (caducous nectaries related to falling floral parts: caducous sepals, petals, and androecium), and (b) **nectaria persistentia** (persistent nectaries related to non-falling floral parts: persistent sepals, receptacle, and gynoecium, including **gynopleural** or septal nectaries), which are associated with non-homologous floral morphomes (Smets et al., 2000). The fate of the organs where the secretory tissue is located was taken into account for this classification.

Turning to more detailed topographic nectary classifications, several have been published (e.g., Bonnier, 1878; Ewert, 1932; Brown, 1938; Fahn, 1953, 1979, 1982; Daumann, 1970), and undoubtedly many more can be formulated.

In my opinion, Schmid's (1988) scheme is simpler than other classifications and, as it is based on strictly positional criteria, extremely practical, covering most requirements to categorize nectaries. Schmid (1988) proposed that only when experimental or observational evidence was available, could a functional classification like Delpino's (1868–1875) be used; otherwise, it seems preferable to adopt a topographical classification. On the other hand, the strict application of rigid definitions is not always easy, and the broader the categories are, the better. Occasionally, nectaries may, for example, be located on the hypanthium and stretch towards the filament bases, or continue from the filament bases onto the receptacle and even the ovary base. In such cases, it will be difficult to place them in any single category. Here, an author should describe the nectaries fully instead of resorting to a category that does not define the situation clearly.

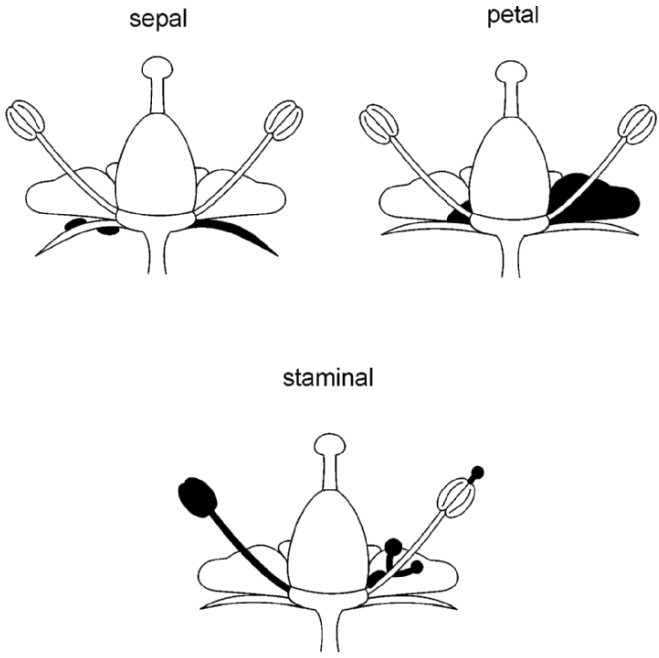

Figure 4. Sepal, petal, and staminal nectaries in a hypothetical flower. Sepal nectaries: *at left* = adaxial and abaxial individual glands on a sepal, respectively; *at right* = a whole sepal modified as nectary. Petal nectaries: *at left* = in the basal part of a petal; *at right* = a whole petal modified as nectary. Staminal nectaries: at left = a whole stamen modified as nectary; *at right* = the basal ventral part of the filament nectariferous, two nectaries at the base of the filament, and an antheral nectariferous appendix, from the base to the top of the stamen, respectively.

Schmid (1988) distinguished the following locations (with a few modifycations proposed here) for what he identifies as reproductive nectaries, i.e., nectaries located on the flowers, inflorescences, and accessory parts. In each case, Schmid (1988) proposed several additional appropriate terminologies; the terms I recommended for their simplicity are highlighted in bold below.

- Inflorescences (**inflorescence** nectaries)
- Peduncles or pedicels (**peduncular** or **pedicellar** nectaries)
- Bracts, bracteoles, or involucra (**bracteal**, **bracteolar**, or **involucral** nectaries)
- Flowers (**floral** nectaries)
- Ovules in gymnosperms (**ovular** nectaries)
- Fruits (**post-floral** or **fruit** nectaries)

Among the floral nectaries in the strict sense, Schmid (1988) recognizes nectaries on the following flower parts:

- Receptacles (**receptacular** nectaries; Fig. 6) with three types, which are discussed in more detail on page 42:
  - **Extrastaminal** nectaries
  - **Intrastaminal** nectaries
  - **Interstaminal** nectaries
- Hypanthia (**hypanthial** nectaries; Fig. 6)
- Tepals (**perigonal** or **tepal** nectaries)
- Sepals (**sepal** or calyx nectaries; Fig. 4)
- Petals (**petal** or **corolla** nectaries; Fig. 4)
- Stamens (**staminal** or **androecial** nectaries; Fig. 4), with three main possibilities:
  - On filaments (**filament** nectaries)
  - On anthers (**anther** nectaries)
  - On staminodes (**staminodal** nectaries)
- Pistils (**gynoecial** nectaries; Fig. 5) with four possibilities:
  - On stigmata (**stigmatic** nectaries)
  - On styles (**stylar** nectaries)
  - On pistillodes (**pistillodal** or **carpellodial** nectaries)
  - On ovaries with two variants:
    - On the outer regions (**ovarian** nectaries, designated as **non-septal** nectaries by Schmid (1988), but as it is a negative way to define them, it may be confusing). According to the type of ovary, these nectaries can be on the ovary wall if it is superior or on its top if it is inferior.
    - In the septal regions (**septal** nectaries) between adjacent carpels that result from incomplete intercarpellary post-genital fusion (Rao, 1975; Schmid, 1985; vanHeel, 1988; Simpson, 1993; Rudall, 2002). Smets and Cresens (1988) and Smets et al. (2000) recommended the use of **gynopleural** instead of septal, because it is more specific and covers inner, outer, and confluent septal nectaries.

Regarding the receptacular nectaries, some comments need to be made. First, the use of the word "disc" or "disk" is discouraged. Although it is (and was) widely utilized in the literature, it has been employed for so many different structures, including nectariferous and non-nectariferous ring-like structures located in diverse floral parts (e.g., ovary, androecium, receptacle), that it is hard to define what a disc is (e.g., Daumann, 1931; Fahn, 1977, 1979; Cronquist, 1981; Smets, 1986; Schmid, 1988; Smets & Cresens, 1988;

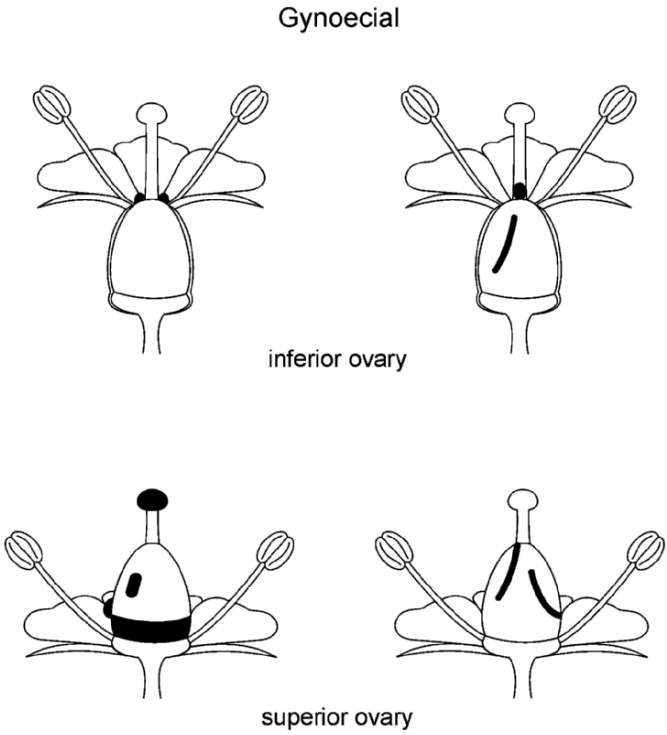

Figure 5. Gynoecial nectaries in a hypothetical flower. Nectaries on inferior ovaries: *at left* = on the top of the ovary; *at right* = septal nectary and stylar nectary, from the base to the top of the ovary, respectively (stylar nectaries can also be found in superior ovaries). Nectaries on superior ovaries: *at left* = a nectariferous ring on the ovary base, glands on the ovary wall, and stigmatic nectary, from the base to the ovary top, respectively; *at right* = septal nectaries.

Vogel, 1998c). In addition and as a consequence, homologies of discs in cladistic analyses may be erroneous (Smets et al., 2000). Moreover, as there is a broad range of potential ways to categorize receptacular nectaries, the use of many subtypes is also discouraged, because it is not easy to devise a classification that considers every potential position.

The exception may be the broad positional terms **intrastaminal** and **extrastaminal** (Fig. 6), indicating nectaries borne on the receptacle between the staminal whorl and the ovary, or between the perianth and the androecium, respectively. Receptacular nectaries located exactly between the stamens can be referred to as **interstaminal**. These words have been widely used in this sense, being easy to understand and apply. Their systematic value, however, must not be overemphasized. On the one hand, both types can be found in the same plant group (e.g., Euphorbiaceae; Webster, 1994)

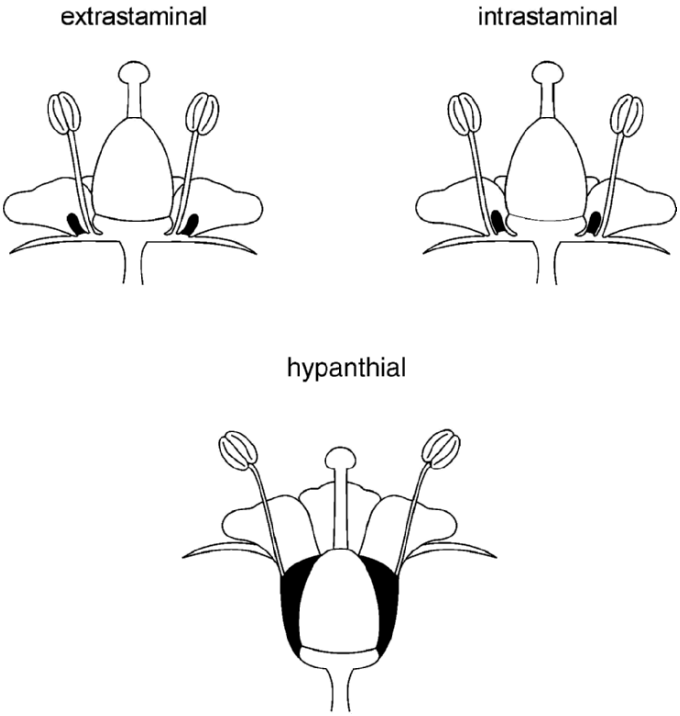

Figure 6. Receptacular (extrastaminal and intrastaminal) and hypanthial nectaries in a hypothetical flower.

and this position may be of systematic interest to delimit species groups. On the other hand, it has to be taken into account that occasionally, nectaries may extend all the way from the corolla to the gynoecium and in these cases (e.g., in some Celastrales; Matthews & Endress, 2005b), the distinction between intrastaminal and extrastaminal is of no use, as is its systematic relevance.

3.4 Nectariferous spurs

Some angiosperm families have nectar spurs (Cronquist, 1981; Judd et al., 2002), i.e., hollow, slender, saclike perianth outgrowths that secrete nectar. These floral structures are thought to have had a strong influence in shaping the evolution of plant and pollinator diversity (Hodges & Arnold, 1994), although sometimes speciation in spurred taxa may be related to other features (von Hagen & Kadereit, 2003). Several investigations have showed that nectar spur morphology often correlates to the mouthparts and foraging habits of pollinators (Nilsson, 1988; Temeles et al., 2002). In addition, there is evidence

that these structures have been important in the evolution of certain plant groups, such as in *Aquilegia* (Ranunculaceae), where the nectar spur represents a key innovation, i.e., a novel adaptation that allowed for diversification (Hodges & Arnold, 1994). In other taxa, however, nectar spur morphology exhibits considerable variation both within and among populations (Herrera, 1988; Travers et al., 2003).

Nectar spurs undoubtedly had independent origins, a hypothesis supported both by large phylogenetic distances between groups with nectariferous spurs, and by the different developmental origins of spurs (Hodges, 1997; APG II, 2003). They should consequently be regarded as convergences. As they generally develop from sepals or petals, it seems logical to regard them as sepal or petal nectaries, after the floral whorl from which they were derived.

There are, however, some genera—e.g., *Delphinium*, *Aconitum* (Ranunculaceae), and *Lobelia* (Campanulaceae)—in which nectar spurs are more complex structures involving both perianth whorls and associated portions of the androecium and gynoecium. In these cases, it seems practical just to name them as nectariferous spurs, and mentioning the floral whorl (or whorls) from which they originated.

A few plant families are characterized by the universal presence of nectariferous spurs (Cronquist, 1981): mostly the Asian and African Balsaminaceae (Travers et al., 2003) and the Central and South American Tropaeolaceae (Fahn, 1979; Fabbri & Valla, 1998)—both families with sepal spurs—and the cosmopolitan insectivorous Lentibulariaceae (Narayana & Satyavathi, 1988; Vogel, 1997), with petal spurs. Some authors regard the spur in Tropaeolaceae as receptacular (either axial or hypanthial; cf. Decraene & Smets, 2001), but the general consensus is that it is calycinal.

In the order Ranunculales, there are several families that have some representatives with spurs:

- Some Papaveraceae—specifically the members of the former Fumariaceae—possess petal spurs (Olesen, 1996).
- In Ranunculaceae, *Aquilegia* has petal spurs, whereas *Delphinium* and *Aconitum* have petal-and-sepal complex spurs (Hodges, 1997; Erbar et al., 1999).
- In Campanulaceae, this kind of complex spur also occurs in some Mexican *Lobelia* species (Koopman & Ayers, 2005).

- Sepal spurs are reported in most genera of Vochysiaceae, except *Amphilochia* and *Euphronia* (Oliveira, 1996; Hodges, 1997), and in *Pelargonium* (Geraniaceae; Fahn, 1979; Vogel, 1998c).
- Petal spurs have been reported in some Valerianaceae *s. str.* (now subsumed under Caprifoliaceae *s.l.*) (*Centranthus*; Fahn, 1979; Wagenitz & Laing, 1984; Rehnberg, 1987), Scrophulariaceae (Vogel, 1998b; Nepi et al., 2003), Violaceae (Vogel, 1998b; Freitas & Sazima, 2003), the genus *Halenia* (Gentianaceae; von Hagen & Kadereit, 2003), and in some *Lonicera* species (Caprifoliaceae; Cronquist, 1981).
- In Cactaceae, hypanthial spurs were reported for a species of *Hildewintera* (Kiesling & Metzing, 2004).

Within the monocots, the tepals of some Orchidaceae, e.g., *Angraecum*, *Gymnadenia*, *Habenaria*, *Jumellea*, *Limodorum*, *Neobathiea*, and *Platanthera* (Nilsson et al., 1987; Dressler, 1990; Figueiredo & Pais, 1992; Galetto et al., 1997; Stpiczyńska & Matusiewicz, 2001; Stpiczyńska et al., 2005) have nectariferous spurs. Spurs can be small and inconspicuous, but can also become the most noticeable part of the flower—the Malagasy hawkmoth-pollinated star orchids are renowned for their 30–40 cm long spurs (Nilsson et al., 1987).

Finally, it is worth mentioning that nectar production in spurs has to be checked carefully, because this simple fact is rarely accurately observed and described. In fact, it has to be confirmed whether nectar is directly produced and accumulated in the spurs. To this end, anatomical studies are required to ascertain whether the spurs contain secreting tissue. Spurs are slender, hollow structures and when nectar is secreted directly in them, the nectariferous tissue is usually composed of unicellular trichomes or small papillae of epidermal nature (e.g., Caprifoliaceae; Fahn, 1979; Orchidaceae; Galetto et al., 1997; Stpiczyńska et al., 2005; Tropaeolaceae; Rachmilevits & Fahn, 1975). Alternatively, nectar may be exuded from nectaries located in other floral parts and, secondarily, be conducted to and accumulated in the spurs, as recorded for some Scrophulariaceae and Violaceae (Vogel, 1998b).

3.5 Patterns of variability in nectaries

Without doubt, the wealth of floral nectaries among the angiosperms is as fascinating as their flowers. Nonetheless, as nectaries are diminutive structures, they are easily overlooked or misinterpreted; as a result, their descriptions are often inaccurate. As Davis et al. (1996) pointed out, it is common for nectaries to be treated superficially or neglected entirely in studies of floral development and morphology. Both microscopical sections and experimental observations

on live plants are necessary to detect nectaries with accuracy. The remarkable diversity within most groups, the inadequate number of taxa studied, and the rare analyses of intraspecific variation available, all contribute to our insufficient knowledge of the distribution and structure of nectaries within higher-level taxonomic groups.

In some plant groups, there is a certain level of homogeneity in terms of the presence (e.g., Brassicaceae, Bromeliaceae, Convolvulaceae, Heliconiaceae, Lamiaceae) or absence of nectaries (e.g., lineages with abiotic pollination as in Betulaceae, Casuarinaceae, Ceratophyllaceae, Cyperaceae, Potamogetonaceae, or with other floral rewards as in Actinidiaceae, Calceolariaceae, Commelinaceae, the former Krameriaceae (now in Zygophyllaceae), some Papaveraceae). In the many nectar-bearing families, nectary structure and position may be reasonably comparable among the members (e.g., Asteraceae, Bignoniaceae, Brassicaceae, Crassulaceae, Rubiaceae), or may vary in morphology and location (e.g., Cucurbitaceae, Euphorbiaceae, Orchidaceae, Ranunculaceae, Thymelaeaceae). On the other hand, nectaries can be present or absent in members of the same plant assemblage (e.g., Amaranthaceae, Cornaceae, Phytolaccaceae, Plantaginaceae, Salicaceae, Solanaceae). As examples of these dissimilar scenarios, some patterns within a few plant groups follow to show the extraordinary variation that floral nectaries can achieve.

3.5.1 Asteraceae

This highly successful, wide-ranging family, with more than 22,000 species, has nectariferous inflorescences (capitula) that also offer pollen as reward. In spite of its diversity, the family is comparatively homogeneous in the presence of a floral nectary. These nectaries are small, annular, and located on top of the inferior ovary surrounding the style base (Brown, 1938; Frei, 1955; Cronquist, 1981; Mani & Saravanan, 1999). It seems more accurate to consider these nectaries as ovarian rather than stylar (as Fahn, 1979), since histologically they are definitely related to the ovary. According to the external morphology, nectary shape and size are extremely variable (Gopinathan & Varatharajan, 1982; Mani & Saravanan, 1999) and may have systematic value. There is also variability within a single capitulum, where some florets may lack or have vestigial nectaries. For instance, disc hermaphroditic florets are usually nectariferous, whereas ray female or neuter florets either lack or have inconspicuous nectaries (Mani & Saravanan, 1999). The situation becomes even more complex when one considers the different types of capitula, the kind of sexuality of the species, and the enormous diversity of the family (Mani & Saravanan, 1999). For instance, in most dioecious taxa only male

florets are usually nectariferous (Mani & Saravanan, 1999), but in some cases, only female florets secrete nectar (Vogel, 1998c).

Nectar is secreted through stomata, whose number can vary among species (Mani & Saravanan, 1999). The type of vascularization of the nectary is variable as well—supplied by both phloem and xylem, only phloem or, more commonly, lacking a special vascular tissue (cf. Wist & Davis, 2006). Several types of vascularization can exist in different species of the same genus (Frei, 1955; Gulyás & Pesti, 1966). In spite of the remarkable practical significance of the Asteraceae, nectary anatomy has rarely been analysed in detail (structure: Frey-Wyssling, 1955; Galetto, 1995c; Torres, 1998; Vogel, 1998c; Sancho & Otegui, 2000; Ma et al., 2002; Visintín & Bernardello, 2005; ultrastructure: Tacina, 1974; Sammataro et al., 1985; Wist & Davis, 2006). More data on the subject with a reasonable taxon sampling may bring additional revealing results.

3.5.2 Brassicaceae

Four types of receptacular nectaries are distinguished in this chiefly entomophilous family, taking into account the location and degree of isolation of the nectariferous tissue (e.g., Norris, 1941; Deng & Hu, 1995; Davis et al., 1986, 1996, 1998, 1999):

- Annular type (a continuous nectariferous zone)
- Four-nectary type (two pairs of glands: the lateral nectaries associated with the short stamens and the median nectaries external to the long stamens)
- Two-nectary type (only the two lateral nectaries)
- Eight-nectary type (two pairs of lateral and two pairs of median nectaries)

In this family, the use of the terms intrastaminal or extrastaminal is not suitable. Effectively, according to the tetradynamous androecium of the Brassicaceae, in a species with four nectaries the median nectaries are opposed to the two outer shorter stamens and located between them and the ovary, whereas the two lateral nectaries are alternipetalous, located between the sepals and the stamens. The lateral sepals are often saccate and serve as reservoirs for accumulating nectar in some species (Davis et al., 1998). Davis et al. (1996) demonstrated that in *Brassica rapa*, a species of the four-nectary type, a percentage of flowers had nectarial connections among the four glands; the nectary would therefore be better described as an annular type. In addition, these authors reported variability in several nectary features according to the ploidy level in two *Brassica* species (Davis et al., 1996). In another investigation of members of the family (Davis et al.,

1998), they recorded differences in the phloem supply of median and lateral nectaries and in the sugar composition of both nectary types. These results show that it is fundamental to determine the morphological variability of nectary structure within a single species. Without doubt, these analyses will have a great impact on the taxonomy of the studied groups, but unfortunately, most published nectary research is based on single individuals and only one or a few flowers.

3.5.3 Cucurbitaceae

In the cosmopolitan gourd family, which is mainly insect-pollinated, there are two different types of nectaries: hypanthial and epidermal.

Hypanthial nectaries are mesenchymatous, exude nectar through stomata, and are typical of most members of subfamily Cucurbitoideae (Brown, 1938; Vogel, 1990, 1997; Nepi et al., 1996; Ashworth & Galetto, 1999; Fahn & Shimony, 2001). The family has characteristic unisexual flowers, and the hypanthial nectaries of male and female flowers show differences in position and size. In male flowers, the nectary forms a concealed nectar chamber at the base of the filaments; in female flowers, it is a circular channel surrounding the style and is usually larger than in male flowers (Nepi et al., 1996; Ashworth & Galetto, 1999; Fahn & Shimony, 2001). The nectary size differences found in species of *Cucumis* and *Cucurbita* are correlated with the comparatively larger volume of nectar secreted by female flowers (Nepi et al., 1996; Ashworth & Galetto, 1999). On the other hand, in a species of *Ecballium* staminate flowers secrete more nectar than pistillate flowers (Dukas, 1987; Fahn & Shimony, 2001), which, accordingly, have an inconspicuous nectary. In species of *Momordica*, *Lagenaria*, and *Luffa* no nectaries were reported for pistillate flowers (Bahadur et al., 1986; Iyer et al., 1989). One can conclude that in species of this and other families with gender dimorphism, sexual differences in nectaries have to be carefully checked and interpreted, because they may be important in the understanding of plant reproductive biology.

The other type of nectary in Cucurbitaceae is epidermal, in the form of glandular trichomes, especially in genera with male flowers with synandria (Vogel, 1981b, 1990, 1997). Nectaries form conspicuous carpets, either on the receptacle or on the hypanthium, and characterize subfamily Zanonioidae and tribes Cyclanthereae and Scycieae of subfamily Cucurbitoideae (Vogel, 1997). The trichomes show a variety of distribution patterns (from loose, sparsely distributed to dense, well-circumscribed cushions, either continuous or separated with different shapes) and composition (from two-celled to

large multicellular heads, often curved "penitent hairs") that have been helpful in the delimitation of taxa (Jeffrey, 1978).

Some authors (Smets, 1986; Vogel, 1997) consider hypanthial nectaries to be ancestral in this family. Nevertheless, recent molecular phylogenies suggest that subfamily Zanonioideae is basal (Jobst et al., 1998), and as members of its sole tribe (Zanonieae) have sparsely distributed nectariferous trichomes with small heads on the petals (Vogel, 1997), this type of nectary can be regarded as primitive. Vogel (1997) regarded the nectaries of Zanonioideae to be anatomically homologous to hydathode hairs, and therefore primitive compared to the trichomes found in other taxa, which show enlargement of glandular heads and concentration in dense carpets.

3.5.4 Euphorbiaceae

The members of this cosmopolitan family show a great variety of flower and inflorescence structures and sexual systems; they are mainly insect-pollinated and have both floral and extrafloral nectaries that are usually morphologically different and have a distinct evolutionary origin (Webster, 1994). Floral nectaries are extrastaminal or intrastaminal receptacular nectaries, either continuous or five-segmented. Extrafloral nectaries occur mainly on the leaves.

Several taxa seem to be wind-pollinated; among them are the dioecious *Mercurialis annua*, in which there is some uncertainty about the existence of reduced nectaries in the female flower (Daumann, 1972), and the monoecious *Ricinus communis*, which has extrafloral nectaries on the leaf petiole producing nectar for ants that discourage predators (Nichol & Hall, 1988). In *Croton* species, both anemophily and entomophily have been reported (Bullock, 1994; Freitas et al., 2001) and nectaries show great variety. For instance, the monoecious *Croton sarcopetalus*, has three types of nectaries (Freitas et al., 2001):

- Extrafloral (located in the leaves as two typical glands near the petiole insertion)
- Floral (five receptacular nectaries in male flowers and ten glands in two whorls, inner and outer, in female flowers)
- Post-floral (the outer whorl of nectaries in female flowers continues to secrete nectar during fruit development).

In the mainly monoecious *Euphorbia* and related genera, male and female flowers are very much reduced and are grouped into bisexual pseudanthial

inflorescences called cyathia (Cronquist, 1981; Webster, 1994). These structures have bracts with tips that alternate with nectary glands (Hoppe, 1985); these are considered extrafloral, but are positioned on an extremely reduced inflorescence (functionally a flower) and certainly attract pollinators (Reddi & Reddi, 1985). Pollinators are mainly small generalist insects (Ehrenfeld, 1976), although in *Pedilanthus* the more or less radially symmetrical cyathium of *Euphorbia* has been highly modified into a bilateral, spurred pseudanthium that is pollinated mainly by hummingbirds (Dressler, 1957).

3.5.5 Ranunculaceae

Except for a few wind-pollinated species (in *Thalictrum*), most taxa of this cosmopolitan family are animal-pollinated (mostly by bees, flies, moths, and birds) and offer easily accessible nectar or pollen as reward (Vogel, 1993; Erbar et al., 1999). *Caltha* is exceptional in possessing nectar-secreting trichomes located on either flank of each carpel (Smets & Cresens, 1988, who consider these nectaries as gynopleural).

In the remaining nectariferous species, nectar is produced by special nectary organs (previously known as honey leaves or nectary leaves; cf. Schmid, 1988) that are located between the perianth and the stamens (Kosuge, 1994); as they are mainly considered to be petals (although some authors treat them as tepals) they are petal nectaries. In some genera (e.g., *Nigella*, *Helleborus*), the whorl of nectaries is arranged to form a revolver blossom (perambulatory apparatus), which intensifies the visitor's anther and stigma contact (Vogel, 1993).

The nectaries in this family are very variable in terms of number, shape (cup-shaped, flat, spurred, peltate, epeltate), and presence, and they have been systematically utilized in the delimitation of subfamilies, tribes, genera, and subgenera (e.g., Tamura, 1966, 1967, 1968; Dahlgren, 1992).

The nectary organs are supposed to have been derived from stamens (cf. Tamura, 1993). After developmental analyses, Erbar et al. (1999) found a presumed relationship of nectary organs and stamens, which does not compellingly imply it. These authors believe that nectaries may be phylogenetically "interpreted as organs which developed during the early evolution of nectar-offering flowers by an overlap of the genetic programs of the perianth members and the following stamens during floral ontogeny".

Hydrastis is the basal genus for the Ranunculaceae (Ro et al., 1997); however, scant information is available on the pollination of this genus, which lacks petals and has caducous sepals. Small bees are the primary pollinators (Sinclair et al., 2000), and it seems to have pollen as reward but no nectar.

3.5.6 Solanaceae

This cosmopolitan family, with its main centre of diversity in South America, offers different kinds of rewards to a broad array of pollinators. It shows a wide adaptive radiation that includes all forms of animal pollination: by birds, moths, butterflies, bats, bees, and flies (Cocucci, 1999; Hunziker, 2001). Similarly diverse are the floral rewards that its members can offer, which include nectar (e.g., Galetto & Bernardello, 1993, 2003), pollen (e.g., Symon, 1979; Lester et al., 1999), scents (e.g., Passarelli & Bruzzone, 2004), and oil (e.g., Simpson & Neff, 1981; Cocucci, 1991).

Solanum is the most diverse and the largest genus in the family, comprising about half of all Solanaceae species (~1,400 spp.). *Solanum* flowers offer pollen as reward and are typically buzz-pollinated (Symon, 1979; Buchmann, 1983; Hunziker, 2001). However, most other genera (e.g., *Capsicum*, *Lycium*, *Nicotiana, Physalis*, *Schizanthus*) and tribes (e.g., Cestreae, Datureae, Lycieae, and most Solaneae) are nectariferous, presenting an annular nectary at the base of the ovary (Bernardello, 1986, 1987; Vogel, 1991; Hunziker, 2001). This widespread nectariferous condition, together with the presence of nectar in the basal Schizanthoideae and Swenckioideae groups (Olmstead et al., 1999; Martins & Barkman, 2005; Perez et al., 2006), suggest that nectar as reward is ancestral in this family.

Taxonomically, the presence or absence of nectaries is useful at the generic and tribal levels. Within a genus, nectary morphology and colour can be variable among the species; nectary colour is a valuable trait used to define assemblages of species (Bernardello, 1987; Cocucci & Galetto, 1992).

3.6 Nectaries and deceit pollination

The presence of a reward in flowers of animal-pollinated plants (nectar in our case) has been thought to allow the evolution of plants with rewardless flowers that only look (or smell) as if they could offer a reward (Willson & Ågren, 1989). Such flowers are said to be pollinated by deceit. Although there are several studies on these kinds of plants (e.g., Williamson, 1982;

Little, 1983; Dafni, 1984; Willson & Ågren, 1989; Nilsson, 1992), many species remain to be recognized and described as such. Some examples follow.

3.6.1 Apocynaceae

In Apocynaceae *s.l.*, most species are nectariferous. They possess either an annular ovarian nectary, a receptacular nectary (either surrounding the ovary or as two glands alternating in position with the two carpels), or have carpellodes transformed into nectaries (Rao & Ganguli, 1963; Boiteau & Allorge, 1978; Galetto, 1997). In some taxa, however (*Plumeria*, *Himatanthus*, *Nerium*, *Aspidosperma*), nectar is not produced, because the nectary becomes inconspicuous and non-functional (Woodson & Moore, 1938; Haber, 1984; Herrera, 1991; Lin & Bernardello, 1999). Despite lacking nectar, other floral features together with massive flowering in these taxa attract naïve visitors, which are deceived into pollinating the flowers. Although data are insufficient, deception in this family seems to be derived and to have arisen several times in the Apocynoideae and Rauvolfioideae clades (Potgieter & Albert, 2001), suggesting that there are factors in common that predispose members of the family to this trend.

3.6.2 Bignoniaceae

The same situation is true for members of the mainly tropical Bignoniaceae—primarily centered in northern South America—most representatives of which have nectar as reward. The nectar is exuded from an annular ovarian nectary (Galetto, 1995a; Rivera, 2000a), a character that can be considered ancestral for the family. Nevertheless, in a few taxa from tribe Bignonieae (*Cydista*, *Clytostoma*, *Phryganocydia*, and some species of *Lundia*; Gentry, 1980, 1982; Rivera, 1996, 2000a), the nectary is non-functional and the species are thought to be pollinated by deceit. As this tribe (Spangler & Olmstead, 1999) and the "mimetic clade" within it are considered to be derived (Lohmann, 2006), this condition can be regarded as derived as well and it seems to have evolved once in the group. In *Lundia cordata*, a species that has lost its ovarian nectary, nectar is secondarily produced from pluricellular corolla trichomes (Lopes et al., 2002); as the presence of an ovarian nectary is considered an apomorphy for the genus, these authors suggested that reversal from deceit to nectariferous flowers has taken place in some taxa of *Lundia*.

3.6.3 Orchidaceae

In the enormous orchid family, most members provide a reward to pollinators, but about one-third of the estimated 18,500 species supply no reward and are pollinated by either gustatory or sexual deceit (van der Pijl & Dodson, 1966; Nilsson, 1992; Bustos Singer & Cocucci, 1999; Soliva & Widmer, 2003; Cozzolino & Widmer, 2005). Odour is employed as the primary long-range attractant in these flowers (van der Pijl & Dodson, 1966). The most common reward in orchids is nectar (van der Pijl & Dodson, 1966; Dressler, 1990), in a variety of nectary structures. Other rewards—such as floral fragrances, oils, and pollen—are also offered (van der Pijl & Dodson, 1966; Dressler, 1990; Neiland & Wilcock, 1998; Cozzolino & Widmer, 2005). The basal Apostasioideae orchids offer pollen (Kocyan & Endress, 2001). In contrast, the other basal groups Vanilloideae and Cypripedioideae have no reward and are pollinated by deceit; this evidence indicates that the absence of nectar might represent the ancestral condition in orchids (Neiland & Wilcock, 1998; Cozzolino & Widmer, 2005). The adoption of nectar production may prove to have been the most effective and frequent means of escaping low pollination success in the Orchidaceae (Neiland & Wilcock, 1998).

3.7 Relictual nectaries in anemophilous species

The lack of a nectar reward is a common feature in chiefly anemophilous plants (Silberbauer-Gottsberger & Gottsberger, 1988). An indication that wind pollination may have been derived from animal pollination in some plant groups may be the relict occurrence of nectaries in flowers of some anemophilous taxa (Faegri & van der Pijl, 1979; Proctor et al., 1996). In the dioecious dwarf palm *Chamaerops humilis*, which is wind-pollinated, Herrera (1989) found that female flowers sometimes secrete nectar. Herrera concluded that nectar which does not attract visitors may represent a trait retained from ancestors with a different floral biology. The current function of floral nectar, not only its presence or absence, should therefore be taken into account when assessing the mode of pollination (Bullock, 1994), as shown in the following examples.

In the Juan Fernandez Archipelago (Chile), there are very few close associations between flowers and insect pollinators, largely because there are few insect pollinators, either native or introduced, on these islands (Bernardello et al., 2000, 2002; Anderson et al. 2001). Consequently, the presence of nectaries and nectar in some species (except for those visited by the two hummingbird species that inhabit the islands) was suggested to be an indication of

the ancestral pollination system of the first colonizers because the nectar does not reward current pollinators (Bernardello et al., 2000, 2002). For instance, the ancestors of *Pernettya rigida* (Ericaceae) seem to have been insect-pollinated, but today this cryptically dioecious species is wind-pollinated and continues to secrete nectar (Anderson et al., 2000a). Similarly, *Wahlenbergia* (Campanulaceae) colonizers are supposed to have been entomophilous, although extant taxa are mostly selfers, with a wind-aided pollination mechanism in *Wahlenbergia berteroi* (Anderson et al., 2000b). A similar situation was reported for *Iris versicolor* in Kent Island, New Brunswick (Zink & Wheelwright, 1997). In another island system, the Hawaiian Archipelago, shifts in *Schiedea* species (Caryophyllaceae) from biotic—pollination or autogamy to wind pollination and sexual dimorphism have been reported (Weller et al., 1998). There, some sexually dimorphic species, which occur in dry habitats, are wind-pollinated, yet show some nectar production from comparatively reduced nectaries (Weller et al., 1998).

Despite these conclusions, Bernardello et al. (2000, 2002) have proposed that the presence of nectar rewards does not necessarily indicate biotic pollination on oceanic islands: studies of reproductive biology need to be carefully done, species by species, before useful generalizations can be made.

3.8 Distribution of nectary types

Although the location of nectaries in flowers is more or less constant within lower order groups such as genera and families, nectary type and location are highly variable in the higher taxonomic groupings of orders and superorders (e.g., Brown, 1938; Fahn, 1979; Cronquist, 1981; Smets, 1986, 1988; Smets & Cressens, 1988; Smets et al., 2000) and our knowledge of their distribution and structure in these higher groups is incomplete (Vogel, 1997).

A survey of the distribution of strictly floral nectaries in an evolutionary context, based on the available literature, is presented here, starting on page 56. For this purpose, the updated classification of the families of flowering plants by the Angiosperm Phylogeny Group (APG II, 2003) is followed, particularly the broader monophyletic family circumscriptions favoured therein (e.g., Alliaceae includes Agapanthaceae and Amaryllidaceae, Buxaceae includes Didymelaceae, etc.). The unplaced families are mentioned first, followed by the orders accepted for each clade. The orders are treated as they are in APG II, and the families are listed alphabetically within each order; to aid identification, these taxa are written in bold face the first time they are mentioned. It has to be remembered that this survey is based on available information, which is generally supported by limited publications

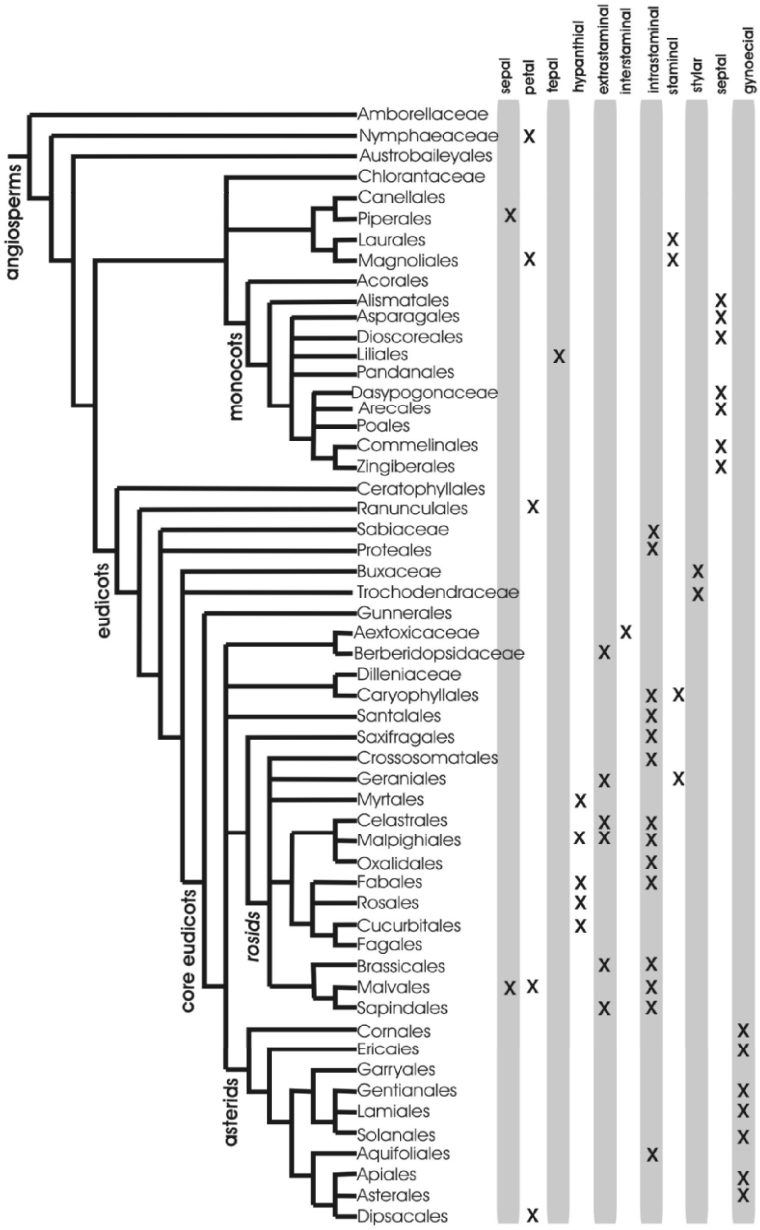

Figure 7. This cladogram shows the interrelationships of the orders and some families based on large-scale analyses of different gene sequences in the angiosperms. The most frequent nectary positions are indicated on the right-hand side. (After APG II, 2003.)

on few individuals and few taxa per family. Even with some flaws or omissions, it will help researchers and students to obtain quick information on the amazing nectary diversity within the flowering plants.

Families in which no nectaries have been observed are indicated as such here, to distinguish them from families for which no data were found. To shorten the list of literature references for each family, recent papers or papers that include extensive literature on the subject were chosen. Most of this information is also presented in the Appendix on page 122, but arranged according to the different nectary types as defined above (see "Basic types of floral nectaries", on page 38); the families in which those nectary types were reported are listed alphabetically and at least one publication is cited.

3.8.1 Early-branching lineages

Amborellaceae. This monotypic dioecious family is the sister group of the rest of the flowering plants (APG II, 2003). Its only species, *Amborella trichopoda*, is endemic to New Caledonia and is pollinated by both insects and wind. Pollen is the only reward for visitors in the absence of detectable floral volatiles and nectar (Thien et al., 2003). Sporadically, free stigmatic secretion was observed in female flowers, but no insects were observed to consume it (Thien et al., 2003). These authors suggested that the presence of a dry stigma would be the plesiomorphic condition for the basal angiosperms, and that a protonectar based on stigmatic secretions evolved independently and along early diverging lineages (e.g., Annonaceae; Endress, 1990; Austrobaileyaceae; Endress, 1990; Chloranthaceae; Tosaki et al., 2001; Magnoliaceae; Allain et al., 1999; Monimiaceae; Endress & Lorence, 1983; Winteraceae; Gottsberger et al., 1980; Thien, 1980; Lloyd & Wells, 1992). This protonectar produced by wet stigmas was believed to be a relictual reward that evolved before the first nectar glands (Endress & Igersheim, 2000), although recent evidence calls into question whether the wet-type stigma was the plesiomorphic condition in angiosperms (cf. Bernhardt et al., 2003).

Nymphaeaceae. The most primitive nectaries in the form of simple petal nectaries are found in this family (Brown, 1938; Schneider & Jetel, 1982; Vogel, 1998a; Schneider et al., 2003). As a whole, extant basal angiosperms have bisexual, protogynous, fragrant, generalist flowers with no nectaries; in addition, floral thermogenesis is widely distributed (Thien et al., 2000; Endress, 2001b). Coleoptera and Diptera are the primary pollinators and wind also seems to be important (Thien et al., 2000; Bernhardt et al., 2003; but see Endress, 1990).

Austrobaileyales. Pollination is performed by wind and insects, with pollen as reward (Endress, 1980, 2001; Thien et al., 2000; Bernhardt et al., 2003), although stigmatic nectar has also been observed (Endress, 1990).

Chloranthaceae. Flowers show great reduction and a trend towards wind pollination; although some taxa are insect-pollinated, no nectaries have been reported so far (von Balthazar & Endress, 1999; Tosaki et al., 2001; Doyle et al., 2003).

3.8.2 Magnoliids

Canellales. Stigmatic nectar has only been reported in some **Winteraceae** (Gottsberger et al., 1980; Thien, 1980; Lloyd & Wells, 1992).

Piperales. No nectaries have been found in **Degeneriaceae** (Kubitzki, 1993c), **Hydnoraceae** (Meijer, 1993), **Lactoridaceae** (Bernardello et al., 1999), and **Saururaceae** (Thien et al., 1994). In **Aristolochiaceae** there is normally a saprophagous fly-pollination system that includes floral scents, sepal nectaries, and trap-and-release mechanisms (Daumann, 1959; Faegri & van der Pijl, 1979; Vogel, 1998a; Sakai, 2002), whereas in **Piperaceae,** Vogel (1998a) recently reported nectariferous bracts, a phenomenon that might be more widespread in the family.

Laurales. The families **Calycanthaceae** and **Siparunaceae** are devoid of nectaries (except for nectariferous tepals in one species of the latter; Vogel, 1998a), whereas **Atherospermataceae**, **Gomortegaceae**, **Hernandiaceae**, **Lauraceae**, and higher **Monimiaceae** taxa have large nectary glands on the filament bases (Fahn, 1979; Cronquist, 1981; Rohwer, 1993; Smets, 1986; Kubitzki, 1993a; Endress & Lorence, 2004). Renner (1999) considered that these filament glands were independently lost in higher Monimiaceae and in Siparunaceae. The loss would have been concomitant with pollinator changes from nectar-foraging flies and bees to beetles and gall midges.

Magnoliales. Most families are beetle-pollinated and have no nectaries (**Degeneriaceae**, **Eupomatiaceae**, **Himantandraceae**, and **Myristicaceae**). Deceit pollination occurs in some cases (Armstrong, 1997; Dieringer et al., 1999). Petal nectaries have been observed only in some **Annonaceae** (Kessler, 1988; Endress, 1990; Silberbauer-Gottsberger et al., 2003) and **Magnoliaceae** (Thien, 1974; Huang et al., 1999). Stigmatic nectaries have been observed in Annonaceae (Endress, 1990) and Magnoliaceae (Endress, 1990; Allain et al., 1999), and staminal nectaries in Annonaceae (Endress, 1990) and **Schisandraceae** (Thien et al., 1983; Endress, 1990). Nevertheless, precise

data on the nectariferous structures modes of secretion in these important plant groups are required.

3.8.3 Early-branching monocots

The unplaced **Petrosaviaceae** (now considered basal in a clade that includes most monocots, except Alismatales) possesses septal nectaries (Rudall, 2002; Remizowa et al., 2006).

Acorales. This monotypic order is a sister group of the monocotyledons. Nectaries are absent, although non-secretory septal slits are present (Rudall & Furness, 1997; Buzgo & Endress, 2000), which are reminiscent of septal nectaries. A droplet that lasts for 1–2 h is secreted at the tip of the stigma, but it is unclear whether it contains sugar and functions as a pollinator attractant or whether it simply catches pollen (Buzgo & Endress, 2000).

Alismatales. Septal nectaries (mostly infralocular; cf. Rudall, 2002) are frequent in families from this order, e.g., **Alismataceae**, **Aponogetonaceae**, **Butomaceae**, **Limnocharitaceae**, and **Toefieldiaceae** (Daumann, 1970; van Heel, 1988; Smets et al., 2000; Remizowa et al., 2006), but absent in **Araceae**, **Cymodoceaceae**, **Hydrocharitaceae**, **Juncaginaceae**, **Posidoniaceae**, **Potamogetonaceae**, **Ruppiaceae**, **Scheuchzeriaceae**, and **Zosteraceae**, most of which have abiotic pollination mainly by water (Cronquist, 1981, 1988; Vogel, 1998d). Other types of nectaries are found as well: in Alismataceae staminal and carpellodial (Smets et al., 2000), in Araceae stigmatic (Vogel, 1983) and staminodial (Vogel, 1998d), and in Hydrocharitaceae staminodial nectaries (Scribailo & Posluszny, 1985).

3.8.4 Monocots

Septal (or gynopleural after Smets & Cresens, 1988) nectaries are exclusive to many monocotyledons (Brown, 1938; Daumann, 1970; Rao, 1975; Schmid, 1985; van Heel, 1988; Smets et al., 2000; Rudall, 2002), resulting from incomplete fusion of a small region of the carpel margins, which are otherwise fused. In dicots, there are non-secretory septal slits in *Saruma* (Aristolochiaceae; Igersheim et al., 2001) and *Koelreuteria* (Sapindaceae; Decraene et al., 2000a) and non-secretory septal cavities in *Cneorum* (Rutaceae; Caris et al., 2006). Septal nectaries have a variety of structural possibilities, since the ovaries can be superior, semi-inferior, or inferior; the nectaries may be infralocular or interlocular/supralocular (terminology of Simpson, 1998), the slits can be located in different positions, and the internal structure can be simple or labyrinthine (Daumann, 1970; Schmid, 1985,

1988; van Heel, 1988; Vogel, 1998d; Smets et al., 2000; Rudall, 2002). Septal nectaries have been lost several times in monocot evolution, probably in association with the development of different pollination systems (in apostasioid orchids, some Tecophilaeaceae, some Xanthorrhoeaceae, some Asparagaceae) or with the development of perigonal nectaries (in Liliales, some Iridaceae, some Orchidaceae) (Daumann, 1970; Vogel, 1981a, 1998d; Dressler, 1990; Smets et al., 2000; Rudall, 2002). In addition, septal nectaries are always absent in taxa with a gynostemium—a compound structure formed by adnation of stamens and style (Rudall & Bateman, 2002).

Asparagales. Septal nectaries are widespread in this order (Daumann, 1970; Meerow, 1987; Vogel, 1998d; Smets et al., 2000; Rudall et al., 2003a; Goldblatt et al., 2004; Nepi et al., 2006; see Rudall, 2002, for a summary of their positions). Even though in most families septal nectaries are always present, in **Alliaceae**, **Asparagaceae**, **Asteliaceae**, **Blandfordiaceae**, **Boryaceae**, **Doryanthaceae**, **Iridaceae**, **Ixioliriaceae**, **Lanariaceae**, **Tecophilaeaceae**, **Xanthorrhoeaceae**, and **Xeronemataceae** they may be absent in some members (Daumann, 1970; Rudall, 1998, 2002; Vogel, 1998d; Smets et al., 2000; Rudall et al., 2003a). Rudall (2002) suggested that the loss of septal nectaries has occurred *de novo* several times in this order, the absence of nectar being related to alternative pollination modes, like buzz pollination in some Asparagaceae, Tecophilaeaceae, Xanthorrhoeaceae, and apostasiod orchids (Vogel, 1981a; Kocyan & Endress, 2001b; Rudall, 2002). In Iridaceae, when septal nectaries are absent, elaiophores, tepal, or staminal nectaries can be found (Rudall et al., 2003a); these authors suggested that perigonal nectaries may have evolved from septal nectaries by heterochrony, i.e., developmentally later formation of nectaries in a more distal position on organ primordia.

Septal nectaries are completely absent in the two monocot families Orchidaceae and Hypoxidaceae. In **Orchidaceae**, Smets et al. (2000) linked this lack to the presence of unilocular ovaries in most species. As already pointed out, orchids may have other floral rewards (pollen, perfume, oil) or deceit pollination; there are also nectariferous flowers that have tepal nectaries (Daumann, 1970; Nilsson et al., 1987; Dressler, 1990; Figueiredo & Pais, 1992; Galetto et al., 1997; Bustos Singer & Cocucci, 1999; Stpiczyńska et al., 2005) and nectaries in the rachis or the pedicels of inflorescences (Fisher & Zimmerman, 1988). On the other hand, **Hypoxidaceae** exclusively presents pollen flowers, mainly with buzz pollination (Vogel, 1998d; Kocyan & Endress, 2001a).

Dioscoreales. The existence of septal nectaries in **Burmanniaceae**, **Dioscoreaceae**, and **Nartheciaceae** appears to be of limited systematic importance, since they are mostly present, but are also lacking in the same group (Daumann, 1970; Smets et al., 2000; Caddick et al., 2002; Rudall, 2002; Remizowa et al., 2006). The frequent loss of septal nectaries in this order was suggested to be related to certain modes of pollinator attraction, such as floral deceit (Smets et al., 2000).

Liliales. The absence of septal nectaries, i.e., complete fusion of carpel margins, represents a highly consistent and usefully predictive synapomorphy for this order (Rudall et al., 2000; Rudall, 2002). At the same time, while tepal nectaries are relatively rare in monocots, they are frequent in Liliales (Brown, 1938) where they have been reported for **Alstroemeriaceae** (Daumann, 1970), **Campynemataceae** (Rudall & Eastman, 2002), **Colchicaceae** (Daumann, 1970; Nordenstam, 1998), **Corsiaceae** (Rudall & Eastman, 2002), **Liliaceae** (Daumann, 1970; Nordenstam, 1982; Kaniki & Persson, 1997; Rudall et al., 2000), **Luzuriagaceae** (Conran & Clifford, 1998a), **Melanthiaceae** (Tamura, 1998), **Philesiaceae** (Conran & Clifford, 1998b), **Rhipogonaceae** (Conran, 1998), and **Smilacaceae** (Conran, 1998). These tepal nectaries represent a synapomorphy linked with the presence of mainly three-traced tepals (Rudall et al., 2000; Smets et al., 2000). Tepal nectaries take various forms in this order: relatively undifferentiated secretory epidermises; small, depressed areas fringed with hairs; or bulbous spur-like sacs (Khaniki & Persson, 1997; Rudall et al., 2000).

Pandanales. Cyclanthaceae, **Pandanaceae**, and **Stemonaceae** lack nectaries (Caddick et al., 2002); Cyclanthaceae is mainly cantharophilous (Gottsberger, 1991), and Pandanaceae either wind-pollinated or insect-pollinated but devoid of nectar. Pollen or food bodies are the offered rewards (Cox, 1990). On the other hand, **Velloziaceae** is reported to have septal nectaries (Menezes, 1973) and **Triuridaceae** taxa often have the inner side of the tepals covered with papillae, trichomes, or a raised glandular tissue along the midrib (Maas-van der Kamer & Weustenfeld, 1998) that may be interpreted as tepal nectaries.

3.8.5 Commelinids

Dasypogonaceae. Members of this family have septal nectaries (Rudall, 2002), although they may be absent or reduced to gynopleural slits (Smets et al., 2000).

Arecales. The only family in this order, **Arecaceae**, presents three main insect-pollination systems, with beetles, flies, and bees as pollinators (Henderson, 1986). Septal nectaries are found in many palms, but their presence is variable even in closely related groups of species (Daumann, 1970; Uhl & Moore, 1971; Rudall et al., 2003b; Stauffer & Endress, 2003), a circumstance correlated with mellitophilous flowers (Henderson, 1986). According to Smets et al. (2000), nectaries have probably been lost in the cantharophilous palms.

Poales. The families in this order are mostly devoid of nectaries, since they are primarily wind- or beetle-pollinated (**Anarthriaceae**, **Centrolepidaceae**, **Cyperaceae**, **Ecdeiocoleaceae**, **Flagellariaceae**, **Hydatellaceae**, **Joinvilleaceae**, **Juncaceae**, **Mayacaceae**, **Rapateaceae**, **Restionaceae**, **Sparganiaceae**, **Thurniaceae**, **Typhaceae**, and **Xyridaceae**). In exceptional cases, some nectaries do occur, as in the floral bracts of some **Poaceae** (e.g., Nicora, 1941; Zuloaga & Sendulsky, 1988). Two families stand out in this regard: **Bromeliaceae**, in which septal nectaries are widely distributed (Daumann, 1970; Böhme, 1988; Bernardello et al., 1991; Sajo et al., 2004), and **Eriocaulaceae**, in which gynoecial glands (in pistillodes or style appendices) have been reported (Stützel & Gansser, 1995; Stützel, 1998; Smets et al., 2000; Ramos et al., 2005).

Commelinales. In this order, **Haemodoraceae** (Daumann, 1970; Simpson, 1990, 1993, 1998), **Hanguanaceae** (Rudall et al., 1999), and **Pontederiaceae** (Daumann, 1970; Strange et al., 2004) have septal nectaries. Only two families do not have nectaries: **Commelinaceae**, with entomophilous pollen flowers (Hrycan & Davis, 2005), and **Philydraceae**, with nectarless and scentless pollen flowers (Hamann, 1998).

Zingiberales. Most families possess septal nectaries: **Cannaceae** (Daumann, 1970), **Heliconiaceae** (Kirchoff, 1992), **Lowiaceae** (Larsen, 1998; Wen & Liao, 1999), **Marantaceae** (Daumann, 1970; Rao, 1975), **Musaceae** (Daumann, 1970; Fahn & Benouaiche, 1979; Kirchoff, 1992), and **Strelitziaceae** (Daumann, 1970; Kronestedt & Walles, 1986). In **Costaceae** (Newman & Kirchoff, 1992) and **Zingiberaceae** (Rao, 1963; Box & Rudall, 2006), septal nectaries are absent; instead, these plants exhibit two, or rarely three, conical epigynous glands, which are closely related in both families. These nectaries have been interpreted as androecial or gynoecial in origin (cf. Rao, 1963), as supragynopleural (Smets & Cresens, 1988; Newman & Kirchoff, 1992; Smets et al., 2000), or as bipartite septal nectaries (Vogel, 1998b).

3.8.6 Ceratophyllales

The monotypic Ceratophyllales is hyphydrophilous, i.e., pollination takes place entirely below the surface of the water (Les, 1993), and its species do not have nectaries.

3.8.7 Eudicots

In the Eudicots, nectaries frequently occur on petals and, to a lesser extent, on receptacles, androecium, and gynoecium parts, excluding ovaries.

Ranunculales. Nectaries may be absent or present even in the same family; when present, they are mostly located in the corolla. **Eupteleaceae** and **Menispermaceae** lack nectaries (Cronquist, 1981; Endress, 1986). In **Berberidaceae**, two nectaries are usually located at the base of the petals (Fahn, 1979; Cronquist, 1981; Suzuki, 1984; Smets, 1986; Bernardello et al., 2000), but staminodes transformed into nectaries have also been observed (Cronquist, 1981; Brett & Posluszny, 1982). Recent floral morphological studies on **Circaeasteraceae** do not mention the presence of nectaries in *Circaeaster* (Tian et al., 2005) or *Kingdonia* (Ren et al., 2004), although no anatomical analyses or experiments with live plants were performed. For *Kingdonia*, Cronquist (1981) points out that there is an outer series of 8–12 apically nectariferous staminodes. **Lardizabalaceae** may not possess nectaries (Kawagoe & Suzuki, 2003) or have nectariferous petals (Cronquist, 1981). The same situation holds for **Papaveraceae**, where nectaries are absent (Cronquist, 1981) or present in the former Fumariaceae as petal nectariferous spurs (Cronquist, 1981; Lidén, 1993; Olesen, 1996). **Ranunculaceae** has a variety of nectaries (see "Ranunculaceae" on page 50 for a detailed discussion) although some taxa have none, e.g., *Thalictrum*. *Caltha* has nectariferous trichomes on the carpels (Peterson et al., 1979; Smets & Cresens, 1988), but most members possess petal nectaries (Fahn, 1979; Cronquist, 1981; Smets, 1986; Kosuge, 1994). Some genera also have nectariferous spurs—*Aquilegia* with petal spurs, *Delphinium* and *Aconitum* with petal-and-sepal complex spurs (Hodges, 1997; Erbar et al., 1999).

Sabiaceae. Data are scarce for the nectaries of this family, other than that Douglas (personal observation in Douglas & Tucker, 1996) indicated that *Sabia* flowers have receptacular nectaries similar to those found in Proteaceae.

Proteales. No nectaries have been observed in **Nelumbonaceae**, in which floral thermogenesis and odours attract a diversity of insect pollinators

(Williamson & Schneider, 1993). In **Proteaceae**, receptacular nectaries are common (although they may be absent in some taxa) in an alternitepalous position between the androecium and the gynoecium (Fahn, 1979; Douglas & Tucker, 1996) and can be considered intrastaminal. Douglas and Tucker (1996), after developmental studies, judged these nectaries as secondary organs, not reduced homologues from "lost" petal or stamen series.

Buxaceae. All members of this family have unisexual flowers. In female flowers of some *Buxus* species, interstylar nectaries occur on the gynoecium (von Balthazar & Endress, 2002). Daumann (1974) interpreted these nectaries as remnants of stamens, whereas Smets (1988) regards them as convergent homologues to septal nectaries in monocots. *Sarcococca* and *Pachysandra* lack interstylar nectaries in female flowers, but male flowers have nectariferous pistillodes (Vogel, 1998c; von Balthazar & Endress, 2002). *Styloceras* has no nectariferous tissues (von Balthazar & Endress, 2002).

Trochodendraceae. Conspicuous dorsal carpellary bulges are differentiated as nectaries (Endress, 1986), similar to the interstylar nectaries reported for Buxaceae.

3.8.8 Core Eudicots

In the Core Eudicots, receptacular nectaries—either continuous or fragmented—are common, located principally between androecium and gynoecium and in association with the filament bases. Sepal, petal, and gynoecial nectaries are less frequent.

Gunnerales. Flowers are wind-pollinated, reduced, and devoid of nectaries (Wanntorp & Decraene, 2005).

Aextoxicaceae. Cronquist (1981) pointed out small receptacular nectary glands alternating with the stamens in an antesepalous position, probably of staminodial origin.

Berberidopsidaceae. The receptacle extends into a lobed ring-like nectary between the androecium and the inner tepals (Decraene, 2004), and could be described as extrastaminal.

Dilleniaceae. No nectar has been observed; flowers have pollen and are buzz-pollinated (Tucker & Bernhardt, 2000).

Caryophyllales. This complex order shows a wide array of nectariferous families, together with others that are anemophilous (**Achatocarpaceae**: Bullock, 1994; **Simmondsiaceae**: Niklas & Buchmann, 1985), have pollen flowers (**Rhabdodendraceae**: Nelson & Prance, 1984), or bear no nectaries (**Drosophyllaceae**: Ortega Olivencia et al., 1995; **Physenaceae**: Dickinson & Miller, 1993). The nectaries are predominantly receptacular, being typically intrastaminal or related to the basal part of the filaments of the stamens. Zandonella (1977), in a comparative study, proposed an evolutionary trend from nectaries surrounding the base of the ovary to nectaries around the androecium. In **Aizoaceae**, a continuous ring of nectariferous tissue coats as the inside of the perianth–stamen tube (Zandonella, 1977; Chesselet et al., 2002). Ihlenfeldt (1960) holds that this nectary is part of the gynoecium, instead of the androecium; the latter view is also held by Zandonella (1977). Chesselet et al. (2002) proposed a new tribal classification supported by floral nectary morphology for the former Mesembryanthemaceae (now placed within Aizoaceae *s.l.*). The basal group (Aizoaceae *s. str.*) has a ring-shaped annular nectary (holonectary), whereas mesembryanthemums possess a hollow or shell-shaped (koilomorphic)—not continuous—nectary (meronectary). On the other hand, the more specialized Ruschioideae is characterized by the following four nectary types: (i) meronectary, (ii) broad, flat holonectary, (iii) lophomorphic (crested or lobed) holonectary, which is considered the most derived type, and (iv) nectary inconspicuous or absent.

In **Amaranthaceae** (including Chenopodiaceae), there is a trend towards anemophily and many taxa possess no nectaries; nevertheless, when present, they are located at the inner base of the filaments, either as a ring or as five glands alternating with the filaments (Zandonella, 1977). **Basellaceae** shows an annular nectary at the outer or inner base of the stamens (Zandonella, 1977). In **Cactaceae**, nectar is secreted by an annular receptacular nectary (*Pereskia*, *Rhipsalis*), or along the basal portion of the hypanthium (Zandonella, 1977; Barthlott & Hunt, 1993; Nassar et al., 1997); in the latter case, distinct nectar chambers may occur, formed by different organs (e.g., filamental or hypanthial appendices). **Caryophyllaceae** shows nectariferous tissue as a ring at the base of the filaments, or in the tube formed by the bases of filaments and petals, or coating the inside of a receptacular cup (Zandonella, 1977). In **Didieraceae**, the bases of the stamens are adnate and form a ring-like nectary surrounding the ovary (Kubitzki, 1993b). For **Droseraceae**, Murza and Davis (2003) found no nectaries or nectar in *Drosera* species, although papillate cells that were reminiscent of secretory tissue were observed at the apices of anthers and ovaries. As there are reports of both the existence and absence of nectaries, it was suggested that the presence of floral nectaries may vary in *Drosera* (Murza & Davis, 2003). In

some cases, the nectaries are found on the petal claws (Kerner von Marilaun & Oliver, 1895), and in other cases nectar was found around the base of the ovary (Lowrie, 2001), so there is no certainty on the exact place of nectar secretion. In **Frankeniaceae**, the five long-clawed petals have five nectar-containing chambers formed by scales on the inner side, forming revolver-type flowers (Brochmann et al., 1995). In **Molluginaceae**, the site of nectar production is the staminal tube of the inner stamens, where the nectariferous tissue lines the inner surface and the region where the staminal tube is adnate to the ovary. In some taxa nectariferous tissue may form a ring surrounding the ovary (Zandonella, 1977). In **Nepenthaceae**, Kato (1993) recorded nectaries wholly distributed on the adaxial surface of the sepals. In **Nyctaginaceae**, the nectariferous tissue is normally located basally on the adaxial surface of the staminal tube (Zandonella, 1977; Vanvinckenroye et al., 1993; López & Galetto, 2002). In **Phytolaccaceae**, which is considered by Zandonella (1977) to be the basal type, the receptacle is nectariferous and forms an intrastaminal ring, either between the inner whorl of stamens and the ovary, or at the base of the inner stamens (Zandonella, 1977; Bernardello et al., 1993); however, no nectar-secreting tissue was found in Rivinoideae (Zandonella, 1977). In **Plumbaginaceae**, the nectaries are always associated with the androecium, mainly with the stamen bases (Galetto, 1993; De Laet et al., 1995). **Polygonaceae** also has receptacular nectaries between the stamen bases and the ovary (Decraene & Smets 1991a; De Melo et al., 2003). Decraene and Smets (1991a) distinguished two nectary types: in Persicarieae there are conspicuous nectar-secreting structures (free or variously fused), whereas in Polygoneae nectarial zones are not developed externally. These authors suggested a centrifugal shift of the nectaries, from a *Rheum*-like ancestor with intrastaminal annular nectaries, through a *Fagopyrum*-like ancestor with free nectaries, to the nectaries of Polygoneae and Persicarieae listed above. **Portulacaceae** possesses nectaries on the adaxial base of the stamens (Zandonella, 1977; Vanvinckenroye & Smets, 1996, 1999). Finally, in **Tamaricaceae** there is a fleshy receptacular nectary where petals, sepals, and stamens are seated, or it may be intrapetalar or intrastaminal (Brown, 1938; Fahn, 1979; Cronquist, 1981); the scales of the petals are not nectariferous (Decraene, 1990).

Santalales. This parasitic order has receptacular nectaries, or if the ovaries are inferior, nectaries are located on the top part of the ovary. In the literature, the nectaries are mostly referred to as discs (e.g., Kjuit, 1969; Cronquist, 1981), and sometimes there is no direct evidence that they secrete nectar, a circumstance that should be investigated. **Loranthaceae** is most often bird-pollinated and flowers produce large amounts of nectar (Kjuit, 1969; Galetto et al., 1990; Aizen, 2005). The nectary is usually located at the top of the

inferior ovary (Kjuit, 1969; Galetto et al., 1990). It was suggested that the petals may be nectariferous at the base (Cronquist, 1981), but anatomical studies demonstrated that they do not have secretory tissue, at least not in the studied species (Galetto et al., 1990). The dioecious **Misodendraceae** has male flowers with a small, lobed nectary disc, but the long feathery stigmata of the female flowers suggest wind pollination (Cronquist (1981). In **Olacaceae**, Cronquist (1981) notes intrastaminal annular nectaries or extrastaminal nectaries, either annular or consisting of separate glands alternate with the petals. For **Opiliaceae**, intrastaminal nectaries have been identified, consisting of distinct or more or less connate nectaries alternating with the stamens (Cronquist, 1981); some species are anemophilous (Bullock, 1994), suggesting that in these cases the receptacle is not nectariferous. In **Santalaceae**, nectaries are well-demonstrated as intrastaminal and commonly surrounding the ovary (Kjuit, 1969; Cronquist, 1981; Bhaskar, 1992; Aronne et al., 1993).

Saxifragales. Some families have no nectaries and inconspicuous flowers, and are probably anemophilous: **Altingiaceae** (Endress, 1993b), **Cercidiphyllaceae** (Endress, 1993a), and **Haloragaceae** (Cronquist, 1981). Alternatively, other families are nectar-secreting and animal-pollinated. In **Crassulaceae**, nectaries are small appendages or scales borne externally at the lower back of the carpels near the base (Cronquist, 1981; Said, 1982); occasionally they can be larger and petaloid (Cronquist, 1981). For **Grossulariaceae**, Cronquist (1981) pointed out a lobed receptacular nectary internal to the stamens. In **Hamamelidaceae**, nectaries occur at different sites: a receptacular ring in the form of ten fleshy knobs that may not always be secretory (Endress, 1993b), on the petal bases (Endress, 1993b), or on staminodes (Mione & Bogle, 1990; Anderson & Hill, 2002); there are also some wind-pollinated, presumably nectarless, taxa (Endress, 1989). **Paeoniaceae** has nectaries on the sepals and floral bracts (Zimmermann, 1932; Elias, 1983; Sánchez-Lafuente, 2002); these nectaries are generally considered extrafloral though located on the flower. For **Saxifragaceae**, the nectary is normally intrastaminal and annular, found on the receptacle between the base of the stamens and the ovary (Bensel & Palser, 1975a, b; Cronquist, 1981; Smets, 1986), but it is considered gynoecial as well (Decraene et al., 1998).

3.8.9 Rosids

Amongst the Rosids, receptacular (intra- or interstaminal) and hypanthial nectaries are most frequent, followed by ovarian and staminal types.

Crossosomatales. Most families—**Aphloiaceae**, **Crossosomataceae**, **Ixerbaceae**, **Geissolomataceae**, **Staphyleaceae**, and **Strasburgeriaceae**—have

a receptacular intrastaminal nectary, which is located in the floral cup (Matthews & Endress, 2005a). There are usually nectary recesses in various positions connecting to the androecium or the gynoecium; they are of systematic importance (Matthews & Endress, 2005a). In Strasburgeriaceae and Ixerbaceae, the nectary surrounds the ovary as a ring. In **Stachyuraceae**, the nectary is ovarian, and forms a ring on the lower part of the ovary wall (Matthews & Endress, 2005a). **Picramniaceae** is poorly known; the presence of small amounts of nectar was reported (Pascarella, 1996), but no data on the nectaries are available. In **Vitaceae**, an intrastaminal receptacular nectary is common, mainly annular or cupulate, or sometimes as five distinct glands (Cronquist, 1981; Gerrath & Posluszny, 1994; Zhang et al., 1999).

Geraniales. Receptacular extrastaminal nectaries are common in this order. Members of **Geraniaceae** usually have five episepal extrastaminal nectaries that can fuse into a ring (Cronquist, 1981; Link, 1994b; Vogel 1998c). In actinomorphic flowers, all glands are similar, but in zygomorphic flowers (e.g., *Erodium*) there is a trend to enlarge the three adaxial nectaries. In *Pelargonium*, the glands have been reduced to a single nectary, situated at the bottom of a spur adnate to the pedicel (Vogel, 1998c). False nectaries—spherical hairs filled with a liquid and resembling nectar droplets—were reported in several *Erodium* species (Aldasoro et al., 2000). **Melianthaceae** (including Francoaceae and Greyiaceae) shows receptacular extrastaminal nectaries that are ontogenetically related (Decraene & Smets, 1999a; Decraene et al., 2001). They may be well-developed and unilaterally located in the flower, or be composed of several radially placed glands. In **Vivianiaceae** (including Ledocarpaceae), *Viviania* has five antesepalous extrastaminal nectaries, but *Balbisia* and *Rhynchotheca* lack them, these genera perhaps being wind-pollinated (Weigend, 2005).

Myrtales. Most families within this order have nectaries located mainly on the hypanthium, the gynoecium, or the junction of the hypanthium with the ovary. **Combretaceae** has nectariferous tissue (usually plicate) covering the internal basal part of the hypanthium (Cronquist, 1981; Bernardello et al., 1994). Both **Heteropyxidaceae** and **Psiloxylaceae** have the nectariferous tissue lining the internal part of the hypanthium (Schmid, 1980). In **Lythraceae**, some taxa are nectarless and others have nectaries in several positions: (i) in some taxa with a superior ovary (although not exclusively), the ovary base is surrounded by an annular nectary; (ii) the nectary may be strictly hypanthial; or (iii) occupy the junction of the hypanthium and the floral tube (Schmid, 1980; Cronquist, 1981; Smets, 1986; Graham et al., 2005); occasionally, (iv) the ovarian nectary can be unilateral (Cronquist, 1981). Within **Melastomataceae**, most species do not produce nectar and

have pollen as the pollinator reward. However, some singular species possess non-structural nectaries on the filaments of the stamens, which show thickened vascular bundles (Stein & Tobe, 1989; Vogel, 1997). Alternatively, petal nectaries on *Medinilla* and stigma nectaries in *Miconia* have been recorded (Stein & Tobe, 1989; Vogel, 1997). Stein and Tobe (1989), taking into account the ancestral myrtalean nectary type (structural, mostly hypanthial), consider that structural nectaries were lost in the lineage ancestral to Melastomataceae; nectaries were acquired again with a different structure and position, a fact correlated with a shift in pollinator interactions: from buzz pollination by bees at lower elevations to vertebrate pollination in higher elevations. **Myrtaceae** is typical for having nectaries on the inner part of the hypanthium, on the top of the ovary, or on both (Cronquist, 1981; O'Brien et al., 1996; Davis, 1997). In **Onagraceae**, the inner, basal part of the hypanthium is commonly nectariferous (Cronquist, 1981; Eyde, 1981; Smets, 1986). In some tubeless species, however, (e.g., species of *Circaea*, *Epilobium*, *Gayophytum*, *Gonylocarpus*, *Lopezia*), nectaries are receptacular, on the appendage side of the junction of appendages and gynoecium or gynoecial and on the top of the ovary (as in *Ludwigia*), either sunken or raised, with trichomes on their epidermises or without them (Eyde, 1981, 1982); staminal nectaries in *Lopezia* were also reported (Eyde, 1982). In **Vochysiaceae**, nectariferous sepal spurs have been observed, with nectar produced directly on the spur (Oliveira, 1996; Hodges, 1997). Concerning **Alzateaceae**, **Oliniaceae**, **Penaeaceae**, and **Rhynchocalycaceae**, there is a recent survey of floral morphology, but unfortunately, there is no mention of the presence of nectaries (Schönenberger & Conti, 2003). These families possess a hypanthium and it may be nectariferous, as in other members of the order.

3.8.10 Eurosids I

The Eurosid I clade has some members without nectaries. When present, nectaries are predominantly receptacular (mostly intrastaminal), followed by hypanthial and staminal nectaries; sepal, petal, and gynoecial nectaries are more rare. In **Zygophyllaceae**, there is a continuous intrastaminal receptacular nectary located around the base of the ovary (Cronquist, 1981; Decraene et al., 1996; Wang et al., 2000; Debandi et al., 2002), except in the former Krameriaceae, which has elaiophores secreting lipids (Simpson, 1982). Data available on **Huaceae** indicate absence of nectaries, if "disc" is assumed to be a synonym for a receptacular nectary (Simmons & Hedin, 1999).

Celastrales. There is a conspicuous annular receptacular nectary (intra- or extrastaminal) in most families in this order. Particularly in **Celastraceae**, the nectary is mostly intrastaminal, extending between the androecium and

gynoecium, either annular or with upturned margins (in most Celastroideae). It may (i) be located between the corolla and androecium (extrastaminal, in Salacioideae, most Hippocrateoideae, some Celastroideae), (ii) extend on the receptacle from the corolla to the gynoecium, or (iii) may form, with the filament bases, a collar with broad interstaminal portions that have been interpreted as staminodes (Simmons, 2004a; Matthews & Endress, 2005b). In **Lepidobotryaceae**, the intrastaminal receptacular nectary extends between stamens and ovary, protruding between the filament bases, which form a collar around the gynoecium (Link, 1991; Tobe & Hammel, 1993; Matthews & Endress, 2005b). In **Parnassiaceae** no receptacular nectaries have been observed, but nectaries have been interpreted as an inner androecial whorl of staminodes because nectar is ventrally secreted from the pad of tissue bearing the staminodial rays (Sandvik & Totland, 2003; Simmons, 2004b; Matthews & Endress, 2005b).

Malpighiales. Some families within the order bear no nectaries, such as **Balanopaceae**, **Elatinaceae**, **Goupiaceae**, **Hypericaceae**, **Lacistemataceae**, **Lophopyxidaceae**, **Ochnaceae**, **Pandaceae**, **Peridiscaceae**, **Picrodendraceae**, **Podostemaceae**, and **Putranjivaceae**, which are either wind-pollinated or have pollen flowers (Cronquist, 1981), while **Malpighiaceae** has typical oil flowers (Simpson & Neff, 1983; Sigrist & Sazima, 2004). Other families are animal-pollinated and show mostly receptacular (intra- or extrastaminal) nectaries, but also hypanthial and staminal. For **Achariaceae**, Bernhard (1999a) found five antesepalous, extrastaminal, vascularized nectary glands; as little secretion was found, however, there is doubt that they are indeed nectaries. In **Bonnetiaceae**, intrastaminal nectarial glands alternating with the stamen clusters at the base of the ovary have double bundles similar to those of stamens (Dickinson & Weitzman, 1998). Based on the vasculature of the nectaries, these authors are of the opinion that they represent transformed stamens. The presence of nectaries is variable in the genus *Archytaea* and this does not seem to follow a geographical pattern (Dickinson & Weitzman, 1998). In the mainly bat-pollinated **Caryocaraceae**, nectariferous tissue was histologically observed surrounding the ovary base (Dickinson, 1990), although previously it was reported to occur on the staminodes (Prance & Freitas da Silva, 1973). **Chrysobalanaceae** has well-developed hypanthia that are nectariferous (Cronquist, 1981; Arista et al., 1997). The flowers of **Clusiaceae** offer either nectar, pollen, or resin as rewards for pollinators (Gustafsson & Bittrich, 2002). In nectar taxa, there are either nectariferous scales or a cupular annular nectary; these have been considered staminodial (Robson, 1961), a presumption confirmed by Decraene and Smets (1991b). In **Ctenolophonaceae**, the nectary is receptacular, extrastaminal and annular, with the filament bases adnate to it, whereas in

Humiriaceae the nectaries are intrastaminal and free from the filaments (Link, 1992a). For **Irvingiaceae**, large intrastaminal receptacular annular nectaries are reported, which are unusual for having 10–15 strictly localized stomata that are deeply sunken in the nectariferous tissue (Link, 1992b). In **Ixonanthaceae**, both prominent intrastaminal annular as well as staminal nectaries (on the filament bases) are known (Link, 1992d). In **Euphorbiaceae**, an extrastaminal or intrastaminal receptacular nectary, either continuous or five-segmented, is present (Cronquist, 1981; Webster, 1994; Freitas et al., 2001), or the typical nectary glands of the bracts of *Euphorbia* and related genera (Hoppe, 1985; Papp, 2004). (See "Euphorbiaceae" on page 49 for a detailed description of the nectary types.) In **Linaceae**, nectaries are staminal, located dorsally at the bases of the filaments or at the inner base of the petals (Brown, 1938; Cronquist, 1981). **Passifloraceae** bears clearly extrastaminal receptacular nectaries (Fahn, 1979; Cronquist, 1981; Bernhard, 1999b), which are not of staminodial origin as previously suggested by Cronquist (Bernhard, 1999b). The nectariferous tissue may also form a continuous ring or several separate glands, as occurs in the former Turneraceae with five glands deeply immersed in the receptacle (Gonzalez, 2001). Variations of systematic importance among species and genera have been found (Bernhard, 1999b; Gonzalez, 2001). In **Phyllanthaceae**, receptacular nectaries, either intrastaminal or extrastaminal, are found as well, although they may be absent in some genera (Webster, 1994). In the mangrove family **Rhizophoraceae**, nectaries are receptacular, intrastaminal, and perigynous (Juncosa & Tomlinson, 1987). In **Salicaceae** (including most Flacourtiaceae), some taxa present reduced flowers, lack nectaries, and are wind-pollinated (e.g., *Populus*, *Xylosma*; Cronquist, 1981; Bullock, 1994). These plants show either (i) a continuous annular receptacular nectary, (ii) separate nectar glands (often extrastaminal), or (iii) interstaminal nectar lobes (Cronquist, 1981; Machado & Oliveira, 2000). Some members have mixed wind and insect pollination (e.g., *Salix,* with nectaries formed by one or two small glands, sometimes unequal, considered calycinal in origin; Brown, 1938; Cronquist, 1981; Smets, 1986). In **Violaceae**, the anterior petal is spurred and nectar is secreted from a pair of basal staminal appendages, specifically from the connective of the inferior stamens, which project the nectar into the spur (Smets, 1986; Vogel, 1998b; Freitas & Sazima, 2003). Occasionally, the glands are stalked and grow into the hollow of the spur, some even reaching the bottom of the spur (Vogel, 1998b); the variation in these glands is systematically useful. On the other hand, there may be striking inter- and intrapopulational variability in the spur and nectaries in some *Viola* species (e.g., Herrera, 1988).

Oxalidales. Receptacular nectaries are present in most families, but notably absent in some **Elaeocarpaceae**, with specialized pollen flowers (Matthews

& Endress, 2002). In **Brunelliaceae**, **Cephalotaceae**, **Cunoniaceae**, and some Elaeocarpaceae the nectaries are annular, intrastaminal, and receptacular around the gynoecium base, protruding as lobes between the stamen filaments (Vogel, 1998a; Matthews & Endress, 2002; Bradford et al., 2004; Humaña & Valdivia, 2004). For Cephalotaceae, Vogel (1998a) noted epidermal nectaries on bracts, tepals, and virtually all other aerial parts. In **Connaraceae**, the nectaries are placed at the base of the stamens (Matthews & Endress, 2002). In **Oxalidaceae** nectaries are located at the base of the epipetalous stamen/staminode filaments and nectar can be foraged by pollinators through a channel formed by each petal claw (Brown, 1938; Matthews & Endress, 2002).

Fabales. Most members of this order have intrastaminal and hypanthial nectaries. **Surianaceae** is an exception and has no nectaries (Cronquist, 1981). **Fabaceae** is biotically pollinated, utilizing mostly bees, birds, and bats (Arroyo, 1981; Schrire, 1989) and offering nectar as a reward. Vascularization of nectaries may be achieved by phloem, xylem and phloem, or no special vascular tissue (Fahn, 1979). As a whole, nectaries are mesenchymatic, receptacular, and intrastaminal; sometimes, the abaxial side of the nectary may be enlarged (Davis et al., 1988; Westerkamp & Weber, 1999). In the basal members of paraphyletic subfamily Caesalpinioideae (Herendeen et al., 2003), nectaries are usually located between the stamens and ovary (Fahn, 1979; Cronquist, 1981; Tucker, 2002; Herendeen et al., 2003). However, in several genera with a hypanthium (e.g., *Balsamocarpon*, *Caesalpinia*, *Cercidium*, *Hoffmannseggia*, *Parkinsonia*, *Zuccagnia*), Cocucci et al. (1992) found hypanthial nectaries, located on the inner surface, from the base up to the region where the filaments are inserted in a long hypanthial tube, as Fahn (1979) reported for *Bauhinia*. In a survey of the nectaries of subfamily Mimosoideae, Ancibor (1969) reported them as receptacular intrastaminal, between the bases of the filaments and either the ovary base or the gynophore; they can also be placed at the fused bases of the filaments, which may also be fused with the corolla. No doubt subfamily Papilionoideae is the most studied because of its economic importance (e.g., Waddle & Lersten, 1973; Fahn, 1979; Cronquist, 1981; Davis et al., 1988; Vogel, 1997; Galetto et al., 2000; Horner et al., 2003; Bernardello et al., 2004). In taxa with free stamens, nectar is easy to reach, whereas in diadelphous taxa, nectar accumulates between the carpel base and the filaments, being sought below the vexillar petal at the base of the filament column, where there are usually two openings (Vogel, 1997); some diadelphous species, like *Coronilla varia* and its relatives, are nectarless (Vogel, 1997). Monadelphous taxa were considered to be lacking in nectar because of their completely fused staminal tube, but current findings indicate that some species (e.g., in *Chamaecytisus*,

Cytisophyllum, *Erinacea*, *Genista*, *Petteria*, *Retama*, *Spartium*, *Spartocytisus*) do secrete nectar (Bisby, 1981; Vogel, 1997; Westerkamp, 1997; Galloni & Cristofolini, 2003). Bisby (1981) described these nectaries as extrastaminal, but Vogel (1997) showed through histological analyses that they are non-structural nectaries located in the filament column. A special case was reported in several species of *Stylosanthes*: as the nectary is destroyed by the elongation of the receptacle into a long tube, an apparently non-homologous nectar gland develops at the distal end of the tube (Vogel, 1997). **Polygalaceae** tends to have highly specialized zygomorphic flowers with secondary pollen presentation (Brantjes, 1982). Members of this family have an intrastaminal nectary surrounding the gynophore or the ovary, but in derived taxa it may be unilateral as an adaxial gland (Cronquist, 1981; Westerkamp & Weber, 1999). No data on nectaries in **Quillajaceae** are available, but nectar has been observed in some species (Bugg, 1987).

Rosales. Nectaries are absent in some wind-pollinated families: **Barbeyaceae**, **Cannabaceae**, **Moraceae** (except *Ficus*, which is insect-pollinated but not nectariferous), **Ulmaceae**, and **Urticaceae** (Dickinson and Sweitzer, 1970; Cronquist, 1981; Judd et al., 2002). When present, nectaries are mainly hypanthial or petal. In **Dirachmaceae**, nectaries are epidermal, as glands associated with the petal bases; in addition, they are covered by trichomes on a protuberance that protects the nectar (Link, 1994a; Decraene & Miller, 2004). **Rhamnaceae** members show hypanthial nectaries that can be attractive parts of the flower in an intrastaminal position: they can be rings around the ovary (resembling receptacular nectaries, but hypanthial in origin), can extend over the inner surface of the lower half of the floral tube, or can be restricted to laminar projections of the hypanthium (Medan & Aagesen, 1995). In some species, the nectary is separated from the gynoecium by intercalary growth. The pubescence that is common in the flower tube can be explained as a hairy barrier that separates the nectary from the outer environment (Medan & Aagesen, 1995). The flowers of **Elaeagnaceae** also have well-developed hypanthial nectaries (Cronquist, 1981; Decraene & Miller, 2004), although some taxa are anemophilous (*Hippophae*). In **Rosaceae**, the inner surface of the hypanthium is commonly nectariferous (Cronquist, 1981; Smets, 1986; Judd et al., 2002; Buban et al., 2003; Evans & Dickinson, 2005). In the literature, Rosaceae nectaries are frequently considered receptacular (e.g., Radice & Galati, 2003; Weryszko-Chmielewska et al., 2003), probably following Fahn's (1979) interpretation.

Cucurbitales. Nectaries are lacking in **Coriariaceae** (Thompson & Gornall, 1995) and **Datiscaceae** (Philbrick & Rieseberg, 1994), which are wind-pollinated, and also in **Begoniaceae**, which has pollen flowers, except for a

few hummingbird-pollinated species in which the site of nectar production is unknown (Vogel, 1998c). The remaining families exhibit a variety of nectaries: receptacular, hypanthial, or staminodial. In **Anisophylleaceae**, receptacular nectaries form hemispherical bulges that protrude between the stamen filaments (i.e., an interstaminal position) and are also connected behind these filaments (Matthews et al., 2001). In **Corynocarpaceae** nectaries are found on staminodes (Narayana et al., 1986; Matthews & Endress, 2004). In **Cucurbitaceae** there two nectary types: (i) hypanthial mesenchymatous nectaries exuding nectar through stomata, typical of most members of subfamily Cucurbitoideae (Brown, 1938; Vogel, 1990, 1997; Nepi et al., 1996; Ashworth & Galetto, 1999; Fahn & Shimony, 2001), and (ii) epidermal nectaries in the form of trichomes, located mostly in the corolla, especially in genera with male flowers with synandria (Vogel, 1981b, 1990, 1997), which characterize subfamily Zanonioideae and tribes Cyclanthereae and Scycieae of subfamily Cucurbitoideae (Vogel, 1997). (See "Cucurbitaceae" on page 48 for a detailed discussion.) There is no certainty on the presence of nectaries in **Tetramelaceae**: Davidson (1973) mentioned the presence of nectar, whereas Matthews and Endress (2004) described no nectaries, indicating that areas on the ovary roof may function as nectaries; experimental studies are needed to clarify this matter.

Fagales. All the families in this order—**Betulaceae**, **Casuarinaceae**, **Fagaceae**, **Juglandaceae**, **Myricaceae**, **Nothofagaceae**, and **Ticodendraceae**—lack nectar-secreting structures, being mostly wind-pollinated or secondarily insect-pollinated (Cronquist, 1981; Judd et al., 2002).

3.8.11 Eurosids II

In the Eurosid II clade, receptacular nectaries are frequent, mainly intrastaminal but also extrastaminal; sepal and petal nectaries are uncommon. In **Tapisciaceae**, the genus *Huertea* possesses an intrastaminal receptacular nectary, whereas *Tapiscia* has no nectaries (Dickinson, 1986a).

Brassicales. Within the order, **Bataceae** and **Gyrostemonaceae** are nectarless and wind-pollinated (George, 2003; Decraene, 2005), and **Setchellanthaceae** also lacks nectaries (Tobe et al., 1999). In the remaining families, nectaries are mainly receptacular but also hypanthial and staminal; sepal nectaries are rare. In **Akaniaceae** (including Bretschneideraceae), *Bretschneidera* has a hypanthial nectary that extends from the base of the filaments to the gynoecium (Decraene et al., 2002), but there are no data on nectaries in *Akania*. The receptacular nectaries in **Brassicaceae** can be annular and continuous, or fragmented into two, four, or eight nectaries (e.g.,

Norris, 1941; Deng & Hu, 1995; Davies et al., 1996, 1998); they are related to the filament bases, and the use of intra- and extrastaminal is imprecise here because of the particular arrangement of the glands. The former Capparaceae, which is now included in Brassicaceae, commonly has extrastaminal annular receptacular nectaries between the sepals and petals, sometimes with three or four appendages that are free or partly adnate to the calyx (Fahn, 1979; Cronquist, 1981; Decraene et al., 2002; Kers, 2003); zygomorphic flowers usually have a gland or nectary appendage (Kers, 2003). (See "Brassicaceae" on page 47 for a detailed discussion.) For **Caricaceae**, it was observed that staminate flowers produced nectar (Baker, 1976; Bawa, 1980); later, and specifically in *Carica papaya*, Decraene and Smets (1999b) demonstrated that nectaries of staminate flowers are located on the central rudimentary pistil (not at the base of the stamens as supposed in earlier reports) and that pistillate flowers produced no nectar but had stigmatic exudates. **Emblingiaceae** has a curved androgynophore with a unilateral receptacular nectary at its base between the two petals (Cronquist, 1981), whereas in **Koeberliniaceae** the bases of the filaments are ventrally (i.e., the region facing the gynoecium) nectariferous (Mehta & Moseley, 1981). On the other hand, in **Limnanthaceae**, nectaries are basal protrusions on the episepalous stamens, and are dorsally located (Link, 1992c). In **Moringaceae**, the hypanthium is nectariferous at its base, which surrounds the gynophore (Cronquist, 1981; Decraene et al., 1998). In **Pentadiplandraceae**, there is an extrastaminal receptacular annular nectary, protected by basal appendages of the petals (Decraene, 2002). **Resedaceae** has extrastaminal receptacular nectaries, except in *Oligomeris* (Cronquist, 1981). Nectaries are cylindrical or infundibular and widen towards the adaxial side of the androgynophore to a fleshy semi-lunate limb that produces nectar from a distinct gland on the lower surface (Kubitzki, 2003). In **Salvadoraceae**, nectaries can be either absent or present as nectar glands alternating with the stamens (Cronquist, 1981). **Tovariaceae** possesses low extrastaminal ring-like nectaries between the filament bases (Fisel & Weberling, 1990; Decraene, 2002). In **Tropaeolaceae**, there is a nectariferous spur (Rachmilevitz & Fahn, 1975; Cronquist, 1981; Decraene & Smets, 2001), which may be either small or well-developed, and is formed by the sepals, although some authors (cf. Decraene & Smets, 2001) consider it receptacular (axial or hypanthial). In some species, the spur is showy and up to five times as long as the calyx lobes (Bayer & Appel, 2003). Some of the nectar is produced by the unicellular trichomes on the inner epidermis of the spur, but most originates from subepidermal tissue (Rachmilevitz & Fahn, 1975; Fabbri & Valla, 1998).

Malvales. The presence of nectaries is common in this order, except in the families **Neuradaceae**, in which no nectaries were found in mature flowers

(Decraene & Smets, 1995), and **Bixaceae**, which has pollen as reward (Poppendieck, 2003a, b). The other families have receptacular nectaries (intra- or extrastaminal), more rarely on calyx or corolla. **Cistaceae** bears an annular intrastaminal receptacular nectary, which is many-lobed (Fahn, 1979; Cronquist, 1981; Manetas & Petropoulou, 2000). A few papers indicate that some **Dipterocarpaceae** species have nectaries and produce nectar (Ghazoul, 1997; Harrison et al., 2005), but do not specify the precise location of the nectariferous tissue; on the other hand, some authors have not observed nectaries (Ashton, 2003). In the broadly circumscribed **Malvaceae** (including Bombacoideae, Byttnerioideae, Dombeyoideae, Grewioideae, Helicterioideae, Malvoideae, Sterculioideae, and Tilioideae), the unusual floral nectaries, composed of densely packed, multicellular, glandular trichomes, have been identified as a synapomorphy (Judd & Manchester, 1997; Vogel, 2000). As there are some nectarless taxa in this family, Vogel (2000) suggested that this loss could be either secondary or a plesiomorphy, considering the basal position of the mostly nectarless taxa (Grewioideae, Byttnerioideae). Trichomes are generally limited to the calyx adaxial surface, but they can also occur on the corolla or the androgynophore (Donato, 1991; Vogel, 2000; Leitao et al., 2005); this barely investigated topographical diversity may provide useful taxonomic characters (Vogel, 2000). The nectar is frequently directly accessible by pollinators because of the flower shape and the way in which the petals are separated, but it can have a secondary presentation (Vogel, 2000). In **Muntingiaceae**, there is an intrastaminal receptacular nectary as part of the broad receptacle of the flower, and nectar is retained by short hairs surrounding the nectary (Bawa & Webb, 1983). In **Sarcolaenaceae**, there is an extrastaminal receptacular annular ring considered nectariferous, which is cupular or deeply quinque-partite (Cronquist, 1981; Bayer, 2003). **Sphaerosepalaceae** possesses a short gynophore that bears an intrastaminal receptacular nectary towards its apex (Horn, 2004). In **Thymelaeaceae**, the nectaries may be absent (in Octolepidoideae), but when present, they are intrastaminal receptacular, either consisting of separate scales, or a cup- or cuff-shaped ring (Cronquist, 1981; Herber, 2003; Cornara et al., 2005; Bandera & Traveset, 2006).

Sapindales. A conspicuous receptacular nectary (Cronquist, 1981) is typical and according to Gadek et al. (1996), is a potentially important morphological synapomorphy for the order. **Anacardiaceae** has intrastaminal receptacular nectaries, generally well-developed and sometimes transformed into short gynophores (Cronquist, 1981; Wannan & Quinn, 1991; Gallant et al., 1998); in *Anacardium occidentale*, nectariferous trichomes were reported on the corolla base (Wunnachit et al., 1992). **Biebersteiniaceae** nectaries are separate extrastaminal nectary glands at the base of the antisepalous stamens,

similar to those of Geraniaceae (Link, 1994b), in which the family was previously included. In **Burseraceae**, there is a prominent, commonly annular, intrastaminal nectary (Cronquist, 1981; Sunnichan et al., 2005). **Kirkiaceae** is reported to have intrastaminal receptacular nectaries (Cronquist, 1981), but no anatomical or biological studies are available to support this assumption. In **Meliaceae**, there is usually an annular intrastaminal nectary, sometimes developed into an androgynophore (Brown, 1938: Cronquist, 1981; Lal, 1994; Moscheta et al., 2002). **Nitrariaceae** also has an intrastaminal nectary, fragmented into five glands (Decraene & Smets, 1991c). In **Rutaceae**, the nectary is intrastaminal, completely surrounding the ovary base, and it may be unilateral, modified into a nectariferous gynophore, or absent (Rachmilevitz & Fahn, 1973; Fahn, 1979; Cronquist, 1981; Souza et al., 2002; Caris et al., 2006); non-secretory septal cavities were found in *Cneorum* (Caris et al., 2006). In **Sapindaceae**, extrastaminal annular receptacular nectaries are widespread between the perianth and the stamens (Cronquist, 1981; Decraene et al., 2000a; Cui et al., 2003), and non-secretory septal slits were found in *Koelreuteria* (Decraene et al., 2000a). A well-developed intrastaminal nectary is common in **Simaroubaceae** (Cronquist, 1981). *Ailanthus glandulosa* has a special nectary with two zones: the intrastaminal apical part and the basal part located in front of the sepals (Bory & Clair-Maczulajtys, 1982).

3.8.12 Asterids

The trend in the Asterids is towards gynoecial nectaries, either on top of inferior ovaries or at the base of superior ones; in exceptional cases, nectaries are located in the calyx whorl.

Cornales. The aquatic submerged wind-pollinated herbs in **Hydrostachyaceae** do not have nectaries (Erbar & Leins, 2004), but the remaining families usually have epigynous nectaries. **Cornaceae** (including Nyssaceae) has inferior ovaries and well-developed nectaries on the top of the ovary around the style, except in *Davidia* (Eyde, 1968, 1988; Cronquist, 1981; Batra, 1999). For **Grubbiaceae**, Cronquist (1981) indicates a nectary atop the pubescent ovary, although no histological studies were done, a situation that holds for **Curtisiaceae** as well (Kubitzki, 2004). In **Hydrangeaceae**, the nectaries are located on the top of the inferior ovary (Bensel & Palser, 1975c; Cronquist, 1981; Hufford, 2001). Floral morphology in **Loasaceae** is particularly complex, with nectariferous floral scales composed of fused staminodia (Brown & Kaul, 1981; Smets, 1988; Hufford, 1990; Weigend & Gottschling, 2006) that are common in Loasoideae (Weigend, 2004). The formation of gynoecial nectaries on the domes of inferior ovaries is also frequent (Brown &

Kaul, 1981; Moody & Hufford, 2000), and these may be large and cup-like, or missing in a few genera (*Cevallia*, *Schismocarpus*), probably related to changes in the pollination system. Petals with a cup-shaped nectary are also found in Gronovioideae and Petalonychoideae (Weigend, 2004). Occasionally, there is a positional change and the nectary is located on the collar of tissue on which the perianth and androecium are inserted, a location considered homologous with the typical epigynous gland (Hufford, 1989). The nectar can be freely accessible (in tilt-revolver flowers, considered plesiomorphic) or hidden by complex structures (in funnel-revolver flowers) (Weigend & Gottschling, 2006).

Ericales. Floral nectaries are usually missing in **Actinidiaceae** (Schmid, 1978), **Diapensiaceae** (Scott, 2004), **Ebenaceae** (Wallnöfer, 2004), and **Myrsinaceae** (Vogel, 1986; Otegui & Cocucci, 1999). However, in Actinidiaceae, Brown (1935) reported nectariferous tissue at the base of the petals in *Saurauia,* and in Myrsinaceae (*Anagallis*, *Elingamita*) trichomes that might be nectariferous were found on the calyx and corolla, and the filaments might produce nectar (Caris et al., 2000; Sthål & Anderberg, 2004). **Balsaminaceae** is characterized by possessing nectariferous sepal spurs, formed by the adaxial sepal (navicular or saccate) or by the three upper sepals which are prolonged backwards (Cronquist, 1981; Smets, 1986; Travers et al., 2003; Fischer, 2004a). For **Clethraceae**, the basal part of the superior ovary is reported as being nectariferous (Brown, 1938; Cronquist, 1981; Schneider & Bayer, 2004). In **Cyrillaceae**, intrastaminal nectaries are present around the base of the ovary (Cronquist, 1981; Dute et al., 2004). In **Ericaceae**, an intrastaminal receptacular nectary is associated with the base of the ovary in taxa with superior ovaries, and is sometimes even attached to it, but when ovaries are inferior to semi-inferior, nectaries are located on top (Brown, 1938; Palser, 1961; Wallace, 1977; Cronquist, 1981; Palser et al., 1991; Anderson et al., 2000b; Freitas et al., 2006). Nectaries may vary considerably in prominence and in overall shape (e.g., simple rings, rings with interstaminal projections, or lobed rings), usually reflecting the morphology and declination of the flowers. In **Fouquieriaceae**, the base of the superior ovary is nectariferous (Cronquist, 1981; Nabhan et al., 1999). **Lecythidaceae** attracts pollinators by different combinations of primary (pollen and nectar) and secondary (colour and scent) attractants (Mori et al., 1978; Prance & Mori, 1979; Knudsen & Mori, 1996): actinomorphic flowers offer pollen as reward, whereas those that are zygomorphic offer nectar. A decrease in the number of stamens and a stronger zygomorphy of the androecium are accompanied by a shift from pollen to nectar as the floral reward (Mori et al., 1978). Nectar flowers are closed by a tightly appressed androecial hood, and sometimes the nectar is located at the apex of an

inwardly coiled chamber (Knudsen & Mori, 1996). Nectar is secreted by an intrastaminal nectary, sometimes enlarged and covering the top of the ovary, or less developed in Lecythidoideae and Napoleonaeoideae, but in Foetidioideae and Planchonioideae there are associated appendages (with anthers or sterile) in the coiled part of the hood that produce nectar (Mori et al., 1978; Cronquist, 1981; Frame & Durou, 2001; Prance, 2004; Prance & Mori, 2004). In **Maesaceae**, there is a gynoecial nectary on the top of the semi-inferior ovary (Vogel, 1997; Caris et al., 2000). In **Marcgraviaceae**, nectaries are present on the floral bracts transformed into variously shaped nectaries, often conspicuously coloured, that are systematically important at the genus level (Elias, 1983; Oliveira & Oliveira, 1991; Dressler, 2004); nevertheless, these nectaries seem not to mediate in the pollination process (Tschapka & von Helversen, 1999). **Pentaphylacaceae** is considered devoid of nectaries, but some observations (that have to be confirmed) suggest that there are nectaries, either as rings around the base of the ovary in *Pentaphylax* and other genera, within the staminal ring in *Cleyera*, or on top of the ovary in *Symplocarpon* (Weitzmann et al., 2004). In **Polemoniaceae**, an intrastaminal receptacular annular nectary, which may be entire to lobed, is found around the ovary base (Cronquist, 1981; Smets, 1986; Wilken, 2004). **Primulaceae** has typical annular gynoecial nectaries at the base of the ovary, but staminodes are not nectariferous (Vogel, 1986, 1997; Caris et al., 2000; Caris & Smets, 2004); in addition, scattered trichomes on the ovary surface were reported as nectariferous in *Glaux* (Vogel, 1997). In **Sapotaceae**, the flowers secrete nectar and the nectary is morphologically poorly differentiated and represented only by a small ring around the ovary base (Pennington, 2004). In **Sarraceniaceae**, only the genus *Sarracenia* yields nectar as floral reward, produced by many small epidermal glands on the external ovary wall (Vogel 1998a). In **Styracaceae**, there is an annular ovarian nectary surrounding its base (Fahn, 1979; Saraiva et al., 1988). In **Symplocaceae**, there are gynoecial nectaries (annular, cylindrical, or five-lobed) located at the base of the style of inferior to semi-inferior ovaries that are often covered by an indumentum (Caris et al., 2002; Nooteboom, 2004). According to Cronquist (1981), the base of the filaments and the ovary base are nectariferous in **Theaceae**; there are no reports on the anatomy, but many on the nectar, including the toxicity for bees of the nectar of a *Camellia* species (Adler, 2000; Rho & Choe, 2003; French et al., 2005). **Theophrastaceae** is reported to have nectar-secreting staminodes (Cronquist, 1981; Vogel, 1986; Caris & Smets, 2004), although secretion was not always noted; glandular trichomes on floral parts may produce small quantities of nectar (Vogel, 1986).

3.8.13 Euasterids I

In the Euasterid I clade, gynoecial nectaries are common, mostly at the base of superior ovaries or on top of inferior ones. **Boraginaceae** possesses annular nectaries at the base of the ovary (Di Fulvio, 1978, 1997; Fahn, 1979; Cosa de Gastiazoro, 1995; Di Fulvio et al., 1997; Hofmann, 1999). In *Phacelia glaberrima* the nectary is located at the base of the tube formed by the corolla tube and the inner whorl of staminodes, a condition considered plesiomorphic for the genus (Cosa de Gastiazoro, 1995). In **Icacinaceae**, most members seem not to have nectaries, at least according to the absence of the so-called disc, but it is present in some of them (Cronquist, 1981; Kårehed, 2001), which would indicate receptacular intrastaminal nectaries if the disc were nectariferous. Anatomical studies showed no nectaries in **Oncothecaceae** (Dickinson, 1986b).

Garryales. Dioecious anemophilous plants characterize this order and consequently, both **Eucommiaceae** and **Garryaceae** have no nectaries (Cronquist, 1981). It should be noted that in Garryaceae, the intrastaminal structure present in the staminate flowers is interpreted as a vestigial nonfunctional nectary (Liston, 2003).

Gentianales. There is a wide array of nectaries within this order—mainly ovarian, but also receptacular, and located on petals or sepals. **Apocynaceae** (Apocynoideae) shows either noteworthy intrastaminal receptacular nectaries (either surrounding the ovary or as two glands alternating in position with the two carpels) or has carpellodes transformed into nectaries (Rao & Ganguli, 1963; Boiteau & Allorge, 1978; Galetto, 1997; Venter et al., 2001). The flower and pollination mechanism of Asclepiadoideae is one of the more complex in angiosperms: flowers have a well-developed staminal and intrastaminal corona, anthers and style form a gynostegium, and pollen is packed in pollinia (Cronquist, 1981). The five nectaries are located inside the anther wings that also secrete nectar (sometimes called stigmatic chamber), directly below the entrance of the anther slit, and can be regarded as androecial nectaries; from there, many genera have highly specialized nectar conducting systems which transfer nectar from the nectaries to secondary holders formed by the staminal corona (Christ & Schnepf, 1988; Kevan et al., 1989; Kunze, 1991, 1997). Interestingly, Kevan et al. (1989) demonstrated that nectar, in addition to being the reward for pollinators, is essential for the germination of the pollen. The enormous morphological variation of these structures is of great systematic and phylogenetic importance (Kunze, 1991, 1997). In **Gentianaceae**, although some taxa may not have nectaries, most genera have nectariferous petals as scales or nectar pits in the adaxial

face at the base of the corolla tube. Other genera (*Gentiana*, *Latouchea*, *Megacodon*, *Oblaria*) present whorls of gynoecial nectaries (continuous or separated) at the base of the ovary (Cronquist, 1981; Chassot et al., 2001; von Hagen & Kadereit, 2003). In addition, Vogel (1998a) reported sepal nectaries on the abaxial face. Petal nectaries may be fimbriate, lamellate, naked, or constitute spurs (*Halenia*) and vary in number (Chassot et al., 2001; von Hagen & Kadereit, 2003); these features have been used systematically, although molecular studies suggest that nectaries cannot be considered reliable synapomorphies at the generic level (Chassot et al., 2001). **Loganiaceae** has superior ovaries; poorly developed annular nectaries are sometimes present on the ovary bases (Cronquist, 1981). In **Rubiaceae**, a family with inferior ovaries, gynoecial nectaries on the top of the ovary surrounding the style base are the rule (Brown, 1938; Cronquist, 1981; Smets, 1986; Galetto, 1998).

Lamiales. The abiotically pollinated members of **Plantaginaceae**—*Callitriche* (Philbrick & Anderson, 1992), *Hippuris* (Leins & Erbar, 2004), and *Plantago* (Primack, 1978)—and the elaiophore-bearing **Calceolariaceae** (Sérsic, 2004) are devoid of nectaries. The remaining members of the order, as a whole, are distinctive for having gynoecial nectaries at the ovary base; this character was considered to be a synapomorphy for the previously recognized Lamiiflorae (Lu, 1990). Some Plantaginaceae present these typical nectaries (e.g., *Aragoa*, *Globularia*, *Linaria*; Cronquist, 1981; Nepi et al., 2003; Bello et al., 2004; Wagenitz, 2004); in some genera, the nectary is non-functional and flowers offer pollen, but nectar can be secondarily produced by glandular trichomes at the dilated base of the filaments (*Penstemon*) or staminodes (*Collinsia*) (Fischer, 2004b). In *Monttea* and *Melosperma* both nectar and oils are secreted; the nectary type is unusual for the family since it is not ovarian, but formed by fusion of the basal part of the filaments and the corolla tube (Sérsic & Cocucci, 1999). In **Acanthaceae**, in addition to gynoecial nectaries (Cosa, 1975; Cronquist, 1981; Piovano et al., 1995), bracteal nectaries are also reported (McDade & Turner, 1997). The nectary of *Thunbergia* is very large and more intrastaminal receptacular than gynoecial, showing interesting differences among the different species (Schonenberger, 1999). **Bignoniaceae** has typical annular ovarian nectaries at the base of the ovary (Fahn, 1979; Cronquist, 1981; Galetto, 1995a; Rivera, 2000a), although in some taxa from tribe Bignonieae nectaries are absent and flowers are pollinated by deceit (Gentry, 1980, 1982; Rivera, 1996, 2000). In some taxa that have lost the ovarian nectary, nectar can be secondarily produced by corolla trichomes (Lopes et al., 2002). The presence of nectaries is frequent on the adaxial part of the calyx tube as well (Rivera, 2000b). **Carlemanniaceae** has inferior ovaries with a nectary on top producing copious

nectar; the nectary can be cylindrical or conical (Thiv, 2004). In **Gesneriaceae**, nectar is produced by glands between the base of the ovary and the corolla; the nectary is either continuous (annular, cylindrical, or cup-shaped) or consists of one to five separate glands, which can be free or adnate to the ovary (Cronquist, 1981; Maldonado & Otegui, 1997; Perret et al., 2001; Weber, 2004); sometimes, they can be reduced and non-functional. The nectary shape is of great importance in defining genera, and trends in shape characterize evolutionary lines in the Neotropical Gesneriaceae: in Beslerieae from a complete ring to a single dorsal gland (through steps involving a dorsally thickened ring and a semilunar structure), and in Gloxinieae, Sinningieae, and Episcieae from a ring to a single dorsal gland (through a sequence including a five-lobed ring, five glands of unequal size, connation of the dorsal glands, and stepwise reduction of the lateral and ventral glands; Weber, 2004). In addition, the quantity and disposition of the glands may show intra- and interspecific variation (SanMartin-Gajardo & Sazima, 2004, 2005). These nectaries are receptacular intrastaminal rather than gynoecial, but developmental studies have to be done to determine their origin properly. In **Lamiaceae**, nectaries are annular at the ovary base, from entire to four-lobed, sometimes with the anterior lobe longer than the others (Cronquist, 1981; Kumari, 1986; Dafni et al., 1988; Zer & Fahn, 1992; Petanidou et al., 2000). In **Lentibulariaceae**, a nectariferous spur is developed from the anterior corolla lobe (Narayana & Satyavathi, 1988; Vogel, 1997). In **Martyniaceae**, the superior ovary is surrounded by an annular hypogynous nectary (Ihlenfeld, 2004a); Thieret (1976) reported that glandular trichomes located on the stamen bases produce nectar. **Phrymaceae** presents the characteristic ovarian nectary of the order (Galetto, 1995b; Hazle & Canne-Hilliker, 2005). In **Plocospermataceae**, the ovary has an annular nectary at its base (Struwe & Jensen, 2004); in addition, functionally male flowers were reported to lack nectaries (D'Arcy & Keating, 1973). In **Scrophulariaceae**, nectaries are as typical for the order and may be unilateral (Cronquist, 1981; Galetto, 1995b; Gaffal et al., 1998). In this family, oil flowers can also exist (Vogel, 1974; Sérsic & Cocucci, 1999). In **Verbenaceae**, the ovary is superior and has the typical basal nectariferous ring (Brown, 1938; Fahn, 1979; Cronquist, 1981; Smets, 1986; Bernardello et al., 2000), as also occurs in **Oleaceae** (Fahn, 1979; Cronquist, 1981), although some members are wind-pollinated (*Fraxinus*, *Olea*, *Phillyrea*; Green, 2004). **Orobanchaceae** (Fischer, 2004b; Bekker & Kwak, 2005), **Paulowniaceae** (Fischer, 2004b), **Pedaliaceae** (Cronquist, 1981; Ihlenfeld, 2004b; Wortley et al., 2005), **Schlegeliaceae** (Fischer, 2004b), and **Stilbaceae** (Linder, 2004) have annular nectaries at the base of the ovary. In addition, in some Pedaliaceae taxa nectar glands recorded at the base of the pedicel are considered to be modified flowers (Monod, 1986).

Solanales. Gynoecial nectaries at the ovary base are the most widespread in this group; only **Byblidaceae**, with pollen flowers and buzz pollination (Conran & Carolin, 2004), and **Sphenocleaceae** have no nectaries (Erbar, 1995). In **Convolvulaceae**, the nectary is a receptacular intrastaminal annular structure surrounding the ovary base, either continuous or lobed (Fahn, 1979; Cronquist, 1981; Stucky & Beckmann, 1982; Pinheiro & Schlindwein, 1998; Galetto & Bernardello, 2004). Sometimes, flowers show a nectar chamber between the nectary and the insertion of the filaments at the corolla, which prevents free access to the nectar; thus, visitors can only reach nectar through five small openings between the filament bases (Pinheiro & Schlindwein, 1998). In **Hydroleaceae**, a gynoecial nectary is developed at the base of the ovary, which is annular and five-humped (Di Fulvio, 1997; Erbar et al., 2005). In **Montiniaceae**, the inferior ovaries of female flowers show a massive nectary at the summit around the style (Decraene et al., 2000b). Nectar-secreting **Solanaceae** (considered basal) presents an annular gynoecial nectary at the base of the ovary, which may be either conspicuous or inconspicuous externally (see "Solanaceae" on page 51 for a detailed discussion; Bernardello, 1987; Vogel, 1991, 1998b; Cocucci & Galetto, 1992; Rabinowitch et al., 1993; Mione & Serazo, 1999).

3.8.14 Euasterids II

In the Euasterid II clade, gynoecial nectaries are the most frequent, followed by petal nectaries. In **Bruniaceae**, gynoecial nectaries occur on the upper parts of the inferior to semi-inferior ovaries in the form of a flat or elevated ring and are quite homogeneous within the family (Quint & Classen-Bockhoff, 2006). According to Cronquist (1981), **Columelliaceae** has no nectaries; on the other hand, *Desfontainia* has nectar (Chalcoff et al., 2006), but the place of secretion has not been determined, although the superior ovary position suggests an annular nectary at its base. In **Escalloniaceae**, a family with inferior ovaries, nectaries are located at the top of the ovary, around the style (Bensel & Palser, 1975c; Bernardello et al., 2000). The same nectary type seems to be valid for **Eremosynaceae** and **Tribelaceae**, which are closely related to Escalloniaceae. Anatomical investigations did not show nectaries in **Paracryphiaceae** (Dickinson & Baas, 1977).

Aquifoliales. In **Aquifoliaceae**, nectar is supposed to be produced by papillose swellings in the petals, either the base or the middle part (Loesener, 1942), but no anatomical or experimental studies were done to confirm this. In **Cardiopteridaceae**, the so-called disc is mostly absent (Cronquist, 1981), but sometimes it may be present, either flat or cushion-like (Kårehed, 2001), and if nectariferous it may represent a receptacular nectary. In **Stemonuraceae**,

the presence of a unilateral scale is frequent in the position of the so-called disc (Kårehed, 2001); it may be a receptacular nectary, as could be the case in **Phyllonomaceae** that has a disc as well (Kårehed, 2001).

Apiales. Ovarian nectaries are dominant in this order. In **Apiaceae** (Brown, 1938; Cronquist, 1981; Smets, 1986) and **Araliaceae** (Cronquist, 1981; Erbar & Leins, 1988; Vezza et al., 2006), families with inferior ovaries, the ovary roof (called stylopodium) is a gynoecial nectary originating from the dorsal base of the carpel primordia (Erbar & Leins, 1995), which is delimited from the styles by a groove at their base. In the two families with superior ovaries, the situation is different: **Pittosporaceae** possesses a gynoecial nectary with a similar origin to that in Apiaceae and Araliaceae, but located at the base of the ovary in their external wall (Fahn, 1979; Erbar & Leins, 1995), whereas **Pennantiaceae** has neither stylopodia nor nectaries (Kårehed, 2003). Stylopodia are present in **Aralidiaceae**, **Mackinlayaceae**, and **Myodocarpaceae** (Kårehed, 2003) and they seem to be nectariferous. The remaining families—**Griseliniaceae**, **Melanophyllaceae,** and **Torricelliaceae**—have no stylopodia (Kårehed, 2003) and probably no nectaries, although Griseliniaceae has a so-called disc that might be nectariferous.

Asterales. Ovarian nectaries are the rule in this group. In **Alseuosmiaceae**, a gynoecial nectary is found as a ring on top of the inferior to semi-inferior ovary, although it may be absent (Cronquist, 1981; Lundberg & Bremer, 2003), as happens in **Argophyllaceae** (Lundberg & Bremer, 2003); on the other hand, these nectaries seem to be missing in **Phellinaceae**, but present in **Rousseaceae** (Lundberg & Bremer, 2003). In **Asteraceae**, nectaries are ovarian and epigynous on top of the inferior ovary surrounding the style base (see "Asteraceae" on page 46 for a detailed discussion; Brown, 1938; Frey-Wyssling, 1955; Galetto, 1995c; Torres, 1998; Vogel, 1998c; Mani & Saravanan, 1999; Bernardello et al., 2000; Sancho & Otegui, 2000; Ma et al., 2002; Visintín & Bernardello, 2005; Wist & Davis, 2006). In **Calyceraceae,** nectaries are formed by stamens and petals: in five areas alternating with the stamens, the nectariferous tissue extends from the base of the filament tube to the top of the stamen–corolla tube (Erbar, 1993). In **Campanulaceae** (including Lobeliaceae), *Lobelia* has complex nectar spurs involving both perianth whorls and associated portions of the androecium and gynoecium (Koopman & Ayers, 2005). Most genera have a voluminous ovarian nectary, forming a ring around the free part of the semi-inferior ovary and, occasionally, extending towards the hypanthium (Fahn, 1979; Cronquist, 1981; Smets, 1986; Erbar & Leins, 1989; Galetto et al., 1993; Vogel, 1998c; Anderson et al., 2000a). In **Goodeniaceae**, there are one or two nectaries on the top of the ovary, with bilateral symmetry (Cronquist, 1981; Leins & Erbar, 1989).

In **Menyanthaceae**, which has superior ovaries, an annular ovarian nectary surrounding the base is present (Cronquist, 1981; Erbar, 1997). In **Pentaphragmataceae**, nectaries are distinctive: unlike most families with inferior or semi-inferior ovaries in the order, which normally have epigynous glands, there are five near-basal nectaries on the ovary wall (in its free part); nectar is accessible by five tubular receptacular channels located between the stamens that have protecting trichomes at their entrances (Vogel, 1998c). In **Stylidiaceae** (including Donatiaceae), two epigynous nectary glands (usually unequal in size) are located at the base of the column (Cronquist, 1981; Erbar, 1992), although they may be absent in some taxa (Laurent et al., 1998).

Dipsacales. Nectaries mostly consist of unicellular trichomes and an underlying nectariferous tissue associated with the corolla tube (Wagenitz & Laing, 1984). In **Caprifoliaceae** *s.l.* (including Diervillaceae, Dipsacaceae, Linnaeaceae, Morinaceae, and Valerianaceae), nectaries are frequently represented by one flat cushion-like nectary in an abaxial position in the corolla tube, which may be saccate or spurred; there can also be five glands or a continuous ring (Brown, 1938; Fahn & Rachmilevitz, 1970; Weberling, 1977; Wagenitz & Laing, 1984; Davis, 2003). On the other hand, **Adoxaceae** is variable: there may be (i) nectaries in groups at the base of each corolla lobe, with multicellular trichomes not accompanied by an underlying nectariferous tissue (*Adoxa*), (ii) a gynoecial nectary on top of the inferior ovary (*Viburnum*), or (iii) no nectaries (*Sambucus*) (Wagenitz & Laing, 1984; Erbar, 1994). Based on these differences, Wagenitz and Laing (1984) proposed that these genera constitute a monophyletic group, a suggestion that was confirmed with molecular data (Bell et al., 2001); the basal nectary type of the order is, however, hard to determine. For *Sambucus*, there are reports of sterile flowers transformed into nectaries and stigmatic exudate as nectar (Vogel, 1997).

3.9 Evolutionary trends

The evolutionary history of nectaries and their potential for elucidating plant phylogenies is still an unexplored field of plant research (Vogel, 1997). In the previous section, the amazing diversity of nectary structures and distributions became evident, a circumstance that suggests that they have had multiple independent origins, as Brown (1938) pointed out (p. 549): "Nectaries appear to have arisen independently in different lines of development and then to have undergone modifications characteristic of various groups". On the other hand, nectaries are easy to lose and reacquire within lineages, and thus it is sometimes difficult to determine the precise evolutionary sequence. Last but not least, determining nectary homologies is essential to gain a clear

knowledge of their evolution, a principle that always has to be borne in mind (Smets et al., 2000).

In Fig. 7, the main nectary types in each clade recognized by APG II (2003) are mapped to follow their fate, summarizing the information given in the previous section, where the appropriate references are cited.

In the early-branching lineages, nectaries are rare, which would indicate that this condition is plesiomorphic. Amborellaceae, the sister group for the flowering plants, has no nectaries, as also happens in Austrobaileyales and Chloranthaceae, and Nymphaeaceae has only simple petal nectaries. In Magnoliids, nectaries are present mainly as sepal (Piperales), petal (Magnoliales), or staminal (Magnoliales, Laurales) nectaries. Stigmatic exudates are also frequent in several families from these lineages—Annonaceae, Austrobaileyaceae, Chloranthaceae, Magnoliaceae, Monimiaceae, Winteraceae—thus forming an alternative reward for the group; nevertheless, their biological significance has to be examined to be sure of their role in the reproduction of the taxa involved.

In monocots as a whole, septal nectaries are characteristic since they are widely distributed and are exclusive to this lineage, although some species-rich groups (such as Liliales and Orchidaceae) lack them entirely. Acorales, the sister group of all monocots, has no nectaries, which would again be the plesiomorphic condition. In Alismatales, the basal Araceae also has no nectaries, as happens in other families as well, but septal nectaries are frequent in many others. In Asparagales (except for Orchidaceae and Hypoxidaceae) and Dioscoreales septal nectaries are widespread, but they are completely absent in Liliales (where tepal nectaries are the rule). Most families of Pandanales are nectarless; only Velloziaceae is reported to have the typical septal nectaries. In Commelinids, septal nectaries are present in Dasypogonaceae, in some Arecales, only in Bromeliaceae within Poales, in most Commelinales, and in the majority of Zingiberales. Other nectary types also found in several lineages are tepal and staminal, but at a lower frequency.

Ceratophyllales, the sister group of the Eudicots and Core Eudicots, has no nectaries, again probably a plesiomorphy. In Eudicots, petal nectaries are widespread, being characteristic of the basal Ranunculales. In addition, intrastaminal receptacular, staminal, and gynoecial (not ovarian) nectaries are less frequent. In Core Eudicots, intrastaminal receptacular nectaries (continuous or fragmented) are common, probably representing the basal condition, followed by sepal, petal, and gynoecial nectaries. In Rosids, both intra- and extrastaminal nectaries are widespread, after ovarian, hypanthial,

and staminal nectaries. Finally, in Asterids, there is a clear trend towards ovarian nectaries; nectaries located in sepals or petals are rare (occurring mainly in the derived Dipsacales).

Looking at the whole sequence, the general trend originally proposed by Brown (1938), and supported by Norris (1941) and Fahn (1953), i.e., an evolutionary acrocentripetal movement of the nectary position in angiosperms (from perianth to gynoecium), may apply. However, as not all nectaries are homologous, the shift in nectary position among the different groups is not always comparable (Smets et al., 2000). Trends must preferably be drawn within each particular lineage and among comparable structures. Unfortunately, in many taxa data are still scarce (in terms of species studied and the variability within and among groups) and developmental analyses are also insufficient, but these are needed to determine the homology of nectaries with confidence.

Recently, our understanding of the control of nectary development at the genetic and molecular level has been providing significant insights into its evolution (e.g., Bowman & Smyth, 1999; Baum et al., 2001; Lee et al., 2005a, b). The observed diversity of nectary structures and distributions within flowering plants and the certainty of their multiple independent origins do not preclude nectaries from sharing developmental genetic machinery (Lee et al., 2005). The gene *CRABS CLAW* (CRC) is one of the key genes for nectary development in *Arabidopsis* (Bowman & Smyth, 1999; Baum et al., 2001; Lee et al., 2005a, b). Lee et al. (2005b) analysed the expression of this gene in several eudicots. Their interesting results indicate that CRC expression is conserved in nectaries from several core eudicot species and that it is required for nectary development in both rosids and asterids, regardless of nectary position and morphology. On the other hand, in a basal eudicot species (*Aquilegia*, Ranunculaceae), no evidence of CRC expression in its nectaries was found. The ancestral function of the CRC gene lies in the regulation of carpel development, a role probably conserved throughout angiosperms (Fourquin et al., 2005; Lee et al., 2005b). These expression analyses suggest that the role of CRC as regulator of nectary development is restricted to a clade within eudicots. In addition, the recruitment of CRC as a nectary regulator might have played a role in localizing floral nectaries near stamens and carpels in core eudicots (Lee et al., 2005b). More data on other key taxa are definitely needed to lend further support to this motivating hypothesis, which helps to explain the acrocentripetal evolutionary trend proposed earlier. Furthermore, identification of additional nectary regulators is required to understand the conservation and

divergence of nectaries within angiosperms (Lee et al., 2005b; Thornburg 2007, Chapter 6 in this volume).

At the end of this chapter, it may be apparent that the world of floral nectaries is certainly fascinating, a world intimately connected to plant–animal interactions and to plant reproductive biology. Also obvious may be the inevitable association of nectaries with plant anatomy, morphology, development, and systematics. All these fields have to be linked so that we may understand the biology and evolution of nectaries, paradoxical structures both simple and complex at the same time. Although a great deal has been done in the last century, much more needs to be added. May the present survey help to increase the attention devoted to nectaries and bury definitely Brown's (1938, p. 550) first sentence in his fundamental work:

> Nectaries have aroused very little interest among botanists and their study has been largely neglected.

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APPENDIX

Examples of nectary positions in angiosperm families (in alphabetical order), based on the information presented in the text. Not all that information is repeated here and only one or two citations are included for each example.

Organ	Family	Selected references
Inflorescence Peduncles, pedicels or rachis	Asparagaceae	Tanowitz & Koehler, 1986
	Bignoniaceae	Elias & Prance, 1978
	Bromeliaceae	Koptur, 1992
	Caprifoliaceae	Vogel, 1997
	Cucurbitaceae	Elias, 1983
	Euphorbiaceae	Vansell, 1940
	Fabaceae	McKey, 1989
	Marcgraviaceae	Oliveira & Oliveira, 1991
	Orchidaceae	Fischer & Zimmerman, 1988; Almeida & Figueiredo, 2003
	Vochysiaceae	Oliveira et al., 1987
Abscission of flower or bract pedicels	Fabaceae	McKey, 1989
	Lamiaceae	Vogel, 1998c
	Pedaliaceae	Monod, 1986
Bracts, bractoles, or involucra	Asteraceae	Durkee, 1987; O'Dowd & Catchpole, 1983
	Acanthaceae	Durkee, 1987
	Bignoniaceae	Elias & Prance, 1978
	Costaceae	Schemske, 1980
	Crassulaceae	Baker et al., 1978
	Euphorbiaceae	Davies, 2001; Hoppe, 1985
	Fabaceae	McKey, 1989
	Liliaceae	Keeler, 1978; Tanowitz & Koehler, 1986
	Marantaceae	Horvitz & Schemske, 1984
	Paeoniaceae	Elias, 1983; Zimmermann, 1932
	Piperaceae	Vogel, 1998a
	Poaceae	Nicora, 1941; Zuloaga & Sendulsky, 1988
Flower Interstaminal	Aextoxicaceae	Cronquist, 1981
	Salvadoraceae	Cronquist, 1981
Intrastaminal	Aizoaceae	Chesselet et al., 2002
	Amaranthaceae	Zandonella, 1977
	Anacardiaceae	Gallant et al., 1998; Wannan & Quinn, 1991
	Aphloiaceae	Matthews & Endress, 2005a
	Apocynaceae	Galetto, 1997; Venter & Verhoeven, 2001
	Basellaceae	Zandonella, 1977
	Bixaceae	Cronquist, 1981

Organ	Family	Selected references
Intrastaminal (continued)	Bonnetiaceae	Dickinson & Weitzman, 1998
	Brunelliaceae	Matthews & Endress, 2002
	Burseraceae	Sunnichan et al., 2005
	Celastraceae	Matthews & Endress, 2005b
	Cephalotaceae	Matthews & Endress, 2002; Vogel, 1998a
	Cistaceae	Manetas & Petropoulous, 2000
	Crossosomataceae	Matthews & Endress, 2005a
	Cunoniaceae	Matthews & Endress, 2002
	Cyrillaceae	Cronquist, 1981
	Didieraceae	Kubitzki, 1993b
	Elaeocarpaceae	Matthews & Endress, 2002
	Emblingiaceae	Cronquist, 1981
	Ericaceae	Anderson et al., 2000b; Freitas et al., 2006
	Euphorbiaceae	Cronquist, 1981; Webster, 1994
	Fabaceae	Herendeen et al., 2003; Waddle & Lersten, 1973
	Geissolomataceae	Matthews & Endress, 2005a
	Gesneriaceae	SanMartin-Gajardo & Sazima, 2004, 2005
	Grossulariaceae	Cronquist, 1981
	Humiriaceae	Link, 1992a
	Irvingiaceae	Link, 1992b
	Ixerbaceae	Matthews & Endress, 2005a
	Ixonanthaceae	Link, 1992c, d
	Kirkiaceae	Cronquist, 1981
	Lecythidaceae	Frame & Durou, 2001
	Lepidobotryaceae	Matthews & Endress, 2005b
	Meliaceae	Lal, 1994; Moscheta et al., 2002
	Molluginaceae	Zandonella, 1977
	Muntingiaceae	Bawa & Webb, 1983
	Nitrariaceae	Decraene & Smets, 1991c
	Nyctaginaceae	Vanvinckenroye et al., 1993
	Olacaceae	Cronquist, 1981
	Opiliaceae	Cronquist, 1981
	Phyllanthaceae	Webster, 1994
	Phytolaccaceae	Bernardello et al., 1993
	Polemoniaceae	Wilken, 2004
	Polygalaceae	Westerkamp & Weber, 1999
	Polygonaceae	Decraene & Smets, 1991a; De Melo et al., 2003
	Portulacaceae	Vanvinckenroye & Smets, 1999
	Rhizophoraceae	Juncosa & Tomlinson, 1987
	Rutaceae	Caris et al., 2006; Souza et al., 2002
	Santalaceae	Aronne et al., 1993; Bhaskar, 1992
	Saxifragaceae	Bernardello et al., 2000
	Simaroubaceae	Bory & Clair-Maczulajtys, 1982
	Sphaerosepalaceae	Horn, 2004
	Staphyleaceae	Matthews & Endress, 2005a
	Strasburgeriaceae	Matthews & Endress, 2005a
	Tamaricaceae	Fahn, 1979; Cronquist, 1981
	Tapisciaceae	Dickinson, 1986

Organ	Family	Selected references
Intrastaminal (continued)	Thymelaeaceae	Cornara et al., 2005; Bandera & Traveset, 2006
	Vitaceae	Gerrath & Posluszny, 1994; Zhang et al., 1999
Extrastaminal	Achariaceae	Bernhard, 1999
	Berberidopsidaceae	Decraene, 2004
	Biebersteiniaceae	Link, 1994
	Brassicaceae	Decraene et al., 2002
	Caryocaraceae	Dickinson, 1990
	Ctenolophonaceae	Link, 1992a
	Euphorbiaceae	Cronquist, 1981; Webster, 1994
	Geraniaceae	Link, 1994b; Vogel, 1998c
	Melianthaceae	Decraene & Smets, 1999a; Decraene et al., 2001
	Passifloraceae	Bernhard, 1999b; Gonzalez, 2001
	Pentadiplandraceae	Decraene, 2002
	Phyllanthaceae	Webster, 1994
	Resedaceae	Cronquist, 1981; Kubitzki, 2003
	Sapindaceae	Decraene et al., 2000
	Sarcolaenaceae	Bayer, 2003; Cronquist, 1981
	Tovariaceae	Decraene, 2002
	Vivianiaceae	Weigend, 2005
Hypanthium	Akaniaceae	Decraene et al., 2002
	Cactaceae	Barthlott & Hunt, 1993
	Chrysobalanaceae	Arista et al., 1997
	Combretaceae	Bernardello et al., 1994
	Cucurbitaceae	Nepi et al., 1996; Vogel, 1990
	Elaeagnaceae	Decraene & Miller, 2004
	Heteropyxidaceae	Schmid, 1980
	Lythraceae	Graham et al., 2005
	Moringaceae	Decraene et al., 1998
	Myrtaceae	Davis, 1997; O'Brien et al., 1996
	Onagraceae	Cronquist, 1981; Eyde, 1981
	Psiloxylaceae	Schmid, 1980
	Rhamnaceae	Medan & Aagesen, 1995
	Rosaceae	Evans & Dickinson, 2005
Tepal	Alstroemeriaceae	Daumann, 1970
	Amaryllidaceae	Endress, 1995
	Campynemataceae	Rudall & Eastman, 2002
	Calycanthaceae	Vogel, 1998a
	Colchicaceae	Nordenstam, 1998
	Corsiaceae	Rudall & Eastman, 2002
	Iridaceae	Rudall et al., 2003a
	Liliaceae	Rudall et al., 2000
	Luzuriagaceae	Conran & Clifford, 1998a
	Melanthiaceae	Tamura, 1998

Organ	Family	Selected references
Tepal (continued)	Orchidaceae	Dressler, 1990
	Philesiaceae	Conran & Clifford, 1998b
	Rhipogonaceae	Conran, 1998
	Smilacaceae	Conran, 1998
	Triuridaceae	Maas-van der Kamer & Weustenfeld, 1998
Sepal	Aristolochiaceae	Vogel, 1998a; Sakai, 2002
	Campanulaceae	Vogel, 1998c
	Gentianaceae	Vogel, 1998a
	Malvaceae	Vogel, 2000; Leitao et al., 2005
	Nepenthaceae	Kato, 1993
	Paeoniaceae	Sánchez-Lafuente, 2002
	Tropaeolaceae	Decraene & Smets, 2001
Petal	Adoxaceae	Erbar, 1994
	Annonaceae	Endress, 1990; Kessler, 1988
	Balsaminaceae	Smets, 1986; Travers et al., 2003
	Berberidaceae	Bernardello et al., 2000
	Caprifoliaceae	Wagenitz & Laing, 1984
	Dirachmaceae	Decraene & Miller, 2004
	Droseraceae	Kerner von Marilaun & Oliver, 1895
	Gentianaceae	von Hagen & Kadereit, 2003
	Lardizabalaceae	Cronquist, 1981
	Lentibulariaceae	Narayana & Satyavathi, 1988
	Magnoliaceae	Huang et al., 1999; Thien, 1974
	Malvaceae	Vogel, 2000; Leitao et al., 2005
	Melastomataceae	Stein & Tobe, 1989; Vogel, 1997
	Nymphaeaceae	Schneider et al., 2003
	Papaveraceae	Lidén, 1993; Olesen, 1996
	Ranunculaceae	Erbar et al., 1999; Hodges, 1997
Stamen	Alismataceae	Smets et al., 2000
	Alliaceae	Meerow, 1987
	Annonaceae	Endress, 1990
	Araceae	Vogel, 1998d
	Atherospermataceae	Cronquist, 1981
	Calyceraceae	Erbar, 1993
	Caryophyllaceae	Zandonella, 1977
	Connaraceae	Matthews & Endress, 2002
	Fabaceae	Vogel, 1997
	Gomortegaceae	Cronquist, 1981; Kubitzki, 1993
	Hernandiaceae	Endress & Lorence, 2004
	Iridaceae	Rudall et al., 2003
	Koeberliniaceae	Mehta & Moseley, 1981
	Lauraceae	Cronquist, 1981; Rohwer, 1993

Organ	Family	Selected references
Stamen (continued)	Limnanthaceae	Link, 1992d
	Linaceae	Brown, 1938; Cronquist, 1981
	Melastomataceae	Stein & Tobe, 1989; Vogel, 1997
	Monimiaceae	Cronquist, 1981
	Onagraceae	Eyde, 1982
	Oxalidaceae	Matthews & Endress, 2002
	Plumbaginaceae	De Laet et al., 1995; Galetto, 1993
	Schisandraceae	Endress, 1990; Thien et al., 1983
	Violaceae	Vogel, 1998b
Staminode	Berberidaceae	Brett & Posluszny, 1982
	Calycanthaceae	Gottsberger, 1991
	Clusiaceae	Decraene & Smets, 1991b
	Hamamelidaceae	Mione & Bogle, 1990
	Hanguanaceae	Rudall et al., 1999
	Hydrocharitaceae	Scribailo & Posluszny, 1984
	Loasaceae	Hufford, 1990
	Parnassiaceae	Matthews & Endress, 2005b
	Saxifragaceae	Sandvik & Totland, 2003
	Theophrastaceae	Caris & Smets, 2004; Vogel, 1986
Gynoecium	Annonaceae	Endress, 1990
Stigma	Araceae	Vogel, 1983
	Austrobaileyaceae	Endress, 1990
	Magnoliaceae	Allain et al., 1999; Endress, 1990
	Monimiaceae	Endress & Lorence, 1983
	Winteraceae	Lloyd & Wells, 1992
Style	Buxaceae	von Balthazar & Endress, 2002
	Eriocaulaceae	Ramos et al., 2005; Stützel, 1998
	Symplocaceae	Caris et al., 2002
	Trochodendraceae	Endress, 1986
Ring at base of superior ovary	Acanthaceae	Cosa, 1975; Piovano et al., 1995
	Bignoniaceae	Galetto, 1995a; Rivera, 2000
	Boraginaceae	Di Fulvio, 1997; Hofmann, 1999
	Clethraceae	Schneider & Bayer, 2004
	Convolvulaceae	Cronquist, 1981; Said, 1982
	Fouquieriaceae	Cronquist, 1981
	Gentianaceae	von Hagen & Kadereit, 2003
	Hydroleaceae	Di Fulvio, 1997; Erbar et al., 2005
	Lamiaceae	Dafni et al., 1988; Petanidou et al., 2000
	Lythraceae	Graham et al., 2005
	Menyanthaceae	Erbar, 1997
	Oleaceae	Cronquist, 1981; Fahn, 1979
	Pedaliaceae	Wortley et al., 2005
	Phrymaceae	Galetto, 1995b; Hazle & Canne-Hilliker, 2005
	Pittosporaceae	Erbar & Leins, 1995

Organ	Family	Selected references
Ring at base of superior ovary (continued)	Plantaginaceae	Bello et al., 2004
	Primulaceae	Vogel, 1997
	Primulaceae	Vogel, 1997
	Sapotaceae	Pennington, 2004
	Sarraceniaceae	Vogel, 1998a
	Schlegeliaceae	Wortley et al., 2005
	Scrophulariaceae	Gaffal et al., 1998
	Solanaceae	Bernardello, 1987; Hunziker, 2001
	Stachyuraceae	Matthews & Endress, 2005a
	Styracaceae	Saraiva & Monteiro, 1988
	Verbenaceae	Bernardello et al., 2000
On an inferior ovary wall	Pentaphragmataceae	Vogel, 1998c
On top of an inferior ovary	Alseuosmiaceae	Lundberg & Bremer, 2003
	Apiaceae	Erbar & Leins, 1995
	Araliaceae	Erbar & Leins, 1988
	Argophyllaceae	Lundberg & Bremer, 2003
	Asteraceae	Mani & Saravanan, 1999
	Bruniaceae	Quint & Classen-Bockhoff, 2006
	Campanulaceae	Erbar & Leins, 1989
	Cornaceae	Batra, 1999; Eyde, 1988
	Costaceae	Newman & Kirchoff, 1992
	Curtisiaceae	Kubitzki, 2004
	Escalloniaceae	Bensel & Palser, 1975c
	Goodeniaceae	Leins & Erbar, 1989
	Grubbiaceae	Cronquist, 1981
	Hydrangeaceae	Hufford, 2001
	Loasaceae	Moody & Hufford, 2000; Weigend & Gottschling, 2006
	Loranthaceae	Kjuit, 1969; Galetto et al., 1990
	Maesaceae	Vogel, 1997; Caris et al., 2000
	Montiniaceae	Decraene et al., 2000b
	Myrtaceae	O'Brien et al., 1996
	Onagraceae	Eyde, 1981, 1982
	Rousseaceae	Lundberg & Bremer, 2003
	Rubiaceae	Smets, 1986; Galetto, 1998
	Stylidiaceae	Erbar, 1992
	Zingiberaceae	Box & Rudall, 2006
Septal	Alismataceae	Daumann, 1970
	Alliaceae	Rudall et al., 2002
	Aponogetonaceae	Daumann, 1970
	Arecaceae	Stauffer & Endress, 2003
	Asparagaceae	Smets et al., 2000; Rudall, 2002
	Asteliaceae	Kocyan & Endress, 2001
	Blandfordiaceae	Kocyan & Endress, 2001

Organ	Family	Selected references
Septal (continued)	Boryaceae	Kocyan & Endress, 2001
	Bromeliaceae	Böhme, 1988; Sajo et al., 2004
	Burmanniaceae	Maas-van der Kamer, 1998
	Butomaceae	Daumann, 1970
	Cannaceae	Daumann, 1970
	Dasypogonaceae	Rudall, 2002
	Dioscoreaceae	Daumann, 1970
	Doryanthaceae	Kocyan & Endress, 2001
	Haemodoraceae	Simpson, 1993, 1998
	Hanguanaceae	Rudall et al., 1999
	Heliconiaceae	Kirchoff, 1992
	Hemerocallidaceae	Rudall, 2002
	Iridaceae	Rudall et al., 2003a
	Ixioliriaceae	Daumann, 1970
	Lanariaceae	Rudall, 1998
	Limnocharitaceae	Daumann, 1970
	Lowiaceae	Wen & Liao, 1999
	Marantaceae	Daumann, 1970; Rao, 1975
	Musaceae	Kirchoff, 1992
	Nartheciaceae	Sterling, 1979; Rudall, 2002
	Petrosaviaceae	Rudall, 2002
	Pontederiaceae	Strange et al., 2004
	Strelitziaceae	Kroenstedt & Walles, 1986
	Tecophilaeaceae	Simpson & Rudall, 1998
	Toefieldiaceae	Smets et al., 2000
	Velloziaceae	Menezes, 1973
	Xanthorrhoeaceae	Rudall, 2002; Nepi et al., 2006
	Xeronemataceae	Chase et al., 2000
Pistillode	Buxaceae	Vogel, 1998c; von Balthazar & Endress, 2002
	Caricaceae	Decraene & Smets, 1999
	Eriocaulaceae	Ramos et al., 2005

Chapter 3

NECTARY STRUCTURE AND ULTRASTRUCTURE

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1 INTRODUCTION

It is easy to define nectaries from a functional point of view: they are plant-secreting structures that produce nectar, but it is difficult to provide a general definition. From the anatomical point of view nectaries vary widely in ontogeny, morphology, and structure (Fahn, 1979a, 1988; Durkee, 1983; Smets et al., 2000), both between species and within species, depending on flower sexual expression or flower morph in heterostylous and heteroantheric species (Nepi at al., 1996; Küchmeister et al., 1997; Fahn & Shimony, 2001; Pacini et al., 2003). Intraspecific morphological differences exist between flowers of the same plant and between plants of the same species with different ploidy (Davis et al., 1996), and morphological characters may be affected by environmental conditions such as water availability. Petanidou et al. (2000) found that nectary structure in Lamiaceae species in a Mediterranean shrub community was largely shaped by phylogenetic and climate constraints. In the course of the flowering season (spring–summer) stomatal opening and nectary size decreased, thus minimizing nectar flow at a time when water was scarce. They hypothesized that very concentrated nectar was secreted via large modified stomata, whereas cuticular secretion was mainly encountered in species with very thin nectars. Petanidou (2007) speculates that the frequency of species with stomatal nectar secretion should be much higher in hot and arid climates like the Mediterranean and deserts than in temperate ones.

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Sometimes the morphological characters of nectaries seem to be correlated with the quantity of the nectar secreted but not its quality. For example, nectar volume depends positively on the volume of nectariferous tissue (Petanidou et al., 2000). Davis and Vogel (2005) noted that in the polymorphic flowers of *Linaria genistifolia* (Scrophulariaceae), where nectar flows from the nectary into a spur, multi-spurred flowers had larger nectaries that produced a greater volume of nectar than single-spurred and spurless flowers. The volume of secreted nectar does not seem to be correlated with the number of nectarostomata (Petanidou et al., 2000; Teuber et al., 1980; Davis & Gunning, 1991) because not all stomata secrete nectar (Gaffal et al., 1988); however, the opposite is true in Bignoniaceae (Galetto, 1995).

Regardless of this enormous variability, Schmid (1988) defines the nectary as "a more or less localized, multicellular glandular structure that occurs on vegetative or reproductive organs and that regularly secretes nectar, a sweet solution containing mainly sugars and generally serving as a reward for pollinators or for protectors (e.g., ants) against herbivores, or, in carnivorous plants, as a lure for animal prey".

Although in some cases the nectary may be an organ (e.g., the rudimentary carpellodia of staminate flowers of *Buxus*; see Schmid, 1988 and references therein), it is commonly only part of an organ and Schmid's definition can be applied correctly when the nectary is conspicuous, continuous, and occupies a well-defined area. Problems may arise when there are small discontinuous nectar-secreting structures scattered over a large area. Vogel (1998a) termed such small secreting structures as nectarioles, and examples have been found among floral and extrafloral nectariferous organs of *Peperomia* (Piperaceae), *Cabomba* (Cabombaceae), *Sarracenia* (Sarraceniaceae), *Cephalotus* (Cephalotaceae), *Chimonanthus* (Calycanthaceae), *Aristolochia* (Aristolochiaceae). In such cases it is unclear whether the term nectary refers to the individual nectar-secreting areas or to all of them as a whole. The term nectarium, introduced by Linnaeus (1735) and used also by Davis et al. (1998) for the complex nectary of Brassicaceae, can be used to describe all separated nectaries in a flower, whereas nectary represents the single unit (see also Bernardello, 2007, Chapter 2 in this volume).

Nectaries may be located at surface level in the organ bearing them, form an outgrowth on the organ, or be concealed deep within the organ (e.g., the septal or gynopleural nectaries of monocotyledons). Unlike other floral structures, the relative positions of which are conserved throughout the angiosperms, the nectary is not located in the same position in all plants (Fahn, 1979a). From the ecological point of view, the diversity in nectary location

is due to the diversity of pollinators and their foraging behaviour. Baum et al. (2001) recently discovered the molecular basis for such great variability: the nectary is independent of the ABC floral homeotic genes that are responsible for floral organ identity specification according to their position. Thus the nectary is potentially "free" to move about the flower during evolution in response to selection imposed by interactions with pollinators.

2 NECTARY STRUCTURE AND ULTRASTRUCTURE

According to Fahn (1979a, 1988, 2000), the nectary is made up of a tissue called **nectariferous tissue**, which consists of an **epidermis** usually overlying a **specialized parenchymatous tissue** (Fig. 1). Durkee (1983) used the terms **secretory tissue** or **glandular tissue** as synonymous to Fahn's **parenchymatous tissue,** but also introduced the term **subglandular** or **nonglandular parenchyma** (also known as ground parenchyma) to describe one or more cell layers that separate the secretory tissue from the vascular bundle (Fig. 1). Merging these two slightly different definitions, I propose the following three terms to describe the general anatomy of the nectary:

- **Nectary epidermis**.
- **Nectary parenchyma** to indicate the layer(s) of small cells with densely staining cytoplasm generally present beneath the epidermis, corresponding to Fahn's specialized parenchymatous tissue.
- **Subnectary parenchyma**, made up generally by larger cells, more loosely packed than those of the nectary parenchyma, corresponding to Durkee's subglandular parenchyma (Fig. 1).

Vascular bundles may be found in the nectary or subnectary parenchyma. With the proposed definitions it is clear that the epidermis and the nectary parenchyma are the tissues involved in nectar production and secretion—thus constituting the functional unit—while the subnectary parenchyma is not directly involved in nectar production, but may have some functions related to nectar production.

All components of the anatomical structure described above are not always recognizable. This is why Zimmermann (1932) distinguished structural (i.e., nectaries with a defined structure) and non-structural nectaries (i.e., without any special differentiated nectariferous structure). Examples of non-structural nectaries are more frequent among extrafloral nectaries (Fahn, 1979a and references therein) than among floral ones (Bernardello, 2007).

Fahn (1979a)	Durkee (1983)	Nepi (this chapter)	
epidermis	epidermis	epidermis	directly involved in nectar production and secretion
parenchymatous tissue with vascular bundles	secretory tissue	nectary parenchyma	
	sub-glandular parenchyma with vascular bundles	sub-nectary parenchyma with vascular bundles	not directly involved in nectar production

Figure 1. Anatomical organization of the nectary according to Fahn (1979a) and Durkee (1983), and that proposed in the present chapter.

On the other hand, in some species the anatomical structure of the nectary can be recognized, but the nectary does not produce nectar. This is the case of the so-called vestigial nectaries found in some Bignoniaceae (*Catalpa, Clytostoma*, *Cydista*, *Phryganocydia*) (Rivera, 2000 and references therein). The lack of a functional nectary has been associated with pollination by deception in Bignoniaceae (Gentry, 1980).

Before discussing the structure of the different components of the nectary, it is necessary to give some definitions and to clarify differences between the terms nectar production, nectar secretion, and nectar release. Nectar production is sometimes considered synonymous with nectar secretion. In my opinion the two terms are different. Nectar production is a phenomenon related to the nectary as a whole. It comprises different events (sugar unloading from the vascular bundle, transport of molecules into the nectary tissue, transformation of molecules, nectar release from the nectary) leading to nectar release (or exudation) from the nectary. Nectar secretion refers to the release of nectar from the protoplasm of the nectary parenchyma cells, thus it describes a phenomenon at the cellular level.

2.1 Epidermis

Epidermal cells are generally smaller than parenchyma cells; they are polyhedric and may have an anticlinal orientation. The vacuole is generally

bigger than in parenchyma cells. Plastids of epidermal cells do not usually store starch (Razem & Davis, 1999), except when a very high rate of nectar secretion is required, as in *Passiflora* sp. (Durkee et al., 1981), *Rosmarinus officinalis* (Zer & Fahn, 1992), and *Cucurbita pepo* (Nepi et al., 1996).

When nectar secretion does not occur through stomata, the epidermis itself is involved in the secretion process via epidermal secreting cells or secreting trichomes. Though this has not yet been demonstrated, the two manners of secretion are not mutually exclusive and may take place contemporaneously (Nepi et al., 2001). In certain plants, the outer walls of the epidermal cells involved in nectar secretion have wall ingrowths (Schnepf & Pross, 1976; Fahn, 1979a; Fahn & Benouaiche, 1979 and references therein; Davis et al., 1988) and are regarded as transfer cells (Pate & Gunning, 1972). Wall protuberances are thought to aid eccrine secretion of individual molecules and are seldom found in systems where secretion by vesicles (granulocrine secretion) has been suggested (Kronestedt-Robards & Robards, 1991).

The anatomical differences in the structure of nectary epidermis concern: cuticle structure and patterning, the presence/absence and structure of secreting trichomes, the presence/absence of stomata (Table 1).

A continuous cuticle is generally present on the surface of the nectary epidermis, although it may be thinner than on the areas adjacent to the nectary (Gaffal et al., 1998 and references therein) or discontinuous as in septal nectaries. A cuticular lining of intercellular spaces in the more peripheral nectary parenchyma and the substomatal chamber has been reported in a number of species (Rachmilevitz & Fahn, 1973; Davis et al., 1988; Maldonado & Otegui, 1997; Razem & Davis, 1999 and references therein) and may enhance nectar movement once in the apoplast.

The patterning, thickness, and permeability of the nectary cuticle vary widely. In the case of nectary trichomes, the cuticle covering the secreting cell seems to be completely impermeable and the nectar accumulates in a subcuticular space formed by separation of the cuticle from the epidermis (Fig. 2). As secretion proceeds, the cuticle stretches and becomes very thin. It has not been determined whether the nectar is released when the cuticle breaks or whether thin areas of the stretched cuticle become permeable to nectar.

Table 1. Main structural features of selected nectaries, both floral and extrafloral. Starch is indicated in arbitrary units according to its abundance in the nectary parenchyma. – = starch absent; + = few starch grains; ++ = many starch grains; +++ = very abundant starch grains.

Species	Nectary type	Anatomy	Starch	Epidermis and parenchyma plastids	Vascular-ization	Nectar secretion	Nectar release	References
Dicotyledons								
Aptenia cordifolia (Aizoaceae)	F	e+p	+	am	nv	e	cp	Meyberg & Kristen, 1981
Arabidopsis thaliana (Brassicaceae)	F	e+p	+	ch	ph		s	Baum et al., 2001
Brassica napus (Brassicaceae)	F	e+p	+	ch	ph	e	s	Davis et al., 1986
Capparis retusa (Brassicaceae)	F	e+p			ph, xy			Di Sapio et al., 2001
Chamelaucium uncinatum (Myrtaceae)	F	e+p				g	s	O'Brien, 1996
Chamelaucium uncinatum	EF	e+p	+	ch		g	cp, s	O'Brien, 1996
Cucurbita pepo (Cucurbitaceae)	F	e+p	+++	am	ph	e	s	Nepi et al., 1996
Digitalis purpurea (Scrophulariaceae)	F	e+p	++	am	ph		s	Gaffal et al., 1998
Ecballium elaterium (Cucurbitaceae)	F	e+p	++	ch	ph, xy		s	Fahn & Shimony, 2001
Eccremocarpus scaber (Bignoniaceae)	F	e+p	+++	am	ph		s	Belmonte et al., 1994
Echinacea purpurea (Asteraceae)	F	e+p	–	p-ob	ph	e	s	Wist & Davis, 2006

Species	Nectary type	Anatomy	Starch	Epidermis and parenchyma plastids	Vascular-ization	Nectar secretion	Nectar release	References
Euphorbia neriifolia (Euphorbiaceae)	EF	e+p	+	ch	ph, xy	g	bc	Arumugasamy et al., 1990b
Glycine max (Fabaceae)	F	e+p	+	ch	ph	ho		Horner et al., 2003
Hibiscus rosa-sinensis (Malvaceae)	F	t, pl, lin	+	am	ph, xy		bc	Sawidis, 1998
Myosotis sylvatica (Boraginaceae)	F	e+p			ph		s	Weryzsko-Chmielewska, 2003
Passiflora spp. (Passifloraceae)	F	e+p	+++	am	ph, xy	e	s	Durkee et al., 1981
Passiflora spp.	EF	e+p	+	ch	ph	e	bc	Durkee, 1982
Pisum sativum (Fabaceae)	F	e+p	+	ch	ph	e	s	Razem & Davis, 1999
Ricinus communis (Euphorbiaceae)	EF	e+p	+	ch	ph, xy	e	bc	Baker et al., 1978
Rosmarinus officinalis (Lamiaceae)	F	e+p	+++	am	ph	e	s	Zer & Fahn, 1992
Sambucus nigra (Caprifoliaceae)	EF	e+p		ch	ph	g	lc	Fahn, 1987
Sanango racemosum (Buddlejaceae)	F	e+p	+++	am	ph		s	Maldonado & Otegui, 1997
Solanum stramonifolium (Solanaceae)	EF	e+p	+	ch	ph, xy		s	Falcão et al., 2003
Tabebuia serratifolia (Bignoniaceae)	F	e+p	+++	am	ph		s	Vinoth & Yash, 1992
Turnera ulmifolia (Passifloraceae)	F	indefinite	–		nv	ho		Elias et al., 1975

Species	Nectary type	Anatomy	Starch	Epidermis and parenchyma plastids	Vascular-ization	Nectar secretion	Nectar release	References
Turnera ulmifolia	EF	e+p	–		ph, xy		bc	Elias et al., 1975
Vicia faba (Fabaceae)	F	e+p	+	ch	ph	g	s, cm	Davis et al., 1988
Vicia faba	EF	t, pl, cap	+	ch	ph, xy	g	pc	Davis et al., 1988
Monocotyledons								
Gymnadenia conopsea (Orchidaceae)	F	t, pl, lin	–	pl-ob		g	cp	Stpiczyńska & Matusiewicz, 2001
Hexisea imbricata (Orchidaceae)	F	e+p	+	ch	ph, xy	g	cp	Stpiczyńska et al., 2005a
Limodorum abortivum (Orchidaceae)	F	e+p	++	am		g	cd	Figueiredo & Pais, 1992
Maxillaria coccinea (Orchidaceae)	F	e+p	–	ch	ph, xy	e	cp	Stpiczyńska et al., 2003
Musa paradisiaca (Musaceae)	F	s	++	am		g		Fahn & Benouaiche, 1979
Platanthera bifolia (Orchidaceae)	F	t, unicel	+	ch	ph, xy	g	bc	Stpiczyńska, 1997
Platanthera chlorantha (Orchidaceae)	F	t, unicel	+	p-ob		g	cm	Stpiczyńska et al., 2005b

Nectary type: EF = extrafloral nectary; F = floral nectary.
Anatomy: e = epidermis; p = parenchyma; s = septal; t = trichomes; pl = pluricellular; unicel = unicellular; cap =capitate; lin = linear.
Plastids: ch = chloroplasts; am = amyloplasts; pl-ob = plastids with osmiophilic bodies.
Vascularization: nv = no vascularization; ph = phloem; xy = xylem.
Nectar secretion: ho = holocrine; e = eccrine; g = granulocrine.
Nectar release: bc = breaking cuticle; cd = cuticle disruption; cm = cuticle microchannels; cp = cuticle permeation; lc = lysigenous cavity; pc = pores in cuticle; s = stomata.

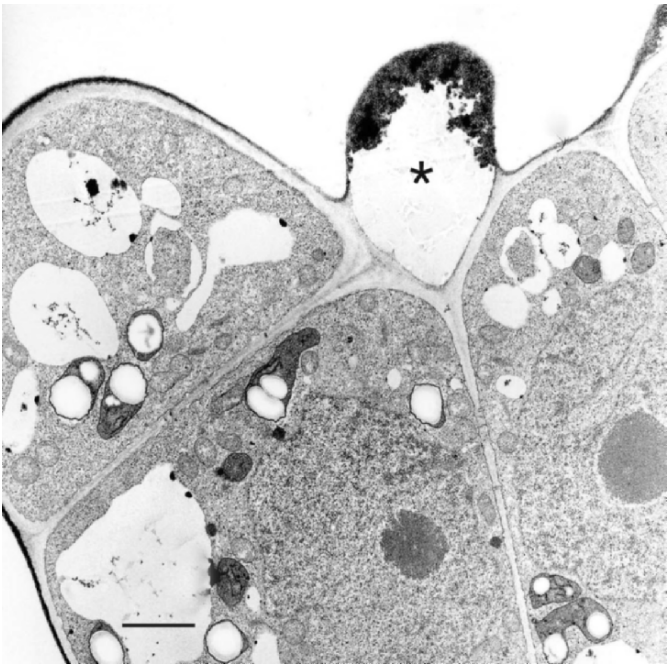

Figure 2. A capitate trichome of the *Cyclanthera pedata* (Cucurbitaceae) nectary at the beginning of nectar secretion. Nectar (*asterisk*) accumulates in a subcuticular space, stretching the cuticle. Bar = 2 μm.

Nepi et al. (1996) hypothesized that the thin cuticle of *Cucurbita pepo* nectaries contains very little wax because it is not stained by auramine O, a specific dye for this substance (see Table 2). Nectar may possibly exude through a permeable cuticle. Cuticle permeability to secretory products has been also postulated in nectaries of the orchid *Maxillaria coccinea* (Stpiczyńska et al., 2003).

In some species of the genus *Euphorbia* the cyathial nectary is covered by a cuticle that is not uniform in thickness, being thinner in the "secretory pits" through which the nectar exudes (Arumugasamy et al., 1990a). Kronestedt et al. (1986) reported pores in the cuticle above the nectar secreting trichomes of *Abutilon* sp. (Malvaceae).

The cuticle may have microchannels from which the nectar exudes (Davis et al., 1988; Stpiczyńska, 2003). In *Platanthera chlorantha* (Orchidaceae), the microchannels appear as fibrillar outgrowths of the outer epidermal cell wall (Fig. 3), as also observed in *Abutilon* sp. (Kronestedt et al., 1986). In *Helleborus foetidus* (Ranunculaceae), microchannels are narrow

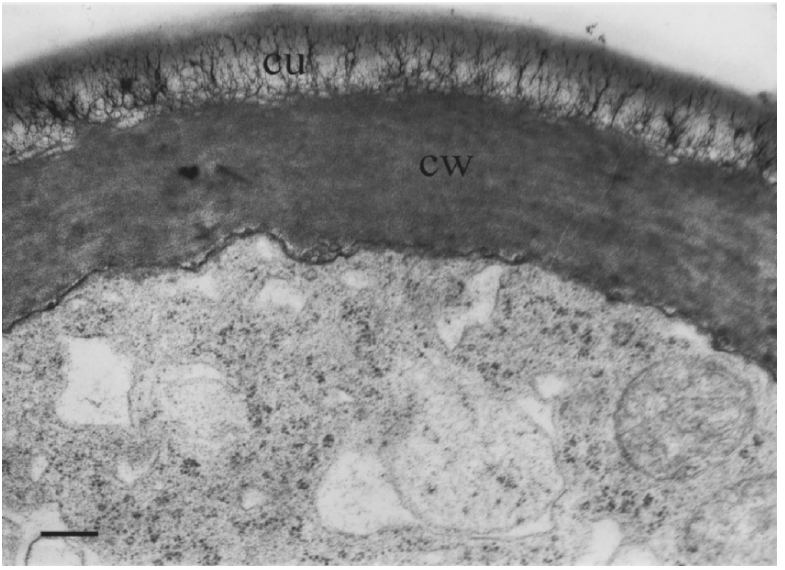

Figure 3. Epidermal cell wall and cuticle of a secretory hair of the *Platanthera chlorantha* (Orchidaceae) floral nectary. Nectar presumably flows out through fibrillar outgrowths of the outer cell wall (microchannels) present in the cuticle. (This picture was kindly provided by Malgorzata Stpiczyńska, Department of Botany, Agricultural University in Lublin, Poland.) Bar = 0.4 μm; cu = cuticle; cw = cell wall.

tubular interruptions of the cuticle in continuity with the cell wall; some of them seem to have direct communication with the outside (Koteyeva, 2005). Very similar microchannels are described in the cuticle of epidermal cells of the *Echinacea purpurea* (Asteraceae) nectary, although they have no direct communication with the outside (Wist & Davis, 2006).

Complex cuticle organization with a lamellar-type outer layer and a reticulate-type inner one has been described in the floral nectary of *Aptenia cordifolia* (Aizoaceae) and *Limodorum abortivum* (Orchidaceae) (Meyberg & Kristen, 1981; Figueiredo & Pais, 1992).

2.1.1 Secretory trichomes

The nectary epidermis may have trichomes as the secretory structures. The morphology of trichomes varies, and includes the following types:

- Unicellular trichomes as in the floral nectaries of *Lonicera* (Caprifoliaceae) (Fahn & Rachmilevitz, 1970)

- Multicellular, linear trichomes as in the floral nectaries of *Abutilon* (Kronestedt-Robards et al. 1986) and *Hibiscus rosa-sinensis* (Malvaceae) (Sawidis et al., 1987a)
- Multicellular, capitate trichomes as in the extrafloral nectaries of *Vicia faba* (Davis et al., 1988) (Table 1)

The detailed ultrastructural development of nectary trichomes has been investigated in *Abutilon* (Kronestedt-Robards et al., 1986) and in *Hibiscus* (Sawidis et al., 1987a). The first event to take place is an outgrowth of epidermal cells followed by periclinal division. Volume increase of epidermal cells is accompanied by cell polarization, manifested by displacement of organelles towards the apical region.

The most specialized cells of pluricellular trichomes are the basal, stalk, and tip cells. The basal cells (situated at the level of the other epidermal cells) have a greater number of plasmodesmata than adjacent cells (Sawidis et al., 1987b). After entering the secreting hairs, pre-nectar flows from cell to cell through plasmodesmata (symplastic route) reaching the tip cell (Sawidis et al., 1987b). The apoplastic route of pre-nectar is impeded by lignification or complete cutinization of the lateral walls of the stalk cells (Fahn, 1979b; Sawidis et al., 1987a; Davis et al., 1988). The tip cells have very elaborate systems of ER, dictyosomes, and vesicles and they are thought to be involved in granulocrine secretion (Fahn, 1979b; Kronestedt-Robards et al., 1986; Sawidis et al., 1987b, 1989).

The floral nectary of *Tropaeolum majus* (Tropaeolaceae) has epidermal hairs, but the main source of nectar is the nectary parenchyma and nectar is exuded through the modified stomata (Rachmilevitz & Fahn, 1975).

2.1.2 Nectary-modified stomata

Nectar exudation through stomata appears to be the most common manner of nectar release (Table 1, Bernardello, 2007). Nectar flow may be so high that the stomatal aperture enlarged (Fig. 4). The nectary stomata may be located on the surface of the nectary or in deep depressions (Fig. 5)

Stomata involved in nectar secretion have been described as “nectarostomata” (Smets & Cresens, 1988). They are considered to be “modified” with respect to leaf stomata because they are not able to finely regulate their aperture (Davis & Gunning, 1992, 1993). In actively secreting nectaries, the stomata are raised slightly above the epidermis, while most stomata of not yet secreting nectaries are open but not raised (Gaffal et al., 1998; Nepi

et al., 1996). After measuring the volume flux of the floral nectar of *Digitalis purpurea* (Scrophulariaceae) through individual stomatal apertures, Gaffal et al. (1998) concluded that only a fraction of the total number of stomata per nectary would be sufficient to release the amount of nectar produced. In *Hedera helix* (Araliaceae; Vezza et al., 2006) and *Echinacea purpurea* (Asteraceae; Wist & Davis, 2006) closed immature stomata were present on the surface of the nectary during the secretion phase.

The stomatal apertures are continuous with intercellular spaces of the nectary parenchyma (Gaffal et al., 1998) and there is evidence to suggest that modified stomata are unable to closely regulate nectar flow through them (Davis & Gunning, 1993; Razem & Davis 1999). For instance, asynchrony in stomatal development (pores wide open a few days before the start of nectar secretion and after secretion has ceased) suggests little coordination between pore opening and nectar release (Davis & Gunning, 1992; Davis, 1997; Razem & Davis, 1999). According to Teuber et al. (1980) and Davis and Gunning (1993), leaf and nectary stomata differ in their response to various stimuli. Nectary stomata remained open under all treatment conditions, suggesting that nectary stomata lack the turgor- and ion-mediated movements generally found in leaf stomata.

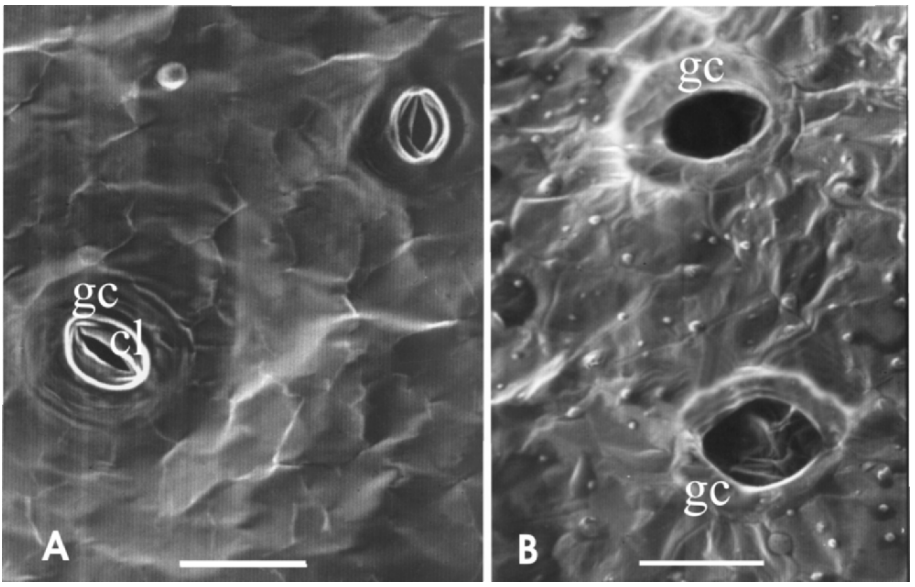

Figure 4. Nectary stomata of *Cucurbita pepo* male flower before (*left*) and after (*right*) nectar secretion. The inner portion of guard cells (gc), where the outer cuticular ledge (cl) is evident before nectar secretion (A), is collapsed at the end of secretion (B). Bar = 50 μm.

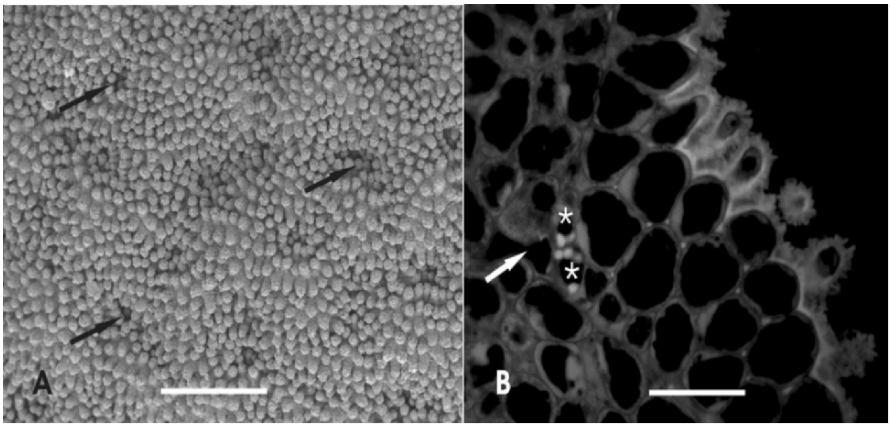

Figure 5. The nectary of *Fatsia japonica* (Araliaceae) A. Electron micrograph (SEM) of the nectary surface shows numerous hollows (*arrows*) that indicate the position of the stomata. Bar = 100 µm. B. Oblique section of the nectary stained with PAS and auramine O and observed by epifluorescence. A thick cuticle with a complex reticulate pattern can be observed. The guard cells of the stoma (*asterisks*), easily recognizable by their small size and starch content, are located at the level of the inner epidermal layer. The arrow indicates the substomatal chamber. Bar = 20 µm.

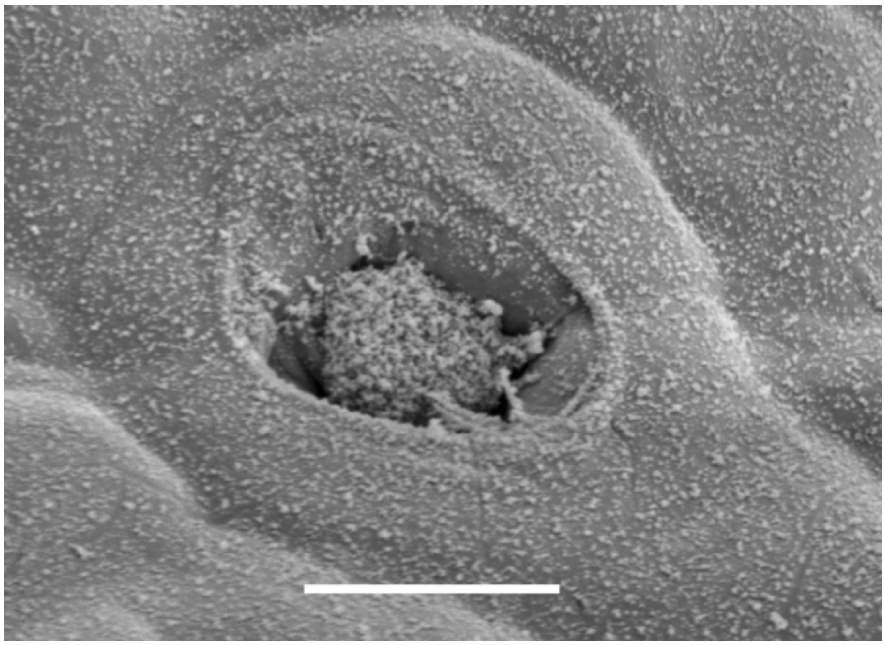

Figure 6. A nectarostoma of *Daphne sericea* (Thymelaeaceae) occluded by granular material. Bar = 10 µm.

Instead of pore closure by guard-cell movements, closure of the modified stomata of the floral nectary may occur exclusively by occlusion in some species (Fig. 6). The occluding material is of uncertain nature (Gaffal et al., 1998). It cannot be excluded that nectar may crystallize in the stomatal aperture. It has been hypothesized that occlusion of the stomatal pores may be a mechanism to seal off potential entry sites for pathogens (Davis, 1997; Razem & Davis, 1999). Micro-organisms have been found in the stomatal apertures (Gaffal et al., 1998) and the nectary has been recognized as the primary site of infection by *Erwinia amylovora*, the agent of fire blight disease in *Malus* and *Pyrus* (Buban et al., 2003).

2.2 Nectary parenchyma

The nectary parenchyma is generally composed of a few to several layers of small, isodiametric cells, generally with thin walls, dense granular cytoplasm, small vacuoles, and relatively large nuclei. Even if there are different types of nectaries and they have a non-uniform structure they always belong to the class of secreting cells. Owing to their secretory activity, all these kind of cells have extra copies of DNA realized by means of multinucleate cells, polyploid nuclei, or polytenic chromosomes (D'Amato, 1984). Nevertheless, even in ultrastructural studies little attention has been paid to the nuclei of nectary secreting cells and multinucleate cells were never observed.

These peculiar cytological characteristics mean that the nectary parenchyma can very often be distinguished easily from the ground parenchyma. Unusually collenchymatous cells with thick walls were observed in the nectary of *Maxillaria coccinea* (Orchidaceae) (Stpiczyńska et al., 2003).

Vacuole size in nectary parenchyma cells varies according to the stage of nectary development: small vacuoles are present in the pre-secretory phase, and may increase in volume at the time of secretion, but generally a sharp increase in vacuole volume takes place after secretion. The cytoplasm is usually rich in ribosomes and mitochondria. These organelles generally increase in number at the moment of secretion, indicating increased energy requirements for nectar production. Intercellular spaces are present and increase at the time of secretion.

It is not uncommon to find cells undergoing cell division in actively secreting nectaries (Gaffal et al., 1998; Nepi et al., 1996). Continued cell division and the lack of subsequent cell extension in small-celled nectariferous tissue are more or less comparable to meristematic tissue (Gaffal et al., 1998 and references therein). This implies that nectary parenchyma cells

maintain the potential of cell regeneration, at least in some species such as *Digitalis purpurea* (Gaffal et al., 1998), *Cucurbita pepo* (Nepi et al., 1996), and *Helleborus* sp. (Vesprini et al., 1999).

The structure and ultrastructure of nectary parenchyma appears to depend mainly on two features: the mechanism of pre-nectar transport (through the apoplast or symplast) and the source of nectar carbohydrates (starch reserves or direct photosynthesis). The term pre-nectar refers to substances transported into nectary tissue to be transformed into nectar by the nectary parenchyma or epidermal cells.

On the basis of numerous plasmodesmata between the cells, Fahn (1979b) proposed the symplast as the main path of pre-nectar transport into the parenchyma cells of *Lonicera japonica*, but evidence is also available for pre-nectar transport via the apoplast (Davis et al., 1988; Peng et al., 2004). The two mechanisms may possibly take place simultaneously (Wergin et al., 1975; Davis et al., 1986; Davis et al., 1988; Stpiczyńska, 1995; Stpiczyńska et al., 2003; Wist & Davis, 2006). Plasmodesmata are generally found between nectary parenchyma and subnectary parenchyma cells; their fine structure in nectaries has been reviewed by Eleftheriou (1990).

Nectar secretion, i.e., the transfer of nectar outside the protoplast of parenchyma cells, may be granulocrine or eccrine. Eccrine secretion involves transport of individual molecules across the secretory cell membrane. In granulocrine secretion molecules are grouped and transported in ER- or dictyosome-derived vesicles that fuse with the plasmalemma and release the molecules outside the nectary cells (Fahn, 1988). When granulocrine secretion occurs, parenchyma cells are rich in ER cisternae, dictyosomes, and vesicles (Rachmilevitz & Fahn, 1973; Fahn, 1987b; Arumugasamy et al., 1990b) and an increase in the number of these organelles indicates imminent nectar secretion. Robards and Stark (1988) demonstrated an extensive "secretory reticulum", i.e., an internal membrane system closely associated with the plasmalemma, within the secretory trichomes in the nectary of *Abutilon*.

On the other hand, when ER cisternae and Golgi vescicles are rare and their number remains almost unchanged during flower development, eccrine secretion is likely (Elias et al., 1975; Eriksson 1977; Nepi et al., 1996; Razem & Davis, 1999; Stpiczyńska et al., 2003).

Different pre-nectar transport mechanisms have been documented in the same family (Eriksson, 1977; Davis et al., 1988; Nepi et al., 1996; Peng et al., 2004), in flowers of the same species (Meyberg & Kristen, 1981) and

are presumably possible in a single nectary (Schnepf & Pross, 1980; Pate et al., 1985).

The source of nectar carbohydrates may be immediate photosynthesis by the nectary itself or by any other part of the plant, or may require temporary starch storage in the parenchyma cells (Pacini et al., 2003). The two modes are strictly related to the rate of secretion: a very high nectar secretion rate requires starch storage in the parenchyma with big amyloplasts differentiating before secretion (Durkee et al., 1981; Belmonte et al., 1994; Nepi et al., 1996; Maldonado & Otegui, 1997), whereas a low rate of nectar secretion is often associated with chloro-amyloplasts with poor thylakoid structure, irregular shape and plastoglobuli (Stpiczyńska, 1997, 2003; Razem & Davis, 1999). Floral nectaries may manifest both modes of carbohydrate supply, whereas in extrafloral ones nectar is always derived from direct photosynthesis.

In plants with a high nectar secretion rate and starch-storing nectary parenchyma, there is a dramatic increase in the number of mitochondria just prior to anthesis, indicating that the comparatively rapid breakdown of stored starch requires more immediate energy than the gradual storage of starch during flower bud development (Durkee et al., 1981).

The source of nectar carbohydrates and the manner of nectar secretion seem correlated: *Passiflora*, *Cucurbita*, and *Rosmarinus* have eccrine secretion and contain a lot of starch; other species with little or no starch at all in the nectary may have granulocrine or eccrine nectar secretion (O'Brien et al., 1996; Nepi et al., 1996) (Table 1).

Although some nectaries are green, presumably due to chlorophyll in their plastids, it seems unlikely that the nectary parenchyma plastids themselves produce the starch grains observed inside them. Nectaries are often concealed and only receive very diffuse light. This may be why the thylakoids and grana are underdeveloped. When nectaries are exposed directly to the light, photosynthesis in the nectary parenchyma cannot be excluded *a priori*. The main photosynthetic activity probably takes place in the subnectary parenchyma where a greater quantity of chlorophyll is located (Fig. 7).

The vacuoles of the nectary parenchyma or subnectary parenchyma cells may contain different types of inclusions. Calcium oxalate crystals in the form of druses or raphides have often been found in floral and extrafloral nectaries (Davis et al., 1988; Horner et al., 2003; Stpiczyńska et al., 2003). It has been demonstrated that Ca^{2+} inhibits plasma membrane ATPase (Leonard

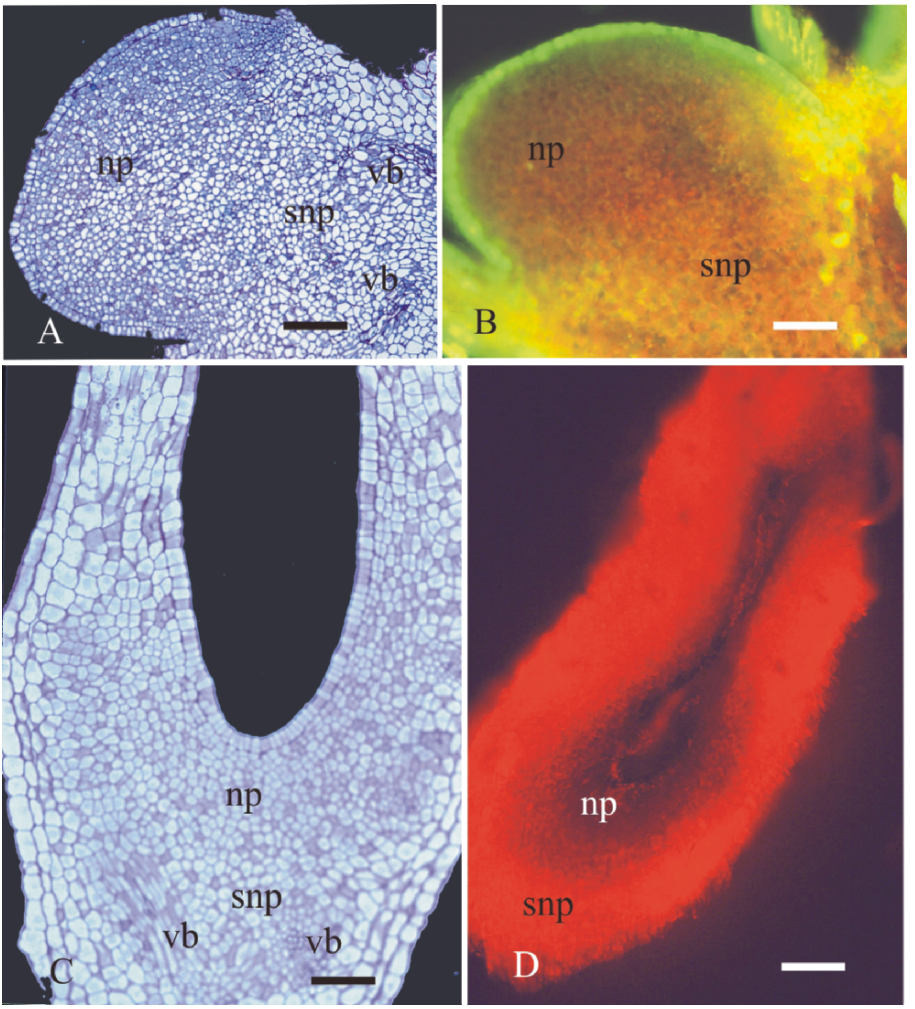

Figure 7. Floral nectary of *Linaria vulgaris* (A, B) and *Helleborus foetidus* (C, D), semithin-sections under bright light (A, C stained with PAS and TBO, see Table 2) and hand sections under UV light (B, D). In both species the main fluorescence of chlorophyll (i.e., the presence of chloroplasts) is located in the subnectary parenchyma where the main branch of vascular bundles is present. np = nectary parenchyma; snp = subnectary parenchyma; vb = vascular bundles. A, B, D bar = 400 µm, C bar = 150 µm.

& Hodges, 1980) and the mechanism of sucrose transport in plants is also known to involve ATPase (Giaquinta, 1979), thus druses and raphides may immobilize calcium in the nectary where active sugar transport is presumably occurring. Another putative function of calcium oxalate crystals in the parenchyma cells of *Glycine max* floral nectaries was descibed by Horner et al. (2003): these crystals sequester calcium during nectary development,

causing the formation of very thin cell walls in the nectary parenchyma. It has, however, also been suggested that they may simply be excretory products and discourage herbivory by invertebrates (Davies, 1999).

Protein bodies, finely granular and irregular in shape, have been found in the vacuoles of *Maxillaria coccinea* (Orchidaceae) (Stpiczyńska et al., 2003) and very similar structures in the floral nectary of *Passiflora* (Durkee et al., 1981; Durkee, 1982). Their role is unclear and requires further investigation. They cannot be precursors of the protein component of nectar because of their presence in secreting cells.

The floral nectary parenchyma and epidermal cells of most plants remain intact throughout secretion (merocrine secretion). In a few cases, secretion of nectar implies cell death (holocrine secretion) as reported for the floral nectaries of *Turnera ulmifolia* (Elias et al., 1975), *Helleborus foetidus* and *H. bocconei* (Vesprini et al., 1999), and *Glycine max* (Horner et al., 2003). A widespread degenerative process occurs in *T. ulmifolia* and *G. max*, but only cell-by-cell in *Helleborus*, probably involving spatial reorganization of secreting cells (Vesprini et al., 1999). This different pattern of cell degeneration is probably related to the very different duration of nectar secretion: short in *Turnera ulmifolia* and *Glycine max* (a few hours and 24 h, respectively) and long in *Helleborus* (about 20 days). The long duration of nectar secretion in *Helleborus* is not compatible with a rapid and massive degeneration of secreting cells.

Horner et al. (2003) reported that in *Glycine max*, before the nectary parenchyma and epidermal cells undergo programmed cell death, they produce compounds of unidentified chemical composition that engorge their central vacuole which has an apparently “discontinuous tonoplast”.

The fate of the nectary parenchyma after secretion may have different patterns when nectar secretion does not cause cell death. The nectary tissue may

- Be involved in nectar reabsorption (Nepi et al., 1996)
- Differentiate into another tissue (parenchyma tissue, as in the case of septal nectaries of certain monocots; see “Gynopleural (septal) nectaries” on page 154)
- Degenerate (Fig. 8)

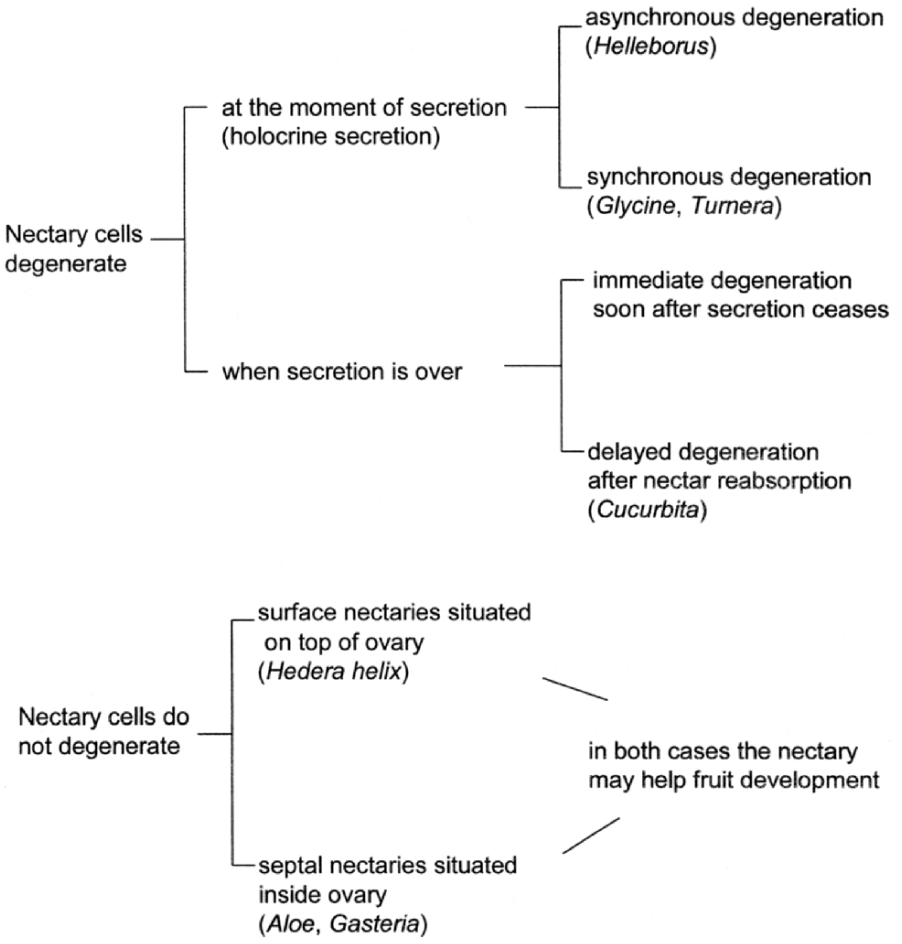

Figure 8. Nectary fates and cell degeneration after nectar secretion.

Several cases of cell degeneration have been reported in nectaries after secretion. Typical evidence of programmed cell death (PCD) such as nuclear disorganization, cytoplasmic condensation, and disruption of ER cisternae was observed in *Arabidopsis thaliana* (Zhu & Hu, 2002). A continuous increase in vacuole volume in post-secretory nectaries is often associated with autophagic events, revealed by the presence of amyloplasts and other organelles in the vacuole (Rachmilevitz & Fahn, 1973; Cecchi Fiordi & Palandri, 1982; Kronestedt et al., 1986; Belmonte et al., 1994; O'Brien et al., 1996). However, examples of nectary tissue degeneration were not reported in a recent review on PCD in floral organs (Rogers, 2006).

In *Rosmarinus officinalis,* the vacuole volume increases after secretion, the cytoplasm darkens and its volume decreases. There is a distinct increase in ER cisternae, which appears to be related to the lytic process of the disintegrating protoplast (Zer & Fahn, 1992). Similar processes of nectary degeneration after secretion were observed in septal nectaries of banana flowers (Fahn & Benouaiche, 1979) and in extrafloral nectaries of *Sambucus nigra* (Fahn, 1987). Multilamellar bodies characterized by membranous conglomeration have been associated with degradative processes in a number of species (Davis et al., 1986 and references therein; Nepi et al., 1996).

According to Durkee et al. (1981), a complete breakdown of the secretory tissue occurs in the floral nectary of *Passiflora* in the post-secretory phase. Intercellular spaces enlarge considerably and cell walls become compressed and collapsed. The cytoplasm becomes electron-translucent and the internal membranes of plastids and mitochondria show signs of considerable disorganization. Collapsed and compressed cells were also observed in the epidermis of *Hexisea imbricata* (Orchidaceae) (Stpiczyńska et al., 2005a).

2.2.1 Patterns of plastid development in nectary parenchyma cells

Plant cell differentiation is a process in which almost all cell compartments are involved, among which plastids always play a crucial role. Proplastids of meristematic cells differentiate into other types of plastids. In vegetative organs, such as leaves, plastid differentiation is commonly unidirectional, while in flower cells plastids may interconvert and dedifferentiation is more frequent than in other plant parts (Pacini et al., 1992; Clement & Pacini, 2001).

Proplastids are the “meristematic” plastid type always encountered in all the young stages of nectaries studied ultrastructurally (Nepi et al., 1996). Generally proplastids undergo some divisions before beginning to differentiate (Pacini et al., 1992; Nepi et al., 1996).

Plastid differentiation may follow different pathways according to the species and the stage of nectar development. The features of plastids in adult parenchyma nectary cells vary widely at the moment of nectar secretion, because of the different development of thylakoids and grana and the different degrees of starch storage (Fig. 9). Undifferentiated plastids (proplastids) are present in the very early stages of nectary development. Close to flower anthesis, chloro-amyloplasts may differentiate and are generally present in nectary parenchyma when secretion begins (Figs. 9 and 10). They contain very few small starch grains per plastid. In some cases, chloro-amyloplasts

lose their thylakoid structure and starch grains increase in size a few days before anthesis (Zer & Fahn, 1992; Fahn & Shimony, 2001). In other cases, proplastids differentiate into amyloplasts and store great amounts of starch in many large grains per plastid before nectar secretion begins (Durkee et al., 1981; Figueiredo & Pais, 1992; Pais & Figueiredo, 1994; Nepi et al., 1996) (Figs. 9 and 10). In *Passiflora biflora, Rosmarinus officinalis*, and *Cucurbita pepo* (Durkee et al., 1981; Zer & Fahn, 1992; Nepi et al., 1996), nectary parenchyma proplastids start to accumulate starch derived from the photosynthesis of other floral parts during pre-anthesis (Pacini et al., 2003 and references therein). In these three species, starch also accumulates in the epidermis, though the number of grains per plastid is fewer than in parenchyma cells. Amyloplasts in the nectar-producing parenchyma are generally almost devoid of stroma and packed with starch (Fig. 10) (Nepi et al., 1996). They also contain many starch grains per amyloplast; this increases starch surface area, facilitating and speeding hydrolysis during nectar production.

The type of plastids and presence of starch are heterogeneous features of orchid floral nectaries (Table 1 and Fig. 9). Plastids may have an undifferentiated appearance and contain osmiophilic bodies (*Gymnadenia conopsea* and *Platanthera chlorantha)*; they may have thylakoid-like membranes that resemble choloroplasts (*Hexisea imbricata, Maxillaria coccinea, Platanthera bifolia),* or they may be amyloplasts *(Limodorum abortivum).*

Undifferentiated plastids and chloroplasts may or may not store starch in the pre-secretory phase. No starch grains were observed in plastids of the nectary cells of the orchids *Gymnadenia conopsea* and *Maxillaria coccinea* (Table 1 and Fig. 9), however starch has been found in plastids of other orchids, such as *Hexisea imbricata, Platanthera bifolia,* and *Platanthera chlorantha.*

The quantity of starch in plastids peaks in mature buds and decreases with the onset of nectar production. Thus many authors infer that hydrolysis of starch in the parenchyma contributes directly to nectar carbohydrate content (Rachmilevitz & Fahn, 1973; Durkee et al., 1981; Zer & Fahn, 1992; Nepi et al., 1996; Pacini et al., 2003; Peng et al., 2004). The sugars derived by starch breakdown can also be used to produce energy for the process of secretion. The general pattern of starch decreasing at the moment of nectar secretion was not found in *Trifolium pratense* (Fabaceae) and *Ecballium elaterium* (Cucurbitaceae). In red clover, starch grains in plastids were actually more numerous and larger in florets at the end of nectar production (Eriksson, 1977). In *Ecballium elaterium,* plastids have well-differentiated thylakoids and grana in the early stage of nectary development; they store starch, reaching

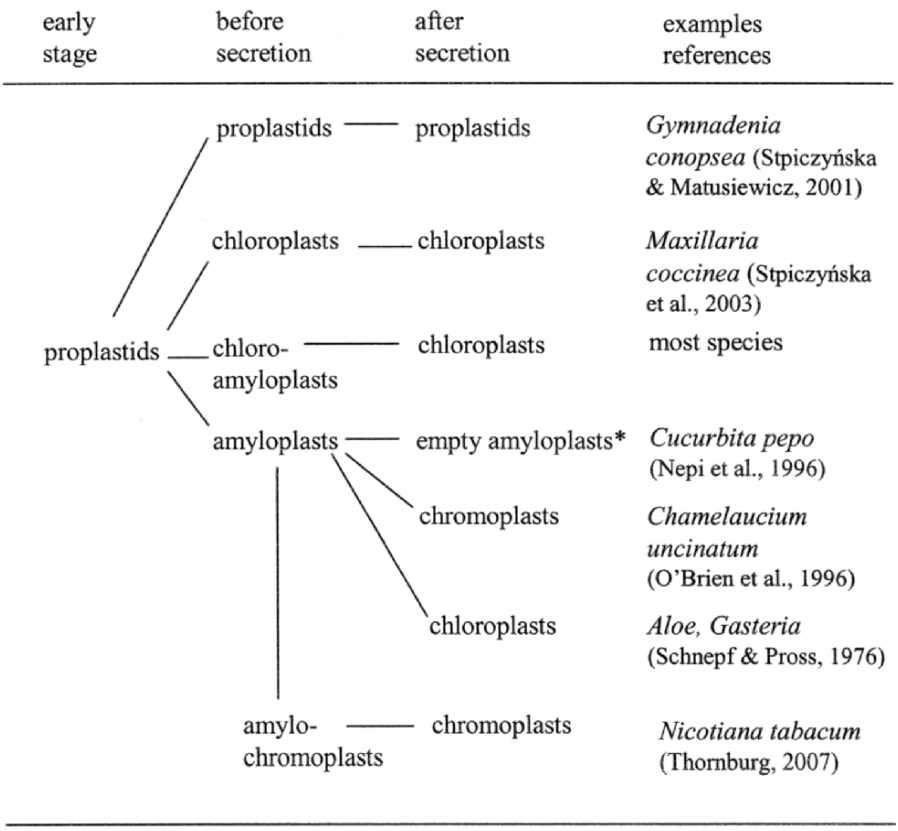

Figure 9. Plastid differentiation pathways during nectary development. In *Gymnadenia conopsea*, proplastids do not differentiate and are probably not so much involved in nectar production. Amyloplasts present a wide range of differentiation. Before nectar secretion they may become amylochromoplasts, after nectar secretion and starch hydrolysis they may remain empty amyloplasts or differentiate into chloroplasts or chromoplasts.

*Empty amyloplasts can be involved in a temporary storage of reabsorbed carbohydrates if nectar was not totally consumed by flower visitors.

peak accumulation in mature buds, but there is apparently no hydrolysis at the time of nectar secretion, probably because of the very small amount of nectar secreted (Fahn & Shimony, 2001).

The amyloplast membrane remains integral during starch hydrolysis in *Passiflora* sp. and *Cucurbita pepo* (Durkee et al., 1981; Nepi et al., 1996). Plastid degeneration generally only occurs after complete starch hydrolysis and/or nectar resorption. The degeneration of the nectary with empty

amyloplasts at the end of secretion or after nectar reabsorption seems to be a general feature.

In *Aloe* and *Gasteria*, which have septal nectaries, dedifferentiation of amyloplasts to chloroplasts has been recorded after nectar secretion (Schnepf & Pross, 1976; Nepi et al., 2005). This dedifferentiation allows secreting cells to transform into fruit parenchyma cells.

Other patterns of nectary plastid development have also been observed. In *Chamelaucium uncinatum* (Myrtaceae), the nectary parenchyma cells have chloroplasts and secrete nectar for 11 days. At the end of the secretion period the nectary becomes red, probably because of transformation of chloroplasts into chromoplasts (O'Brien et al., 1996). The pattern of nectary plastid development is more complicated in *Nicotiana tabacum*, where the nectary parenchyma cells differentiate into chloroplasts in the early stages. Later they accumulate starch, becoming amyloplasts, and when starch is hydrolysed they accumulate β-carotene becoming amylochromoplasts (Fig. 9) (Thornburg, 2007).

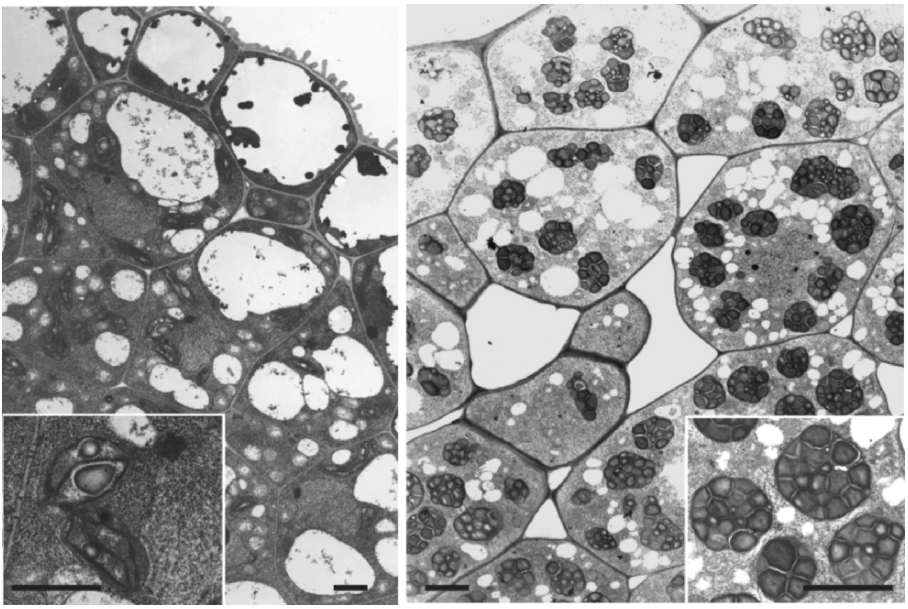

Figure 10. Floral nectaries of *Arabidopsis thaliana* (*left*) and *Cucurbita pepo* (*right*). Inserts show the details of plastid structure in the nectary parenchyma cells. In *A. thaliana* there are chloroamyloplasts with few thylakoids and very few starch grains. In *C. pepo* there are very large amyloplasts with many large starch grains. Bars = 5 μm.

2.3 Subnectary parenchyma

The subnectary parenchyma is located below the nectary parenchyma, from which it is generally easily distinguished because it consists of larger cells, with bigger vacuoles, less dense cytoplasm, and larger intercellular spaces. Durkee (1982) reported plasmodesmata between nectary and subnectary parenchyma cells in the extrafloral nectary of *Passiflora*, suggesting that these tissues cooperate in the secretion of nectar. Generally neither, the ER nor Golgi apparatuses in the subnectary parenchyma cells show the unusual degree of development and swelling found in the nectary parenchyma cells (Durkee, 1983). Insignificant ultrastructural changes take place in subnectary parenchyma cells approaching secretion, and generally the vacuole increases in size at secretion.

As described earlier, subnectary parenchyma is generally richer in chloroplasts than nectary parenchyma. Vascular bundles are always present in subnectary parenchyma. In most cases the xylem vessels stop in this tissue while phloem strands branch into the nectary parenchyma (Fig. 11).

Oil and mucilage cells were described by Sawidis (1998) in the subnectary parenchyma of *Hibiscus rosa-sinensis*. Because of the water-binding capacity of mucilage, with rapid water uptake and slow release, it was hypothesized that in this species mucilage cells offer an ideal regulation mechanism for water balance during nectar secretion and efficient protection of nectary tissue against water stress damage. Oil cells, on the other hand, are supposed to be involved in nectary protection against herbivores.

2.4 Nectary vasculature

The vasculature brings raw materials for nectar production to the nectary. Frey-Wyssling and Agthe (1950) suggested a correlation between the vascular supply of the nectary and the concentration of nectar. Nectaries that secrete very concentrated nectar are vascularized by phloem only. Nectaries secreting nectar with low sugar concentrations are vascularized equally by phloem and xylem or primarily by xylem. This hypothesis was supported by observations in *Gossypium* (Wergin et al., 1975), *Abutilon* (Gunning & Hughes, 1976), and *Hibiscus* (Sawidis et al., 1987a), but was not always confirmed in subsequent studies (Dafni et al., 1988; Zer & Fahn, 1992).

Although some nectaries are reported to be vascularized by xylem and phloem, the last branches reaching the nectary parenchyma are generally phloem elements, which may reach the area of the epidermis. This feature is

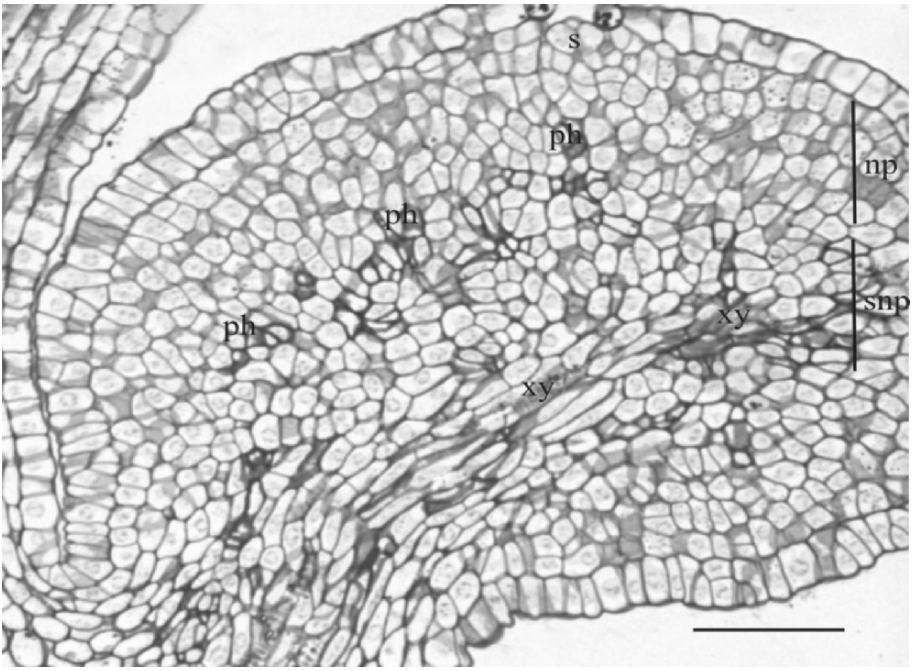

Figure 11. The floral nectary of *Daphne sericea* (Thymelaeaceae). Xylem vessels (xy) stop in the subnectary parenchyma (snp) while phloem strands branch into the base of the nectary parenchyma (np). s = stoma. Bar = 80 µm, stained with PAS.

often encountered in nectaries of Asteraceae (Wist & Davis, 2006). Phloem alone supplies the floral nectaries of most species of Brassicaceae and a direct relation has been demonstrated between the abundance of phloem supply and nectar carbohydrate production (Davis et al., 1998).

Well-developed wall ingrowths, reminiscent of those of transfer cells (Pate & Gunning, 1972), have been detected in the companion cells and are common in nectary phloem (Davis et al., 1988; Belmonte et al., 1994; Razem & Davis, 1999; Wist & Davis, 2006). The increased surface area of the companion cell membrane around these ingrowths is thought to enhance unloading of pre-nectar components from sieve tube elements and their direct transfer to adjacent phloem parenchyma and intercellular spaces (Davis et al., 1988; Razem & Davis, 1999; Wist & Davis, 2006). Unusually large companion cells, characterized by large membrane-bound protein bodies, were reported in the floral and extrafloral nectaries of different species of *Passiflora* (Durkee et al., 1981; Durkee, 1982). In these “intermediary cells”, wall ingrowths are not evident, but the unloading process may be favoured by their large surface area. The function of the membrane-bound protein bodies is obscure.

3 GYNOPLEURAL (SEPTAL) NECTARIES

Fahn (1979a) formulated a topographical classification of floral nectaries, differentiating nine different types. Among them, the "ovarial nectary" type includes nectaries that are situated in the septal region between adjacent carpels, known as septal or, more recently, gynopleural nectaries (Smets & Cresens, 1988). Gynopleural nectaries are largely absent in dicotyledons, although there are non-secretory septal slits in *Saruma* (Endress, 1994), *Cneorum tricoccum*, *Koelreuteria paniculata*, *Ruta bracteosa* and a few other dicotyledons (Schmid, 1985). On the other hand, they are the most common type of floral nectary in monocotyledons (Smets et al., 2000, Table 1) and are therefore considered separately from the other types of floral nectaries. According to Rudall (2002), septal nectaries have been lost several times in monocot evolution, probably in association with the emergence of different pollination syndromes.

The gynopleural nectary, being a cavity inside the ovary, is not directly exposed to nectar-feeding animals and the site of nectar emission is often different from the site of nectar production (secondary presentation). Nectar must therefore flow through auxiliary ducts—up to 13 cm long in *Milla biflora*—to reach its site of emission (Vogel, 1998b; Bernardello, 2007; Pacini & Nepi, 2007). The morphological characters of gynopleural nectaries were reviewed from a systematic point of view by Daumann (1970) and Smets et al. (2000) and from a functional perspective by Schmid (1985). A very thin and sometimes apparently discontinuous cuticle is present on the surface of epithelial cells (Fahn & Benouaiche, 1979; Nepi et al., 2005). The nectar cavity is lined by a layer of secretory epithelial cells that may overlie a subsidiary glandular tissue, characterized by smaller cells with denser cytoplasm than the ground parenchyma cells, thus resembling the nectary parenchyma of floral nectaries. Wall ingrowths are very common in epithelial cells that, for this reason, are regarded as transfer cells. The differentiation of transfer cells in septal nectaries is supposed to be an anatomical device to increase nectar output via eccrine secretion (Schmid, 1985). Cell wall ingrowths are highly developed in *Aloe* and *Gasteria* (Schnepf & Pross, 1976; Nepi et al., 2005), but are not so abundant in the nectaries of banana and *Tillandsia* (Fahn & Benouaiche, 1979; Cecchi Fiordi & Palandri, 1982), where predominantly granulocrine secretion seems likely. Different extents of the subsidiary tissue were observed in different species of *Tillandsia* (Cecchi Fiordi & Palandri, 1982) and were related to nectar production rates.

The development of septal nectaries follows two patterns that differ mainly in the fate of the nectary after the secreting phase. A breakdown of

the nectary epithelium after secretion was demonstrated in male and female *Musa paradisiaca* flowers (Fahn & Kotler, 1972), where the cytoplasm became very electron-dense, plastids and mitochondria degenerated and the vacuole increased gradually in volume until it occupied most of the cell (Fahn & Benouaiche, 1979). On the other hand, transformation of nectary tissue into parenchyma, by elongation of epithelial cells and occlusion of the nectary cavity by acidic polysaccharides, has been reported in *Aloe, Gasteria,* and *Tillandsia* (Schnepf & Pross, 1976; Cecchi Fiordi & Palandri, 1982). Schnepf & Pross (1976) demonstrated differentiation of transfer cells in the epithelium of septal nectaries in some *Aloe* species. A short time before anthesis, they formed an elaborate system of wall protuberances along their outer walls. They redifferentiated in the developing fruit; losing the wall protuberances, increasing in size, and becoming parenchymatous cells. Rearrangement of these cells was accompanied by transformation of amyloplasts into chloroplasts, probably involved in photosynthesis to help fruit development.

A very complex type of secretion has been reported in several species with septal nectaries. A mixture of protein and polysaccharides was found in the septal nectaries of banana (Fahn & Benouaiche, 1979). Sajo et al. (2004) reported a ring of mucilage canals around the infralocular nectary of some Bromeliaceae. Poor nectar production and the presence of amorphous, hydrophilic, acid polysaccharides suggest that the nectariferous tissue may have a role in water and nutrient accumulation in *Tillandsia*, where nectaries are more developed in species growing in dry habitats (Cecchi Fiordi & Palandri, 1982).

4 EXTRAFLORAL NECTARIES

Extrafloral nectaries differ from floral nectaries in position and function. Extrafloral nectaries may be situated on virtually any vegetative structure, but most often on the upper half of the petiole or near the base of the leaf blade (Elias, 1983). They may be associated with floral structures: on the rachis of the inflorescence, near the base of flowers or their pedicel, on the calyx, or on the corolla. Regardless of position, extrafloral nectaries are never directly involved in pollination and their main function is to feed ants that protect the plant against herbivores (Beattie, 1985; Heil et al., 2001; Falcão et al., 2003; Ness, 2003; Vesprini et al., 2003). In some cases they are active during the flower bud stage (Anderson & Symon, 1985) or during fruit development (Vinoth & Yash, 1992; Morellato & Oliveira, 1994). It was recently demonstrated that the total number of extrafloral nectaries on a plant may be

affected by the intensity of herbivory (Wäckers et al., 2001; Mondor & Addicott, 2003) and that herbivore-induced plant volatiles are responsible for increased extrafloral nectar production (Choh et al., 2006; Kost & Heil, 2006).

According to Elias (1983), who modified the early classification of Zimmermann (1932), seven morphological types of extrafloral nectary can be observed: formless nectaries, flat nectaries, elevated nectaries, scale-like nectaries, hollow nectaries, pit nectaries, and embedded nectaries. They are usually small protuberances, which may be covered by protecting non-secretory hairs (Sousa e Paiva et al., 2001; Falcão et al., 2003). Different morphological types of extrafloral nectaries may co-occur in different positions even on the same leaf of a plant such as *Passiflora* sp. (Galetto & Bernardello, 1992; Blüthgen & Reifenrath, 2003). Two types of extrafloral nectaries—differing in morphology, anatomy, function, and nectar composition—were described in *Vigna unguiculata* by Pate et al. (1985). Four extrafloral nectary sites (petiole, calyx, corolla, fruit) can be recognized in *Campsis* (Bignoniaceae), which also has floral nectaries (Elias & Gelband, 1976).

As happens among floral nectaries, some extrafloral nectaries are also devoid of vascularization and lack the anatomical organization typical of nectaries. Elias (1983) described this type of nectary as non-vascularized, non-structural; examples are those located in the outer verticel of petals in certain Bromeliaceae, Zingiberaceae, Paeoniaceae, and Cactaceae (Galetto & Bernardello, 1992 and references therein). More frequently, extrafloral nectaries have a structure not very different from that of floral nectaries. The most frequent vascularization consists of phloem or phloem and xylem. A continuous thick cuticle covers the epidermal cells of the extrafloral nectaries and nectar release generally takes place through cuticle rupture. In some cases, such as in some Bromeliaceae species (Galetto & Bernardello, 1992) and *Solanum stramonifolium* (Solanaceae) (Falcão et al., 2003), nectar can be secreted through stomata. Secretory cells located under the epidermis may occur in one or several layers and are usually elongated and orientated along a vertical axis. Plastids in extrafloral nectaries are generally chloro-amyloplasts (Pacini et al., 2003, see also Table 1) with very few starch grains. In fact, extrafloral nectaries show less evident starch formation and degradation processes than floral nectaries (Durkee et al., 1981).

Extrafloral nectaries also generally have merocrine secretion, though holocrine secretion has been described for *Ailanthus glandulosa* (Clair-Maczulajtys & Bory, 1983). Holocrine secretion has also been reported in the

extrafloral nectaries of *Sambucus*, but the cells die and disintegrate after they have ceased to secrete nectar in the usual merocrine manner, thus cell disintegration in this species can be regarded as tissue degeneration after secretion (Fahn, 1987).

Compared to floral nectaries, a wider range of inclusions has been found in the vacuoles of the parenchyma cells in extrafloral nectaries:

- Dense osmiophilic material in *Euphorbia neriifolia* (Arumugasamy et al., 1990b).
- Tannins in *Euphorbia neriifolia* and *Ailanthus glandulosa* (Arumugasamy et al., 1990b; Clair-Maczulajtys & Bory, 1983).
- Calcium oxalate raphides or druses in *Turnera ulmifolia*, *Passiflora* sp. (Elias et al., 1975; Durkee, 1982; Elias, 1983).
- Anthocyanin in *Ricinus communis* (Baker et al., 1978).
- Crystalline protein bodies in *Ricinus communis*. Since they were not observed in very young nectaries, they are presumably associated with storage of retained nitrogen (Baker et al., 1978). There is no evidence that they are subsequently hydrolyzed; thus their participation in nectar production is unlikely.

In a recent survey in an Australian rainforest, Blüthgen & Reifenrath (2003) found 34 plant species bearing extrafloral nectaries. Plant organs with extrafloral nectaries were mostly leaves and leaf petioles. Both adaxial and abaxial positions were commonly involved.

5 NECTARY HISTOCHEMISTRY

Although not sufficient for a detailed study of nectary structure and function, light microscopy and histochemistry may provide a general view of the sites and organization of the various parts of nectaries.

Active floral nectaries may be located by staining inflorescences with neutral red. Nectary cells selectively accumulate this stain. However, this does not seem to work with extrafloral nectaries (Kearns & Inouye, 1993). Common histochemical techniques for the study of nectaries are listed in Table 2. Toluidine blue O is frequently used as general nectary stain. The periodic acid–Schiff (PAS) reaction is a simple informative staining technique that stains cell walls and starch in amyloplasts or, temporarily, in chloroplasts. It must be preceded by blockage of free aldehyde groups (e.g., with a saturated dimedone solution) to avoid artefacts (O'Brien & McCully, 1981).

Table 2. Histochemical techniques used for nectaries. bf = bright field; UV = ultraviolet light.

Specificity	Stain—optics	Cell components stained	Reference
General stains			
	toluidine blue O (TBO)—bf	cell walls, nucleus, cytoplasm	Beardsell et al., 1989; Link, 1991; O'Brien et al., 1996; Maldonado & Otegui, 1997; Stpiczyńska et al., 2005a
	acid fuchsin—bf	nucleus, cytoplasm	Maldonado & Otegui, 1997
Polysaccharides			
Total insoluble polysaccharides	PAS (periodic acid-Schiff)—bf	cell walls, cytoplasm, amyloplsts	Beardsell et al., 1989; Nepi et al., 1996; Maldonado & Otegui, 1997; Nepi et al., 2003
Starch	IKI (iodine-potassium-iodide)—bf	starch grains inside amyloplasts	Nepi et al., 1996; Stpiczyńska et al., 2003; Maldonado & Otegui, 1997
Acid polysaccharides	ruthenium red—bf	cell walls	Maldonado & Otegui, 1997; Fahn & Benouachie, 1979; Stpiczyńska et al., 2003; Stpiczyńska et al., 2005a
Lipids			
	sudan III—bf	cytoplasm, lipid droplets	Stpiczyńska et al., 2003
	sudan IV—bf	cytoplasm, lipid droplets	Davis et al., 1988; Fahn & Benouachie, 1979
	auramine O—UV	cuticle	Beardsell et al., 1989; Nepi et al., 1996; O'Brien et al., 1996; Nepi et al. 2003
Proteins			
	comassie brilliant blue—bf	cytoplasm, nucleus, vacuole	Maldonado & Otegui, 1997; Stpiczyńska et al., 2003; Stpiczyńska et al., 2005a
	bromophenol blue—bf	cytoplasm, nucleus, vacuole	Nepi et al., 1996;
Phenols			
	Millons reagents—bf	vacuole	Sawidis, 1998
Tannins			
	DMB (dimethoxy-benzaldehyde)—bf	vacuole	Sawidis, 1998

Bright-field and epifluorescence techniques can be useful to study nectary structure. Autofluorescence of chlorophyll can be used to highlight the distribution of chloroplasts in the nectary and subnectary parenchyma (see Fig. 5). Phenolic compounds, the lignin of xylem vessels, and cuticles can be located by autofluorescence. Details of the cuticle can be highlighted using Auramine O. When the fluorescence is strong enough, UV and visible light of an appropriate intensity can be used simultaneously. This makes it possible to observe samples treated with conventional stains and fluorochromes at the same time.

As far as electron microscopy is concerned, the zinc iodide–osmium tetroxide (ZIO) method is suitable for general impregnation of the endomembrane system of many plant, algal, and fungal tissues. It has also been used for staining subcellular compartments of the nectary (Machado & Gregorio, 2001), where it facilitated observation of membranes and helped to elucidate the role of nectary regions and cytoplasmic organelles in nectar secretion.

Conventional chemical fixation can damage cell components and any results from studies using this technique must be considered with caution, especially when applied to highly dynamic systems such as those operating during nectar secretion. To overcome such problems, the freeze-substitution technique was recently applied to the study of nectary ultrastructure and nectar secretion (Robards & Stark, 1988; Zhu et al., 1997; Zhu & Hu, 2002; Stpiczyńska et al., 2005b). According to Zhu et al. (1997), the membranes of organelles, vacuoles, and nuclei showed less shrinkage than with chemical fixation. With this technique, Robards and Stark (1988) observed an open extracytoplasmic space external to all the cells of the secretory hairs of *Abutilon*. According to these authors, the endomembrane system of nectar secretory cells is not appreciably affected by chemical fixation.

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Chapter 4

NECTAR PRODUCTION AND PRESENTATION

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1 INTRODUCTION

Nectar secretion is complicated to study from the ultrastructural point of view because it is a dynamic process involving many tissues simultaneously. Study may also be affected by artefacts created by chemical fixation procedures, although this problem can be overcome by freeze-drying and freeze-substitution techniques (Zhu & Hu, 2002; Stpiczyńska et al., 2005b). Previous research has focused on the ultrastructure of secretory cells, especially secreting trichomes (Robards & Stark, 1988), and a general model of nectary function as a whole is still lacking.

Nectar is secreted with particular rhythms and can be reabsorbed over the life of the flower. The temporal patterns of secretion, cessation, and reabsorption, if any, define nectar production dynamics. This parameter is usually linked to the foraging behaviour of visiting animals (see also Nicolson, 2007, Chapter 7 in this volume) whose activity, together with changing environmental parameters, is responsible for the amount of nectar found in a flower at a certain moment, known as nectar standing crop (Galetto & Bernardello, 2005). Knowledge of all these parameters is fundamental to understanding the reproductive biology of plant species, and complex, interdependent plant–animal relationships.

This chapter also highlights the relationship between the ecological features of nectar and the cyto-physiological characters of nectaries that were considered in earlier literature. The ultrastructure of nectary parenchyma cells is closely related to the manner and rate of nectar secretion. Búrquez

S.W. Nicolson, M. Nepi, and E. Pacini (eds.), Nectaries and Nectar, 167–214.

and Corbet (1991) stated that the supply of material for nectar sugar may depend on immediate photosynthesis or carbohydrate reserves, the latter being essential in the case of plants producing nectar at night. Nectary parenchyma cells may serve as a storage site for starch that will be hydrolysed at the moment of secretion or they may photosynthesize because of the presence of chloroplasts. The source of nectar components, especially sugars, is closely related to the rate of nectar production: a high rate requires storage of reserve material in nectary parenchyma cells. The rate of nectar production and its total quantity is, in turn, linked to the type of foraging animals, their behaviour and food requirements. These features also influence the manner and site of nectar exposure (nectar presentation).

This chapter closes with a look at the enormous variability in nectar features (volume, concentration, and composition) existing at flower, plant, species, and population levels. Nectar variability within or among plants may result from the interaction between the pattern of nectar secretion by the plant and pollinator foraging strategies. Nectar secretion patterns may be genetically determined (Mitchell, 2004; Leiss and Klinkhamer, 2005b) and also influenced by macro- and micro-environmental conditions (Nicolson, 1994; Nicolson & Nepi, 2005; Petanidou, 2007). It is fundamental to identify the plant effects before studying other potential sources of variability (Galetto & Bernardello, 2005).

2 NECTAR SECRETION MECHANISM AND MODELS OF NECTARY FUNCTION

Two main types of secretion can be recognized in animals and plants: the holocrine type, in which the process involves cell death at the moment of secretion, and the merocrine type, in which the secreting cells survive and continue their secretory activity.

In most cases nectar secretion is merocrine, but in a few cases it is holocrine, implying the death of the cells (Elias et al., 1975; Vesprini et al., 1999; Horner et al., 2003; Nepi, 2007) (Fig. 1).

There is a general consensus in considering phloem sap the "raw" material of nectar. The pre-nectar is unloaded from the sieve elements to the adjacent phloem parenchyma cells and sometimes even in the intercellular spaces. Pre-nectar unloading is favoured by phloem companion cells that often have wall ingrowths of the transfer cell type as observed in *Vicia faba*,

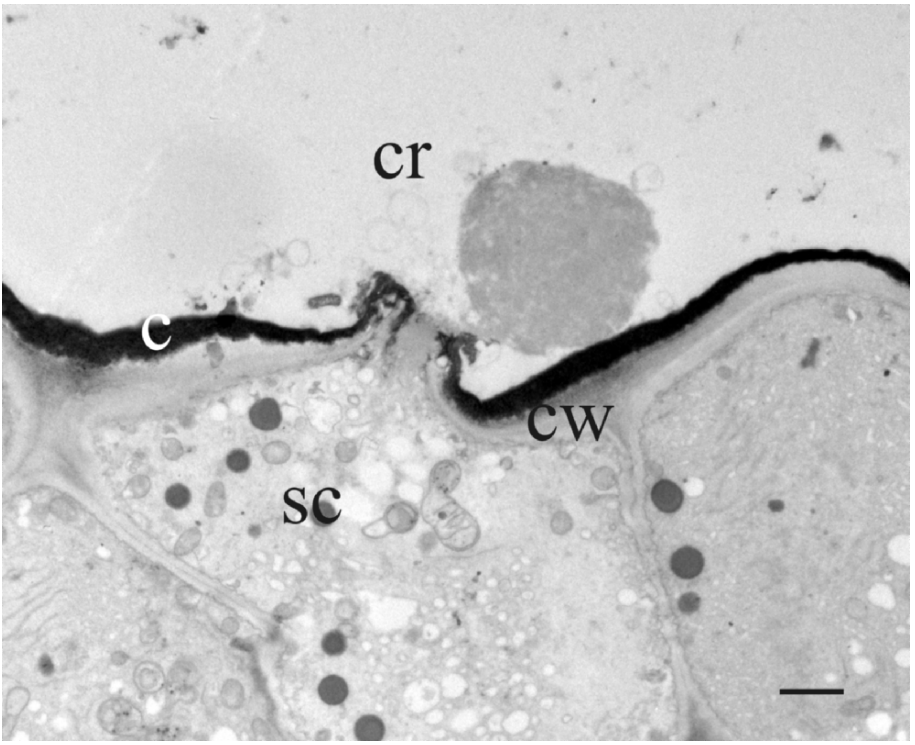

Figure 1. Detail of epidermis of floral nectary of *Helleborus foetidus* (Ranunculaceae). Nectar secretion is holocrine and involves degeneration of cell contents and rupture of the outer wall (cw). Degeneration of secreting cells is not synchronous and secretion lasts several days. Cells that have already secreted nectar appear almost empty while cells not yet involved in secretion are still intact. c = cuticle; cr = cytoplasmic remnants; sc = secreting cell; Bar = 8 μm.

Pisum sativum (Fabaceae) (Davis et al., 1988; Razem & Davis, 1999), and *Eccremocarpus scaber* (Bignoniaceae) (Belmonte et al., 1994).

Pre-nectar passes through plasmodesmata from the phloem parenchyma cells to the nectary parenchyma cells by the so-called symplastic route (Fig. 2). Alternatively, pre-nectar flows from sieve elements and companion cells via intercellular spaces and cell walls to the secretory cells, by the so-called apoplastic route (Fig. 2). Nectar secretion, i.e., the transport of nectar outside the protoplast of the secretory cells, may occur by two mechanisms as described by Fahn (2000):

- Eccrine secretion is molecular transport of individual sugar molecules across the cell membrane, possibly by a carrier molecule.

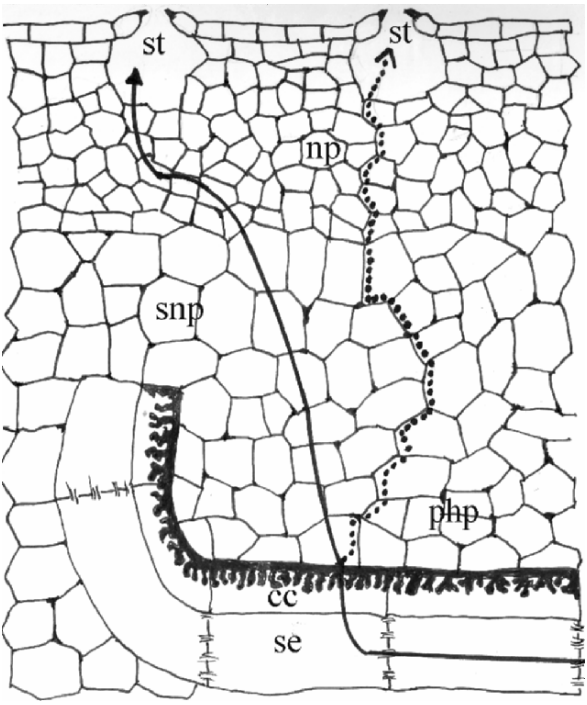

Figure 2. Semi-diagramatic drawing representing the possible pathway of phloem sap and pre-nectar from the sieve element (se) to the stomata (st): *continuous line* represents the apoplastic route, and the *dotted* one the symplastic route. Phloem sap is transferred from sieve element (se) to the phloem parenchyma (php) by wall ingrowths of companion cells (cc). It is then transferred as pre-nectar through the subnectary parenchyma (snp) and subsequently transformed into nectar in the nectary parenchyma (np).

- Granulocrine secretion is transport of a sugar solution into vesicles derived from dilated cisternae of ER or from dictyosomes that fuse with the plasmalemma, releasing nectar into the wall area.

According to Fahn (2000), pre-nectar flows from the phloem endings, through the nectariferous cells, to the secretory cells which exude the nectar externally. Fahn also reports different transport mechanisms for pre-nectar and nectar. Transport of pre-nectar mainly takes place by the symplastic route, whereas nectar secretion occurs by active molecular transport through membranes (eccrine secretion) or by transport via vesicles whose membranes fuse with the plasmalemma (granulocrine secretion). These two distinct mechanisms of pre-nectar and nectar transport are not confirmed by other studies, in which eccrine or granulocrine pre-nectar transport are postulated to occur in parenchyma and secretory cells (Wist & Davis, 2006). Nor can it be excluded that symplastic and apoplastic transport of pre-nectar

take place simultaneously (Wergin et al., 1975; Davis et al., 1986, 1988; Stpiczyńska, 1995; Stpiczyńska et al., 2003c; Wist & Davis, 2006).

Pre-nectar transport follows the symplastic path in nectaries with trichomes as secretory structures. The apoplastic route of pre-nectar is impeded by lignification or complete cutinization of the radial walls of the stalk cell, i.e., the second cell of the hair (Fahn, 1979b; Sawidis et al., 1987a; Davis et al., 1988). In *Hibiscus rosa-sinensis* (Malvaceae) the basal cells of the trichomes (i.e., the first cells of the trichomes), situated at the level of epidermal cells, have a greater number of plasmodesmata, implying a role in the collection and conveyance of pre-nectar from nectary parenchyma cells towards secretory hairs (Sawidis et al., 1987b). Great density of plasmodesmata also occurs in the walls of stalk cells, which, besides providing a barrier to apoplastic transport, favour symplastic flow of pre-nectar. After entering the secreting hairs, pre-nectar flows from cell to cell through plasmodesmata to reach the tip cell (Sawidis et al., 1987b). Here nectar accumulates in spaces between the cell membrane and the cuticle prior to its "pulsed" release (Sawidis et al., 1987b).

In their study based on freeze-substituted material from the nectary of *Abutilon* (Malvaceae), Robards and Stark (1988) postulated an apoplastic route of pre-nectar transport at all levels along the hairs: there seemed to be an open extracytoplasmic space outside all cells of the secreting hair. They observed "secretory reticulum" (SR) in cells of secreting hairs. This complex internal membrane system is sometimes also observed in chemically fixed material. The SR is very abundant and its total surface area may be 23×103 μm^2 in a single trichome (Robards & Stark, 1988). The authors did not study SR ontogeny, but saw it as similar to ER. They did not observe fusion sites of SR cisternae with cell plasma membranes. With these limitations they proposed a model of nectar secretion through *Abutilon* hairs:

> It is envisaged that pre-nectar moves into the symplast of the hair via the numerous plasmodesmata in the transverse walls of the stalk cells. In each of the hair cells some of the pre-nectar is loaded from the cytoplasm into the SR. It is at this stage that a filtration effect takes place, so defining the chemical composition of the secreted product. The sucrose is partially hydrolyzed to glucose and fructose (*Abutilon* nectar contains all three sugars) but evidence is not yet available as to whether this takes place at the membrane or within the cavity of the SR. As loading into the SR continues, a hydrostatic pressure builds up until, ultimately, a minute pulse of nectar is forced into the freely permeable apoplastic space bounded by the plasmalemma to the interior and the cuticle to the exterior. The continuing build up of pressure within this compartment

ultimately reaches the level where pores in the cuticle over the tip cell become patent and release a pulse of nectar to the exterior (Robards & Stark, 1988).

This model combined the classical symplastic transport of nectar in secretory hairs with a new system of apoplastic transport where the SR is primarily involved.

A detailed plausible hypothesis for the function of the nectary as a whole is only available in sporadic cases of parenchymatous nectaries.

According to Fahn (1988), hydrolysis of sucrose in the nectary cells maintains a sucrose concentration gradient that could cause a passive flow of sucrose from sieve elements to nectary secreting cells. This model may also explain the preferential flow of pre-nectar towards secretory cells rather than neighbouring cells; it is, however, not applicable to nectaries where sucrose is the dominant sugar secreted.

A somewhat similar model with new hypotheses was formulated by Nichol and Hall (1988). Excised nectaries of various species cultivated *in vitro* continue to secrete nectar for a period if sugars are supplied (Matile, 1956; Findlay et al., 1982; Bieleski & Redgwell, 1980; Nichol & Hall, 1988). This experimental approach is important to understand the function of nectaries, especially their status as autonomous organs with respect to other parts of the flower. Nichol and Hall (1988) studied nectar secretion in excised extrafloral nectaries of *Ricinus communis*, finding that sucrose, glucose, and fructose can each sustain secretion of nectar, unlike other sugars, and that the nectar secreted in each experiment contained all three sugars. These results indicate the presence of sucrose synthase and sucrose invertase activity in the nectary parenchyma. On the other hand, the phloem vessels of *in situ* extrafloral nectaries of *R. communis* exclusively translocate sucrose (Baker et al., 1978).

Nichol and Hall (1988) demonstrated that hydrolysis of sucrose occurs as a final step of secretion during transport across the membrane of the tonoplast of secreting cells, and no invertase activity was found in the nectar. They also questioned why nectar consistently contains sucrose as well as hydrolysis products. They postulated two transport pathways in *R. communis* extrafloral nectaries. One involves sucrose hydrolysis to the monosaccharides glucose and fructose; it is energy-dependent (ATPase activity was found in the secretory epidermis) and inhibited by anaerobic conditions. The second pathway only involves sucrose; it does not require energy because it is not inhibited by anaerobic conditions and contributes to the sucrose component of

nectar. According to this model the sucrose-only route may take place in the apoplast through which sucrose passes unaltered, while the symplastic route involves active transport and sucrose hydrolysis. The proposed model of nectar secretion for *Ricinus* is not applicable in those cases where an apoplastic barrier between the parenchyma and the epidermis conveys the pre-nectar from the apoplast into the symplast.

Zhu et al. (1997) proposed a model for the nectary function of *Arabidopsis thaliana* (Brassicaceae), a species with a caducous floral nectary, characterized by the development of "densely stained cells". These cells are quite different from normal nectariferous cells because they show signs of degeneration long before secretion. "Densely stained cells" in nectariferous parenchyma appear before secretion and gradually increase in the early secretory stage, reaching a maximum with very few normal cells at the heavy secretion stage. "Densely stained cells" of nectaries of *A. thaliana* are therefore suggested to function as a transferring tissue, despite the absence of wall ingrowths. Although some vesicles are also observed in nectary parenchyma cells during secretion, according to Zhu et al. (1997) it is not clear what kind of substance is actually transported in these vesicles so that their role in nectar secretion is obscure. According to the proposed model, pre-nectar is transferred from sieve elements to nectary parenchyma cells by plasmodesmata and multivesicular structures, where it presumably accumulates and is stored as a few starch grains in chloroplasts. The starch is then degraded in the chloroplasts and is modified in nectary parenchyma cells. Next, the nectar is transported by plasmodesmata or occasionally by vesicles, finally gathering in the "densely stained cells", which connect with modified stomata forming a "corridor" for transferring nectar. The development of "densely stained cells" is reported to be a process of programmed cell death (PCD) leading to loss of function of the semipermeable plasmalemma, so that nectar is easily and quickly transported to the top of the nectary (Zhu & Hu, 2002).

Peng et al. (2004) observed a peculiar pattern of amyloplast degeneration in the nectary parenchyma of *Cucumis sativus* (Cucurbitaceae) from 3 days before anthesis to the day of anthesis. During this period the starch grains become irregular in shape, indicating starch hydrolysis. Amyloplast membranes stretch towards the tonoplast of the closest vacuole. The whole amyloplast then intrudes into the vacuole and the electron density of the degenerating amyloplasts changes from high to low (Peng et al., 2004). On the day of anthesis, the starch grains and amyloplasts disappear totally, while vacuole volume increases significantly. From these observations the authors conclude that a combined amyloplast–vacuole complex acts as the centre of

dynamic nectar transformation. This intimate relationship between amyloplasts and nectary cell vacuoles before nectar secretion has only been reported in this case. Although characterized by amyloplasts very similar to those of *C. sativus*, the allied species *Cucurbita pepo* does not show this close association throughout nectary development and nectar secretion (Nepi et al., 1996a). This dissimilarity could be related to differences in the rate of nectar secretion.

Starch hydrolysis products may be transformed in the vacuole by specific enzymes, such as invertase. Peng et al. (2004) also demonstrated that pre-nectar transport in *C. sativus* follows the apoplastic route. ATPase activity in nectary parenchyma cells is required for transport of pre-nectar from secretory cells to intercellular spaces and also for secretion of nectar on the surface of the nectary. The authors found ATPase activity not only in the plasmalemma of secreting cells, but also in vesicle membranes in intercellular spaces during nectar secretion.

The presence of invertase activity in nectar is still debated. According to early studies reported by Baker and Baker (1983b and references therein) invertase occurs in the nectar of *Tilia* (Malvaceae) and other species. Invertase is not found in nectar from the extrafloral nectaries of *R. communis* (Nichol and Hall, 1988). Pate et al. (1985) noted that diluted extrafloral nectar of *Vigna unguiculata* (Fabaceae) contained inverted sucrose whereas undiluted nectar did not. This finding suggests that nectar contains freely soluble invertase, the activity of which is inhibited osmotically at high sugar levels, or that invertase is associated with nectariferous cells and cell debris in nectar, being leached from these materials when nectar is diluted.

It is clear that we are far from understanding the coordinated activity of all parts of the nectary. The studies cited above are restricted to a few species and approach the topic from either an ultrastructural or a physiological perspective, which are never combined; sometimes they only consider part of the nectary. It is probable that there is no general model of nectary function—a certain grade of variability can be expected on the basis of nectary structure and ultrastructure and nectar production rate and composition.

3 DYNAMICS OF NECTAR PRODUCTION

It is generally accepted that the dynamics of nectar production coevolved with the requirements of plant pollinators. For example, flowers pollinated by diurnally active animals produce nectar and expose it during the day.

Flowers pollinated by nocturnally active animals expose nectar at night (Cruden et al., 1983). As far as the quantity of nectar produced per flower is concerned, numerous works demonstrate that flowers pollinated by high-energy requiring animals, such as bats, hawkmoths, and birds, produce significantly more nectar containing more sugar than flowers pollinated by low-energy requiring animals, such as butterflies, bees, and flies (Cruden et al., 1983 and references therein). Considering the nectar secretion rate, i.e., the quantity of nectar secreted in a unit of time (generally an hour), Cruden et al. (1983) recognized three classes of nectar producers:

- Slow producers secrete 5–10% of their maximum accumulation per hour.
- Fast producers secrete 22–68% of their maximum per hour.
- Super producers secrete two or three times as much as fast producers.

It is reasonable to suppose that the different classes of nectar producers have different nectary parenchyma features. According to Pacini et al. (2003), nectar sugars can be derived directly from photosynthesis or from storage material. In species whose pollinators require rapid unloading of large quantities of nectar, storage of starch in nectary parenchyma cells undoubtedly provides the most efficient means of accumulating nectar constituents (Belmonte et al., 1994). Amyloplasts in nectaries may not only serve as a source of nectar sugars, but also of energy through starch hydrolysis (Belmonte et al., 1994). These plants may start to produce nectar at any hour of the day or night. Most plants that produce nectar at night have storage tissues in the nectary itself, other floral organs or the stem, leaves, or roots. Since many of these species are crassulacean acid metabolism (CAM) plants, the timing of nectar secretion may be linked to stoma opening (Búrquez & Corbet, 1991). On the other hand, the nectar of many plants is derived directly from phloem exudates, i.e., directly from photosynthesized products, especially when the nectary parenchyma has a reduced mass. In this case a very high nectar secretion rate cannot be expected. Defoliation experiments conducted with *Impatiens glandulifera* (Balsaminaceae) demonstrated that only a fraction of the day's nectar secretion depends on that day's photosynthesis, while another fraction must be mobilized from stored photosynthates in storage organs (Búrquez and Corbet, 1998). Some Rosaceae that flower before leaf emission certainly use vegetatively stored substances for nectar production. In fact Radice and Galati (2003) observed amyloplasts in the nectary parenchyma of *Prunus persica* before nectar production.

In most cases flowers begin to secrete nectar before pollinators start their foraging activity and in some cases before the flowers open (e.g., Pleasants,

1983; Witt et al., 1999; Nepi et al., 2001). In *Mandevilla pentlandiana* (Apocynaceae) most nectar is produced during the flower bud stage (Torres & Galetto, 1998). Slow producers are generally early initiators and their nectar is commonly protected by a thick corolla or calyx to prevent predation or evaporation. In contrast, fast producers are late initiators and offer less protection to the nectar (Cruden et al., 1983).

Nectar secretion can be continuous over the flower lifespan until senescence or may cease at certain times. Such differences in nectar production dynamics were recently found in six *Ipomoea* (Convolvulaceae) species (Galetto & Bernardello, 2004). Nectar production dynamics could, however, not clearly be related to the pollinator guild and it was suggested that structural constraints may play a major role in the determination of nectar traits.

Cessation of nectar secretion may take place at two different times: when a maximum is reached or during a period of pollinator inactivity (Cruden et al., 1983 and references therein). In the first case nectar removal may or may not induce resumption of secretion. Secretion does not resume after nectar removal in species where a single pollinator visit is sufficient to maximize seed set. Cruden et al. (1983) pointed out that if nectar secretion resumes after its removal, measuring the nectar of bagged flowers may lead to a gross underestimation of the amount of nectar that can be produced by flowers during the day.

Cessation of nectar secretion may also occur between the two sexual phases of dichogamous plants, as demonstrated in *Carum carvi* (Apiaceae). Nectar is produced in the male and female phases but there is no nectar secretion in the "neutral" phase between them: the male and female phases are both followed by nectar reabsorption (Langenberger & Davies, 2002).

When nectar secretion relies on the accumulation of starch, the quantity of sugar produced is already determined before secretion by the quantity of starch accumulated in nectary parenchyma: the nectary produces a fixed amount of sugars. In these types of nectaries, nectar removal has no effect on total sugar secretion, as in the two cucurbits *Cucurbita maxima* (Ashworth & Galetto, 2002) and *C. pepo* (Nepi et al., 2001). On the other hand, when nectar secretion is derived directly from photosynthesis, nectar removal may increase or decrease total nectar secretion or it may have no effect (Galetto & Bernardello, 1992, 1993, 1995; Bernardello et al., 1994; Galetto et al., 1994, 1995, 2000; Rivera et al., 1996; Torres & Galetto, 1998; Vesprini & Galetto, 2000).

An increase in total secretion after nectar removal can be explained by the fact that nectar secretion stops after a maximum is reached and nectar production can only be re-activated after nectar removal. This is probably related to the necessity for several pollinator visits to deposit enough pollen on the stigma. Alternatively, increased total nectar production in repeatedly sampled flowers is considered to be evidence that nectar secretion proceeds in conjunction with nectar reabsorption: the effect of reabsorption is reduced by sampling a flower repeatedly at short intervals, minimizing the quantity of nectar available in the flower for reabsorption (Corbet, 2003).

3.1 Nectar reabsorption: resource recovery and homeostasis

Búrquez and Corbet (1991) proposed a model to explain changes in the apparent secretion rate, defined as the rate of change of solute content of nectar in undisturbed, unvisited flowers. The apparent secretion rate can be resolved into two components: gross secretion rate (rate of change of solute content in nectar of repeatedly sampled flowers) and apparent reabsorption rate. In fact, for some species there is evidence that reabsorption of nectar proceeds in conjunction with secretion (Búrquez & Corbet, 1991; Nicolson, 1995; Corbet, 2003) so that the quantity of nectar present in a bagged flower at a given time is a function of the relative rates of secretion and reabsorption.

Since Bonnier's (1879) finding that flowers of *Platanthera* (Orchidaceae) reabsorb nectar, several direct and indirect demonstrations of nectar reabsorption have been published (Table 1). Nectar reabsorption is not an unusual floral feature. It is reported in many plant species and occurs irrespective of the age or sexual expression of the flower, and does not depend on pollination. Also, reabsorption of nectar occurs regardless of nectary structure and the manner of nectar exudation via modified stomata or unicellular hairs. Burqúez and Corbet (1991) reported nectar reabsorption in *Brassica napus* by net solute loss in unvisited flowers. Masierowska and Stpiczyńska (2005) demonstrated nectar reabsorption in *Sinapis alba*, another species of Brassicaceae. Reabsorption of uncollected nectar has been noted in *C. pepo* and *C. maxima* (Cucurbitaceae; Nepi et al., 1996a, b, 2001; Ashworth & Galetto, 2002), *Eucalyptus* sp. (Myrtaceae; Davis, 1997), *Aerangis verdickii* (Orchidaceae; Koopowitz & Marchant, 1998), *Mystacidium venosum* (Orchidaceae; Luyt & Johnson, 2002), *Linaria vulgaris* (Scrophulariaceae; Nepi et al., 2003), and *Aloe castanea* (Asphodelaceae; Nicolson & Nepi, 2005). Nectar is also reabsorbed in *C. carvi*, after each male and female flower stage (Apiaceae; Langenberger & Davis, 2002), and near the end of the flower lifetime in *Combretum fruticosum* (Combretaceae; Bernardello et al., 1994),

Ligaria cuneifolia (Loranthaceae; Rivera et al., 1996), *Mandevilla pentlandiana* (Apocynaceae; Torres & Galetto, 1998), and *Platanthera chlorantha* (Orchidaceae; Stpiczyńska, 2003a, b). These examples concern species with exposed nectar (*C. carvi*, *Eucalyptus*), species with hidden nectar (*C. pepo*, *C. maxima*, *A. castanea*), and species with nectar stored in a spur (*P. chlorantha*, *A. verdickii*, *L. vulgaris*).

It is now evident that secretion may occur concomitantly with reabsorption and that sometimes reabsorption continues after secretion has ended (Bielesky & Redgwell, 1980; Búrquez & Corbet, 1991; Nicolson, 1995; Nepi et al., 2001; Corbet, 2003).

Reabsorption of unconsumed nectar seems quite a common phenomenon, especially when the nectary is large in volume and the quantity of produced nectar is not negligible. This process is more difficult to explain when the nectary is persistent and contains chloroplasts, because these organelles continue their anabolic activity and cannot store material coming out of a cell. Reabsorbed substances are not temporarily stored in chloroplasts and must be immediately transported into other parts of the flower or plant.

Two main functions of nectar reabsorption can be recognized: recovery of resources invested in nectar production and a homeostatic mechanism during nectar secretion and presentation (Table 1). The two functions may not be mutually exclusive in a given species: in *C. pepo,* nectar reabsorption has a homeostatic function during flower anthesis and subsequently a resource recovery function after flower closure (Nepi et al., unpublished data).

Nectar production requires considerable expenditure of energy. Pyke (1991) reported that removal of nectar from flowers of *Blandfordia nobilis* (Blandfordiaceae) increased net nectar production but reduced the plant's ability to produce seeds, which can result in reduction of plant growth and reproduction during the following season. Resource recovery is therefore an important reason why plants try to re-utilize this source of carbohydrates not collected by pollinators. This strategy of resource recovery has recently been demonstrated or postulated in several species (Búrquez & Corbet, 1991; Koopowitz & Marchant, 1998; Luyt & Johnson, 2002; Stpiczyńska, 2003a, b). In species such as *C. pepo* and *P. chlorantha*, all unconsumed nectar is reclaimed regardless of pollination, maximizing the recovery of energy invested in nectar production (Nepi & Stpiczyńska, 2007). In some plant species nectar reabsorption is evidently induced by pollination. In the

Table 1. Current knowledge of species (listed alphabetically) with clear evidence of nectar reabsorption and its possible functions. RR = resource recovery; H = homeostasis.

	Methods used	Function	Reference
Dicotyledons			
Brassica napus (Brassicaceae)	Cumulative production	RR	Búrquez & Corbet, 1991
Carum carvi (Apiaceae)	Volume, sugar decrease	RR	Langenberger & Davis, 2002
Combretum fruticosum (Combretaceae)	Volume, sugar decrease	RR	Bernardello et al., 1994
Cucurbita maxima (Cucurbitaceae)	Volume, sugar decrease	RR	Ashworth & Galetto, 2002
Cucurbita pepo (Cucurbitaceae)	Volume, sugar decrease Nectar substitution Radioactive tracer	RR, H	Nepi et al., 1996b, 2001; Nepi & Stpiczyńska, 2007 Nepi et al., unpublished data
Echinacea purpurea (Asteraceae)	Volume, sugar decrease		Wist & Davis, 2006
Eucalyptus sp. (Myrtaceae)	Volume, sugar decrease	RR	Davis, 1997
Grevillea robusta (Proteaceae)	Volume, sugar decrease	H	Nicolson, 1995
Ligaria cuneifolia (Loranthaceae)	Sugar decrease	RR	Galetto et al., 1990
Linaria vulgaris (Scrophulariaceae)	Volume, sugar decrease	RR	Nepi et al., 2003
Mandevilla pentlandiana (Apocynaceae)	Volume decrease	RR	Torres & Galetto, 1998
Medicago sativa (Fabaceae)	Radioactive tracer	RR	Pedersen et al., 1958
Prunus sp., *Pyrus communis* (Rosaceae)	Radioactive tracer	H	Bieleski & Redgwell, 1980
Silene sp. (Caryophyllaceae)	Volume decrease	RR	Witt et al., 1999
Sinapis alba (Brassicaceae)	Radioactive tracer	RR	Masierowska & Stpiczyńska, 2005
Sophora fernandeziana (Fabaceae)	Volume, sugar decrease	RR	Bernardello et al., 2004
Streptosolen jamesonii (Solanaceae)	Radioactive tracer	RR	Shuel, 1961
Trifolium repens (Fabaceae)	Volume, sugar decrease	RR	Jakobsen & Kristjansson, 1994
Monocotyledons			
Aerangis verdickii (Orchidaceae)	Volume, sugar decrease	RR	Koopowitz & Marchant, 1998
Allium cepa (Alliaceae)	Sugar content decrease	RR	Kumar & Kumar Gupta, 1993
Aloe castanea (Asphodelaceae)	Volume, sugar decrease		Nicolson & Nepi, 2005
Mystacidium venosum (Orchidaceae)	Volume, sugar decrease	RR	Luyt & Johnson, 2002
Platanthera chlorantha (Orchidaceae)	Radioactive tracer	RR	Stpiczyńska, 2003a, b

African orchids *A. verdickii* (Koopowitz & Marchant, 1998) and *M. venosum* (Luyt & Johnson, 2002), the nectar from unpollinated flowers is not reabsorbed and after anthesis it is probably lost.

Using radio-labelled sucrose, Shuel (1961) demonstrated that part of the stigma exudate is derived from reabsorbed nectar and, conversely, sugar reabsorbed from the stigma exudate may appear in nectar. This suggests a general recycling of substances within the flower.

Nectar sugar reabsorption also plays an important ecological function, being involved in nectar homeostatic mechanisms (Galetto et al., 1994; Nicolson, 1995), the molecular basis of which is far from clear (Castellanos et al., 2002). Nectaries are supposed to have a "sugar sensing" mechanism for regulating nectar concentration. Sugar secretion may occur passively on the basis of a concentration gradient, while regulation of concentration in the apoplast could be achieved by sucrose hydrolysis and/or sugar reabsorption (Castellanos et al., 2002). It was hypothesized that reabsorption could be a response to modifications of cell turgor which in turn respond rapidly to changes in osmolality (Castellanos et al., 2002 and references therein).

The nectar homeostatic mechanism enables regulation of nectar volume, concentration, and thus viscosity, by reducing the effect of water loss due to evaporation. Since nectar composition and concentration are adapted to the type of animal visitor (Baker & Baker, 1983a), the nectar homeostatic mechanism may be important to ensure visits by the most efficient pollinator. Reabsorption of sugars reduces viscosity, which may facilitate nectar probing, as in *Penstemon gentianoides* (Scrophulariaceae; Cruden et al., 1983) and bird-pollinated flowers (Baker, 1975; Nicolson, 1995; Nicolson & Nepi, 2005). The nectar homeostatic mechanism is presumed to be more pronounced in plants with a long period of nectar presentation because they are presumably exposed to variations in weather conditions and pollinator visits that affect nectar characteristics. The homeostatic mechanism is in any case not very precise. A regulation system that compensates precisely for variations in nectar volume and concentration owing to pollinator activity, temperature, evaporation, water stress, light stress, etc., would presumably be metabolically expensive (Castellanos et al., 2002).

One would expect that plants living in dry habitats could compensate for water evaporation with solute reabsorption, thus maintaining a constant nectar concentration. This was demonstrated for *Grevillea robusta* (Proteaceae; Nicolson, 1995), but in *A. castanea* constant nectar concentration was

mainly maintained by very high production of dilute nectar throughout anthesis (Nicolson & Nepi, 2005).

On the other hand, the capacity to vary sugar concentration may be an adaptive character to ensure visits by a wider spectrum of pollinators, as in the case of *Catalpa speciosa* (Bignoniaceae; Cruden et al., 1983). Moreover, if a pollinator does not remove nectar, reabsorption may be a form of compensation for nectar theft. In *M. venosum*, nectar reabsorption at night followed a decline in pollinator activity (Luyt & Johnson, 2002). Nectar reabsorption may also reduce the negative effects of post-pollination visits, which have the potential to damage already pollinated flowers (Burqúez & Corbet, 1991).

Whereas several papers tackle the cyto-physiological mechanism of nectar production and secretion (see "Nectar secretion mechanism and models of nectary function" on page 168), very few consider nectar reabsorption (Nepi et al., 1996a; Nepi and Stpiczyńska, 2006). Nectar is generally reabsorbed by the nectary itself and according to Búrquez and Corbet (1991) this is why some plants that accumulate nectar in spurs or other types of reservoirs do not reabsorb nectar. However, reabsorption takes place in *L. Vulgaris* (Nepi et al., 2003), in which nectar flows from the nectary to the spur.

Nectar is not reabsorbed by all plants in which nectar remains in contact with the nectary. According to Búrquez and Corbet (1991), reabsorption seems to occur mainly in flowers whose nectaries remain attached to the plant after the corolla has fallen, or when the fall is delayed or the corolla wilts.

Epidermal cells are most involved in nectar reabsorption (Nepi et al., 1996a). Nectary stomata, from which nectar often exudes, do not seem to be involved in the process (Nepi & Stpiczyńska, 2007). Transport of reabsorbed nectar sugars from the epidermis to parenchyma cells may occur via apoplast or symplast (Nepi & Stpiczyńska, 2007) and reabsorbed sugars may be stored temporarily as starch grains in amyloplasts (Nepi et al., 1996a; Wist & Davis, 2006). Reabsorbed sugars from a flower nectary are mainly translocated to the nearest developing ovule or ovary (Nepi & Stpiczyńska, 2007). They may go even longer distances—in *P. chlorantha*, reabsorbed sugars seem to be utilized in the whole inflorescence, which has flowers at different stages of development; they can be translocated upward or downward as far as 12.5 cm towards growing ovaries (Nepi & Stpiczyńska, 2007).

In *Medicago sativa*, reabsorbed sugars can be found in roots and leaves (Pedersen et al., 1958).

We do not know if soluble substances in nectar are reabsorbed simultaneously or at different times. The molecular weight and chemical nature of the substances may influence the temporal pattern of reabsorption.

3.2 Nectar standing crop

Different environmental parameters, dynamics of nectar production and reabsorption, animal foraging activity, and their interactions contribute to nectar standing crop. This can be considered the recent and current interaction between a population of flowers, a population of foragers and environmental parameters such as temperature and relative humidity (RH) (Corbet, 2003).

Nectar standing crop is defined by Kearns and Inouye (1993) as the "quantity and distribution of nectar determined by randomly sampling flowers, that have not been protected from pollinators by bagging, at a given moment". From the ecological point of view this parameter is fundamental; in fact, there is a reciprocal dependent relationship between the nectar standing crop and animal visits: the foraging behaviour of visiting animals is affected by standing crop, which is in turn affected by animal activity. The distribution of the standing crop within a plant or within a population may show some spatial patterning (Kearns & Inouye, 1993 and references therein; Corbet, 2003 and references therein). Nectar standing crop was patchily distributed within individual plants of two boraginaceous species, *Anchusa strigosa* (Shmida & Kadmon, 1991) and *Echium vulgare* (Leiss & Klinkhamer, 2005a). This implies that nectar volumes of neighbouring flowers were positively correlated with each other and that differences in volume of nectar between pairs of neighbouring flowers were significantly higher than differences between flowers of the same pair. Because there is evidence that foragers may selectively visit the more rewarding flowers (Corbet et al., 1984; Kadmon, 1992), the spatial patterning of the standing crop has a great influence on pollinator foraging movements among flowers of a plant or among plants of the same population (Kearns & Inouye, 1993; Corbet, 2003). Shmida and Kadmon (1991) discussed the effect of within-plant patchiness in nectar standing crop on the foraging behaviour of pollinators. They argued that if nectar is patchily distributed within plants, foragers encountering nectar-rich flowers will move to neighbouring flowers, while foragers encountering nectar-poor flowers will move longer distances in order to avoid visits to neighbouring flowers. Under such circumstances the

encountered crop, i.e., the standing crop encountered by pollinators foraging systematically, is likely to exceed the mean standard crop measured by an unselective ecologist who randomly samples flowers (Kadmon, 1992; Corbet, 2003). Shmida and Kadmon (1991) pointed out that the mean nectar standing crop per flower, the variance in nectar standing crop, and the scales of variation contributing to the total variance, may all vary considerably at different times during the day, so that a forager returning to the same patch after a short time may encounter a completely different pattern of nectar distribution.

The spatial distribution of nectar production in a population may have a genetic basis (Leiss et al., 2004; Mitchell, 2004) and it may also be affected by small-scale variations of environmental parameters (water availability, air humidity, temperature, and light) (Leiss & Klinkhamer, 2005a).

4 THE SOURCE OF NECTAR COMPONENTS

Sexual reproduction represents a strong investment of resources. Vegetative growth slackens or ceases when sexual reproduction is starting. This is particularly evident in annual plants. Resources for basal metabolism, on the other hand, are always kept constant (Wardlaw, 1990).

The demand for resources for reproduction, in the absence of predation, increases continuously until fruit ripens, because of increases in biomass. The plant invests in the different parts of the flower and later fruit, but some of these parts (pedicel, calyx, corolla, stamens, ovary, fruit integuments) are green, contain chloroplasts and may photosynthesize and self-sustain, sparing the mother plant resources.

Hoch (2005) demonstrated experimentally that developing fruits of some European trees "exhibited complete carbon autonomy of fruiting at the level of whole, undisturbed branchlets". We do not know how much this self-sustainability applies to other phases of reproduction, types of plants, and environments; nevertheless, it represents a matter for speculation. The presence of some green and photosynthesizing reproductive structures in flowers induces us to think that they may be autonomous, at least for part of the carbohydrates.

Nectary formation and nectar secretion have a high energy demand. Southwick (1984) and Pyke (1991) quantified the energy necessary to produce nectar and demonstrated that more than 30% of daily photosynthate is

used by the nectary to produce nectar. Notwithstanding the problem of partial autonomy of the flower, the various researchers working on nectar biology commonly have not considered the sources of the different nectar components. Pacini et al. (2003) showed that nectar components may have different sources, depending on the type of nectary and its cytological structure.

Certain previous researchers (Frey-Wyssling, 1955; Fahn, 1979b) working on nectary cytology and physiology attributed much importance to phloem sap as precursor of pre-nectar. According to these authors, pre-nectar is transformed into nectar by nectary parenchyma cells.

The two main soluble nectar components are carbohydrates and amino acids/proteins. These have different origins according to secretion type. If secretion is of the merocrine type, at least part of the two main components are derived from phloem or xylem sap transformed by nectary parenchyma. In the case of holocrine secretion, nectar components may be derived from xylem and phloem sap and nectary cell cytoplasmic content.

Nectar carbohydrates are directly or indirectly derived from the photosynthetic activity of the nectary itself or of other floral or vegetative parts, generally close to the flower. If the nectary is responsible for the formation of part of the nectar carbohydrates it must have a photosynthesizing part. This photosynthesis may occur in the parenchyma or in cells close to it (subnectary parenchyma, Nepi, 2007). The parts most often involved in the production of nectar carbohydrates by photosynthesis are the flower pedicel, calyx, ovary, and even adjacent leaves. This is, however, only a hypothesis, as no conclusive demonstration is yet available.

These inner carbohydrate sources of nectar (coming from the nectary parenchyma itself) and outer sources (coming from tissues situated outside the nectary, mentioned previously) cannot generally be considered mutually exclusive.

The presence of phloem or xylem in the floral nectary complex may indicate whether a nectary is partially or totally autonomous, but the little research on this topic is inconclusive. Authors sometimes omit to describe the type of vascular bundle when they describe the structure of a nectary. The presence of phloem only, or both xylem and phoem, their relative abundance and disposition have a physiological meaning, i.e., transport of complex molecules such as sucrose, amino acids, and soluble proteins. Only phloem is present in *B. napus* (Brassicaceae; Davis et al., 1986), *V. faba* (Fabaceae;

Davis et al., 1988), *Digitalis purpurea* (Scrophulariaceae; Gaffal et al., 1998), and *Cyclanthera pedata* (Cucurbitaceae; Pacini et al., 2003). Phloem and xylem are present in *Capparis retusa* (Brassicaceae; Di Sapio et al., 2001), *Ecballium elaterium* (Cucurbitaceae; Fahn & Shimony, 2001), *Solanum stramonifolium* (Solanaceae; Falcão et al., 2003), and *Hexisea imbricata* (Orchidaceae; Stpiczyńska et al., 2005a) (see also the examples reported by Fahn (2000) and Nepi (2007)).

Only phloem is present in the extrafloral stipular nectaries of *Passiflora* sp. (Passifloraceae; Durkee, 1982) and *Sambucus nigra* (Caprifoliaceae; Fahn, 1987). Both phloem and xylem are present in those of *R. communis* (Euphorbiaceae; Baker et al., 1978), *Euphorbia neriifolia* (Euphorbiaceae; Arumugasamy et al., 1990), and *V. faba* (Fabaceae; Davis et al., 1988) .

No definitive conclusions can be drawn from the site of origin of the nectar components; however, nectary parenchyma is certainly the site where these components are transformed into nectar, and nectar carbohydrates are undoubtedly unloaded from the phloem or partly produced by chloroplasts of the subnectary parenchyma cells.

There are several reasons why nectar components may have different anatomical and histological origins:

- Location of nectaries in different parts of the flower
- Production of nectar for different types of consumers, with different compositions
- The environment in which the plant grows
- Time of flowering during the year
- Time of exposure of the nectary during the day

Table 2 shows the different components of nectar and their probable origins and functions.

It is reasonable to believe that the more the nectary is exposed to sunlight, the higher the contribution of nectary photosynthesizing parenchyma to nectar carbohydrates. In Araliaceae and Apiaceae, nectaries are located in the upper part of the ovary (Bernardello, 2007, p. 83). Vezza et al. (2006) studied the nectary biology of ivy (*Hedera helix*, Araliaceae), which has an exposed nectary, and demonstrated that photosynthetic pigments in the nectary and in leaves adjacent to the inflorescence are qualitatively similar but quantitatively diffferent: leaves contain a larger amount of photosynthetic

Table 2. Common substances contained in nectar and their possible origins and functions. Some of these substances may have different origins according to the type of nectar secretion (i.e., holocrine, granulocrine, or eccrine) because they involve the activity or degeneration of different cell types. The presence of antioxidants becomes a necessity in the case of exposed nectar presented for long periods.

Substances	**Possible origin**	**Functions**
Water	Xylem and/or phloem sap	A medium in which soluble substances can dissolve A reward, especially in dry ecosystems
Ions	Xylem and/or phloem sap	
Carbohydrates	Phloem sap, photosynthesizing and/or starch storing parenchyma	A reward for pollinators
Amino acids and soluble low molecular weight proteins	Phloem sap and/or a product of the activity of certain nectary parts (parenchyma or epidermis)	A reward for pollinators
Enzymes	Certain nectary parts (parenchyma or epidermis)	Responsible for secretory and post-secretory modifications
Lipids	Certain nectary parts (parenchyma or epidermis)	A reward for pollinators and a way to reduce water evaporation when lipids form a surface layer
Volatile substances	Certain nectary parts (parenchyma or epidermis)	Involved in attraction or repulsion of visitors
Toxic compounds (e.g., alkaloids, phenols)	Certain nectary parts (parenchyma or epidermis)	To discourage certain consumers and to reduce the potential visitors
Antibiotics	Certain nectary parts (parenchyma or epidermis)	To reduce growth of moulds and bacteria
Antioxidants	Certain nectary parts (parenchyma or epidermis)	To avoid oxidation of substances such as lipids

pigments than the nectary. Nevertheless, this research did not demonstrate whether this type of nectary is completely autonomous for nectar carbohydrates.

When a nectary lies below the ovary (Ericaceae, Scrophulariaceae) or forms a protuberance at the base of the ovary (Brassicaceae, Fabaceae), where it is often covered by the calyx, corolla, or stamen filaments, it is more difficult to believe that it is totally self-sustaining, even if its parenchyma cells contain chloroplasts. This could be possible only if the photosynthetic pigments "concentrate" the little available light. In this case we expect to find

nectary parenchyma photosynthetic pigments different from those of adjacent leaves.

If we consider recent research on these topics, it is clear that there have been no conclusive studies on the sources of nectar components. Defoliation or light deprivation experiments make it possible to determine the relative contribution to nectar of recently produced photosynthates, as opposed to stored assimilates. In *I. glandulifera* (Balsaminaceae), Búrquez and Corbet (1998) used exclusion of nectar consumers and defoliation experiments to determine that nectar carbohydrates have two sources: a part derived directly from daily photosynthesis and another derived from stored carbohydrate photosynthate. However, they do not say whether photosynthesis occurs inside or outside the flower, and also do not consider that photosynthetic parts may be present in the flower or even in the nectary itself.

5 ECOPHYSIOLOGICAL SIGNIFICANCE OF PARENCHYMA PLASTIDS

The nectary parenchyma may have amyloplasts or chloroplasts, containing only a few stacks of grana and small starch granules, most probably involved in low photosynthesis. This is the site where pre-nectar is transformed into nectar and subsequently released outside. The subnectary parenchyma may also contain chloroplasts or amyloplasts. Nectaries with amyloplasts in the nectary and subnectary parenchyma cells seem less common than those with chloroplasts. In addition, all extrafloral nectaries described ultrastructurally have chloroplasts in their parenchyma (Pacini et al., 2003).

There are several papers on the ultrastructure of nectaries in which the authors show chloroplasts, recognized by the presence of grana stacks and plastoglobules. Chloroplasts may contain small or large starch grains (Razem & Davis, 1999; Baum et al., 2001; Horner et al., 2003) or may even be empty (Stpiczyńska & Matusiewicz, 2001; Stpiczyńska et al., 2003). Nevertheless, the authors of these studies do not approach the problem of whether nectar sucrose, or the other carbohydrates derived from chloroplast photosynthesis are:

1. Immediately conveyed to form nectar without being polymerized to starch
2. Polymerized to small starch grains to be hydrolysed later in the day
3. Polymerized to form big starch grains hydrolysed during the night

The third hypothesis is the mechanism commonly invoked for leaf chloroplasts, but the first and the second are possible modifications for rapid production and use of nectar carbohydrates derived immediately and directly from photosynthesis.

Chloroplasts and amyloplasts differ in morphology and physiology during development and nectar secretion. Amyloplasts change during their development because the starch content increases before secretion and decreases during secretion. Secretion may only last a few hours. Chloroplasts change during the day and night because they store starch during the day and starch is hydrolized during the night. This implies that ultrastructural observations must be done at different times of day in order to determine the pathway of plastid differentiation. Available ultrastructural observations rarely consider plastid morphology changes in time. Night observations are totally absent.

The environment may affect nectar production irrespective of plastid type in the nectary parenchyma (chloroplasts without starch, chloroplasts with starch grains, amyloplasts), but the effects of environmental conditions act at different times. If nectary parenchyma cells have chloroplasts, nectar production is affected by immediate environmental conditions, whereas it is affected by the environmental conditions of previous days when nectary parenchyma cells contain amyloplasts.

An advantage to having nectary parenchyma with chloroplasts is that it continues to function when nectar secretion is over (persistent nectary), conveying photosynthate to other parts of the flower or fruit. Other advantages and disadvantages of the two types of plastids in nectary parenchyma are listed in Table 3.

On the other hand, an advantage of nectaries with amyloplasts in the nectary parenchyma cells is that nectar may be available for consumers, and in large quantities, at any time of the day or night. The nectar may also have a high sugar concentration and can be produced in a short time. In the case of *C. pepo*, nectar becomes available from 6 am and has a high sugar concentration (30–40%). This high rate of nectar production is impossible with nectary parenchyma containing chloroplasts. In *C. pepo*, nectar is secreted for only 3–4 h but the nectary does not immediately degenerate. Empty amyloplasts may be involved in temporary storage of reabsorbed carbohydrates if the nectar is not totally consumed by flower visitors (Nepi et al., 1996a, b). Unconsumed nectar carbohydrates are temporarily polymerized to spherical electron dense bodies which react positively to the PAS test for total insoluble

Table 3. Advantages and disadvantages of photosynthetic and non-photosynthetic nectaries. The same volume of nectary parenchyma has a higher nectar production rate when nectar is produced by hydrolysis of stored starch than by direct photosynthesis.

		Advantages	Disadvantages
Nectary parenchyma	**Photosynthetic** (chloroplasts)	Nectar can be produced for days or months as in extrafloral nectaries	Nectar carbohydrates are produced and presented for consumption only in the day if starch is hydrolysed only at night
		Nectar carbohydrates at least partly originate from photosynthesis by nectary parenchyma	Nectar production rate is low and proportional to nectary parenchyma volume
		Nectar carbohydrates are produced close to the presentation site	Nectar sugar concentration rarely reaches high values
		Nectary may continue photosynthesis when nectar production is over, to the benefit of developing fruit	The nectary can be a portal for pathogens because of long nectar production*
		Nectar removal by insects may lead to further nectar production	
	Non photosynthetic (amyloplasts)	Nectar may be exposed to consumers at any time of day	Nectar is derived from photosynthesis by green floral parts, bracts or leaves close to the flower which are affected by environment
		Large amounts of nectar can be produced in a short period of time	Nectaries commonly fall after nectar secretion and any unconsumed nectar has been reabsorbed
		It is possible to produce nectar with a high sugar concentration	No further nectar can be produced after consumption
		Reabsorption of unconsumed nectar may occur by the nectary itself and empty amyloplasts may temporarily store nectar carbohydrates	

*The presence of antimicrobial proteins avoids this disadvantage.

polysaccharides, but do not show the blue colour of the IKI test, i.e., the typical reaction for starch (Nepi et al., 1996a). Afterwards these plastids empty and the carbohydrates are probably totally reabsorbed by contiguous parts of the flowers. The nectary then abscisses. In this case it seems that empty amyloplasts are important as storage sites during the reabsorbing process.

This pattern of nectary plastid development (from proplastids to amyloplasts) could be more common in the tropics, where more animals are active at night, than in temperate zones. No research has been done into these aspects. Degeneration of nectaries with amyloplasts at the end of secretion or after nectar reabsorption seems to be a general feature. In *Aloe* and *Gasteria*, which have septal nectaries, dedifferentiation of amyloplasts to chloroplasts is recorded (Schnepf & Pross, 1976; Nepi et al., 2006). This dedifferentiation enables transformation of nectary parenchyma into fruit parenchyma.

Research on plastid development mainly concerns floral nectaries. As far as is known extrafloral nectary parenchyma cells have only chloroplasts (Table 4). The presence of chloroplasts in extrafloral nectaries allows production of low quantities of nectar over long periods: several weeks to months, like the age of the leaf where they are situated. This nectar is consumed by ants that protect the plant from predators. Floral nectaries differ widely according to the dynamics of nectar production and plastid differentiation patterns, because they are visited by a wider spectrum of consumers and may reabsorb unconsumed nectar (Table 4).

6 NECTAR PRESENTATION

6.1 Floral nectaries

Fahn (1979a) recognized three different positions of floral nectaries with respect to the organ bearing them:

- At surface level
- Forming an outgrowth, as in Brassicaceae and Fabaceae
- Sunken, as in the ovary septal nectaries of monocots

These different positions influence the structure of the nectary and its persistence, the manner of nectar secretion, nectar presentation, and the

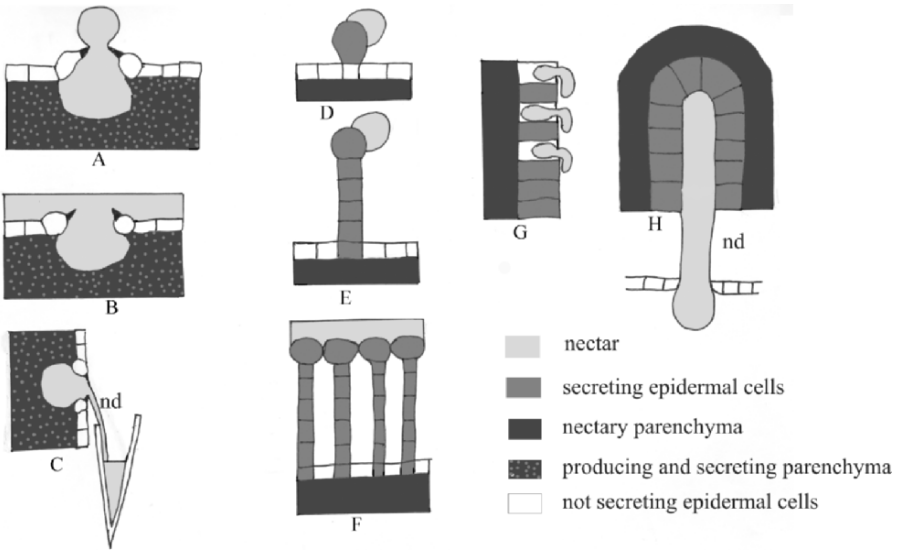

Figure 3. Common types of nectar secretion and presentation. In A, B, and C, parenchyma cells produce and secrete nectar. In G and H, the secretory function is performed by epidermal/epithelial cells, and by trichomes in D, E, and F. Nectar may be presented as drops (A, D, E) or as a continuous layer of variable depth (B, F, G). Nectar may be presented outside the nectary (secondary presentation) in a spur (C) or at the end of a nectary duct (nd) as in H. A: *Fatsia japonica* (Araliaceae; Nepi & Pacini unpublished data); B: *Cucurbita pepo* (Cucurbitaceae; Nepi et al., 1996a, b); C: *Linaria vulgaris* (Scrophulariaceae; Nepi et al., 2003); D: *Lonicera japonica* (Caprifoliaceae; Fahn, 1979a); E: *Zeyheria* (Bignoniaceae; Bittencourt & Semir, 2004); F: *Hibiscus* (Malvaceae; Sawidis, 1987a); G: *Helleborus* (Ranunculaceae; Vesprini et al., 1999); H: *Aloe* (Asphodelaceae; Nepi et al., 2006).

foraging pathways of pollinators. Figure 3 illustrates the main types of nectar secretion and presentation.

Pacini et al. (2003) proposed the new term **nectar presentation** in analogy to the better-known term **pollen presentation** (Faegri and van der Pijl, 1979), to indicate how and where nectar is offered for consumption. Nectar presentation can be further subdivided into **primary presentation**, when nectar is offered in the nectary itself, and **secondary presentation** when it is presented elsewhere, e.g., stored in spurs or other reservoirs.

Spurs are cavities commonly derived from the corolla and are present in at least 15 angiosperm families (Hodges, 1997; Bernardello, 2007). In certain families, such as Scrophulariaceae, spurs are typical of almost all members; in other families, such as Ranunculaceae, they may occur in some members only (the genera *Aconitum*, *Aquilegia*, *Delphinium*, *Consolida*, and *Nigella*).

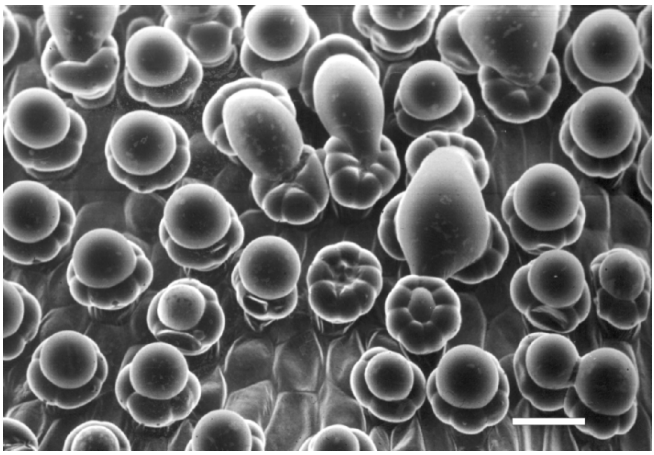

Figure 4. Nectar exudation in *Cyclanthera pedata.* Nectar is secreted asynchronously by multicellular capitate trichomes and exuded through ruptured cuticles. Bar = 180 μm.

Spurs may be directed upwards as in Ranunculaceae, or downwards as in *L. vulgaris* (Scrophulariaceae) and many orchids.

The "cuculli" of *Asclepias* (Kevan et al., 1989) are another type of nectar reservoir—here the nectar flows from the nectary by a capillary system. Vogel (1998) describes auxiliary structures, named nectar ducts, the function of which is to conduct nectar from the source towards the site of presentation, as in septal nectaries (Fig. 3). Nectar flows along these ducts driven by capillary forces, secretion pressure, and gravity, depending on the orientation of the nectary and the organs bearing it.

The presence of spurs may imply one or more of the following:

- Protection against evaporation
- Consumption by a limited number of animals with long sucking mouthparts that can reach into the spurs
- Protection against contamination by fungal spores and bacteria
- Long exposure (several days), increasing the chances of the flower being visited, especially when pollinators are few as is the case of many orchids (Neiland & Wilcock, 1995)

These hypotheses hold not only for nectar stored in spurs, but also when nectar is hidden in corolla tubes or septal nectaries. Nectar presentation may be a species-specific characteristic and may vary even in allied species or members of the same family. Different types of nectar presentation are associated with certain advantages and disavantages (Table 5).

Table 4. Flush diagram showing some differences between floral and extrafloral nectaries. The type of plastid in the nectary parenchyma cells is associated with different investments of the plant for insect attraction to the flower.

<table>
<tr><td colspan="4">Nectary parenchyma with chloroplasts (photosynthetic parenchyma)
Nectar carbohydrates are at least partially derived from photosynthesis</td><td colspan="2">Nectary parenchyma with amyloplasts
(non-photosynthetic parenchyma)
Nectar carbohydrates are derived from amyloplast starch hydrolysis</td></tr>
<tr><td colspan="2">Extrafloral nectaries
(visited mainly by ants)</td><td colspan="2">Floral nectaries (visited mainly by bees and allied Hymenoptera, Diptera, Lepidoptera)</td><td colspan="2">Floral nectaries (visited mainly by bees and allied Hymenoptera, Diptera, etc.)</td></tr>
<tr><td>On leaves</td><td>On ovary/fruit</td><td rowspan="2">Nectaries fall or degenerate after secretion lasting only a few days (Brassicaceae, Fabaceae, Helleborus sp.)

Nectar flows into a spur; if not consumed it is reabsorbed by the spur wall (Linaria vulgaris)</td><td rowspan="2">Nectaries persist after the end of secretion, which lasts from a few hours to several days, chloroplasts continue to photosynthesize but their products are presumably conveyed to developing fruit

Nectar is offered to consumers in the nectary; unconsumed nectar is lost to the plant (Hedera helix)</td><td rowspan="2">Unconsumed nectar is reabsorbed by the nectary itself or other floral parts (Cucurbita pepo)</td><td rowspan="2">Unconsumed nectar is not reabsorbed and is lost to the plant (Fatsia japonica)</td></tr>
<tr><td>Secretion lasts from several weeks (annual plants) to several months or more (perennial plants) (Ricinus communis)</td><td>They become active and produce nectar during fruit growth (Tabebuia serratifolia, Guarea macrophylla)</td></tr>
</table>

Table 5. Advantages and disadvantages of exposed and partially or completely protected nectar. In the case of exposed nectar, evaporation may positively or negatively affect the response of pollinators, thus facilitating different types of consumers.

	Advantages	**Disadvantages**
Exposed	• Nectar may be collected by several types of pollinators because it is accessible. • Nectar concentration varies with temperature and RH and is therefore suitable for different pollinators. • Photosynthetic nectary parenchyma receives enough light to be autonomous for carbohydrate synthesis.	• Nectar can be easily plundered. • Nectar concentration is profundly affected by environmental parameters; nectar may crystallize due to evaporation and cannot be collected. • Fungal spores, bacteria, and airborne material may fall into the nectar, triggering infections and damage in the absence of antibiotic devices. • Rain and heavy mist may remove nectar or dilute it. • Nectar reabsorption is difficult or impossible because of evaporation.
Inside the nectary and/or the flower	• Nectar concentration is relatively unaffected by environmental parameters. • Nectar is protected from fallout of airborne material. • Nectar is not removed by rain or mist.	• Nectar guides become necessary to advertise its presence. • Nectar may be accessible to only one type of pollinator: species-specific pollination. • Photosynthetic nectary parenchyma receives little light if shaded by floral parts.
Inside a spur	• Nectar concentration is relatively unaffected by environmental parameters. • Nectar reabsorption may occur over a long period because evaporation is limited. • Nectar is not removed by rain or mist.	• Nectar collected in the spur may be stolen by animals with mouthparts that can pierce the spur wall. • Nectar is accessible only to a few pollinators having specialized mouthparts. • Number of potential pollinators decreases with increasing spur length.

Nectar always comes to the surface of the nectary in microdrops, irrespective of its manner of secretion, whether secreted by modified stomata that have lost their capacity to open and close (Davis, 1997), formed at the tip of secreting hairs (as in many Malvaceae and some Cucurbitaceae, e.g., *Cyclanthera*, *Sechium*), or derived from degeneration of single cells as in *Helleborus* (Vesprini et al., 1999) (Fig. 3). These microdrops successively fuse to form bigger drops that remain *in situ* (Fig. 4), slide down a vertical surface, or form a flat surface.

The different ways of nectar emergence and presentation may be related to one or more of the following:

- Nectary position in the flower
- Nectary accessibility from outside
- The path of flower visitors
- Mouthparts of flower visitors
- Number of ovules per ovary

6.2 Extrafloral nectaries

Extrafloral nectar, unlike floral nectar, is always presented on the surface of the nectary (primary presentation), and there are no reports of secondary presentation. The most common extrafloral nectaries are situated on leaves and stems, rarely on fruit. Irrespective of position, the nectar secreted is always derived directly from photosynthesis by the nectary or other contiguous tissues, generally without storage of starch. This means that the nectar is always produced during the day and in small quantities. Floral nectar can often be seen as drops by the naked eye, whereas extrafloral nectar, owing to its reduced volume, is not perceived as drops but as a shiny surface. The nectar generally does not flow and is rarely lost. In at least some species, feedback occurs—no further nectar is produced if that present is not collected (Cruden et al., 1983 and references therein).

7 FATE OF NECTAR AND NECTARIES

Secreted nectar has different fates. It can either

1. Be consumed by a pollinator
2. Be consumed by a nectar thief
3. Drop from the flower
4. Remain in the nectary or flower if not removed

The nectar investment is sucessful in the first case, but unsuccessful in cases 2 and 3 where the environment benefits from the nectar. In case 4 there are different possibilities depending on the programme of the plant. Nectar theft is more common when the nectary is exposed, although nectar predation is not unknown in hidden nectaries or when nectar has a secondary presentation.

When nectary parenchyma has chloroplasts, there are two possibilities at the end of secretion: the nectary either abscisses or persists. Abscission occurs if the nectary is a small inconspicuous protuberance, as in many members of Brassicaceae, Fabaceae, and Asteraceae (Horner et al., 2003). Persistence means that photosynthesis by the nectary parenchyma continues, though products of photosynthesis are shifted to benefit the developing fruit. This calls for a rearrangement in the manner of conveying parenchyma photosynthate, i.e., a reorientation of cell polarity and flux must occur in phloem cells. Table 6 presents some examples of modes of nectar secretion, nectar reabsorption, and nectary and nectar fates.

8 VARIABILITY OF NECTAR CHARACTERISTICS

The physicochemical characteristics of nectar vary with biotic and abiotic parameters. In general, the longer the flowering period of a species, the wider the variations in nectar physicochemical properties (Nepi et al., 2003). Similarly, the longer the period of flower anthesis, the wider the variations in nectar properties (Vesprini et al., 1999).

There is a mass of literature demonstrating interspecies variability of nectar properties in terms of volume, solute concentration (and thus total sugar production), and composition. These differences have been interpreted as adaptive: pollinator behaviour favours certain traits over others (Faegri & van der Pijl, 1979; Baker & Baker, 1983a; Cruden et al., 1983; Cnaani et al., 2006). Differences in nectar volume are explained on the basis of a cost–benefit balance: plants are most fit when they produce the lowest volume of nectar that will attract their pollinator (Lanza et al., 1995 and references therein). In this respect, species pollinated by large animals such as birds and bats produce more nectar than species pollinated by smaller animals such as bees, bumblebees, butterflies, and moths (Baker & Baker, 1983a). Differences in nectar concentration are interpreted as a balance between different factors: pollinator preference for high concentration, pollinator difficulty in handling viscous solutions and plant energy allocation patterns that minimize the cost of nectar production (Baker and Baker, 1983a, Zimmerman, 1990). Differences in composition are attributed to pollinator preferences linked also to alternative sources of food (Baker & Baker, 1983a, 1986).

Table 6. Different pathways of floral nectar production, secretion and post-secretion in some representative species. Nectar reabsorption may occur in photosynthesizing and non-photosynthesizing nectaries; the latter continue photosynthesis even when nectar secretion is over to produce substances for ovary growth.

Species	Presecretory phase	Secretory phase	Nectar presentation	Post-secretory phase
Brassica (Brassicaceae) (Davis et al., 1986)	Parenchyma cells photosynthesize. The flower has two types of nectaries	Nectar is derived partly from photosynthesis by the parenchyma cells producing dimorphic nectar for 2–3 days. Nectar flows from stomata	Nectar, irrespective of nectary type, is presented on the surface of the nectary (primary presentation)	Nectar may be reabsorbed. The two types of nectary fall at the end of anthesis
Cucurbita pepo (Cucurbitaceae) (Nepi et al., 1996a)	Parenchyma cells accumulate starch inside amyloplasts	Amyloplast starch is hydrolysed and nectar flows through stomata, this phase lasts about 3–4 h Nectary cells behave synchronously	Nectar is exposed inside the flower, in a thin continuous layer for 6 h (primary presentation)	Unconsumed nectar is reabsorbed via the epidermis and parenchyma plastids re-accumulate polysaccharides, nectary falls after reabsorption
Eucalyptus sp. (Myrtaceae) (Davis, 1997)	Parenchyma cells photosynthesize	Secretion through stomata lasting 10–15 days according to species	Nectar is presented as coalescing drops on the surface of the nectary (primary presentation)	Unconsumed nectar can be reabsorbed
Hedera helix (Araliaceae) (Vezza et al., 2006)	Parenchyma cells photosynthesize	Nectar is derived partly from photosynthesis by parenchyma cells that produce nectar for 5–6 days. Nectary cells behave synchronously	Nectar is directly exposed to the environment and easily collected, predated, or concentrated by evaporation (primary presentation)	Unconsumed nectar can crystallize if not collected, especially if the environment is dry. Photosynthesis of nectary continues even when nectar secretion is over but photosynthate is probably used for ovary growth

Species	Presecretory phase	Secretory phase	Nectar presentation	Post-secretory phase
Helleborus foetidus (Ranunculaceae) (Vesprini et al., 1999)	Parenchyma cells photosynthesize	Nectar is derived partly from photosynthesis by parenchyma cells that produce nectar for about 10 days; it flows from epidermal cells that die during holocrine secretion Parenchyma cells are synchronous, epidermal cells are not	Nectar fills the nectary cavity; some is lost because it falls down (primary presentation)	Photosynthesis of the nectary continues even when nectar secretion is over but photosynthate is probably used for ovary growth
Linaria vulgaris (Scrophulariaceae) (Nepi et al., 2003)	Parenchyma cells photosynthesize	Nectar is derived partly from photosynthesis by parenchyma cells, which produce nectar for 2–3 days; it flows from stomata Nectary cells behave synchronously	Nectar flows in the spur from where it may be collected by pollinators or stolen (secondary presentation)	Unconsumed nectar is reabsorbed by the spur inner epidermis. Photosynthesis by the nectary continues even when nectar secretion is over and photosynthate is presumably used for ovary growth
Lonicera japonica (Caprifoliaceae) (Fahn, 1979b)	Starch accumulates in the secretory papillar cells	Starch is hydrolysed and nectar accumulates between cuticle and cell walls that have wall protuberances (transfer cells). Secreting cells are asynchronous and secretion lasts only a few days	Nectar is secreted through unicellular trichomes and accumulates at the base of the tubular corolla (primary presentation)	Nectary degenerates

Species	Presecretory phase	Secretory phase	Nectar presentation	Post-secretory phase
Vicia faba (Fabaceae) (Davis & Gunning, 1992; Stpiczyńska, 1995)	Parenchyma cells photosynthesize	Nectar is derived partly from photosynthesis by parenchyma cells and is secreted through stomata for 1–1.5 days	Nectar is presented on the surface of the nectary (primary presentation)	Nectary falls at the end of anthesis
Aloe sp. (Asphodelaceae) (Nepi et al., 2006)	Parenchyma cells photosynthesize and starch accumulates in chloroplasts	Nectar is derived from starch hydrolysis and is secreted through transfer cells Nectar accumulates in the septal nectary and flows through nectar ducts	Nectar is presented in the corolla tube (secondary presentation)	Photosynthesis by nectary continues even when nectar secretion is over but photosynthate is used to feed ovary growth
Platanthera chlorantha (Orchidaceae) (Stpiczyńska, 2003a)	Parenchyma cells accumulate starch in amyloplasts	Nectar is secreted by trichomes for 14–17 days	Nectar is contained in a spur covered by the secretory tissue (primary presentation)	Unconsumed nectar may be reabsorbed by the nectary

8.1 Environmental variables

Enviromental parameters can affect nectar properties. Nectar, especially from exposed nectaries, tends to reach a concentration in equilibrium with the RH of the air (Corbet et al., 1979): low RHs tend to cause water evaporation and concentrate nectar, while very high RHs tend to dilute nectar. However, the nectar of unprotected flowers of several species does not reach the high concentration values that would be in equilibrium with low RH (Corbet et al., 1979). At a given RH the rate at which evaporation elevates solute concentration is inversely related to the size of the nectar drop (Corbet, 2003). The effect of RH can be reduced by the following:

- Morphological features of flowers that offer more protection to the nectar (e.g., long corolla tubes, spurs).
- Waterproofing lipid monolayers on the nectar surface, as hypothesized by Corbet et al. (1979) in *E. vulgare.*
- Reabsorption of sugar (Nicolson, 1995).
- Constant high-secretion rate of diluted nectar (Nicolson, 1995; Nicolson & Nepi, 2005).
- A combination of these factors—reabsorption of sugar and a very high secretion rate of diluted nectar seem to be common strategies to maintain low sugar concentration in dry habitats (Nicolson, 1995; Nicolson & Nepi, 2005).

Temperature is the environmental variable that is most often cited as related to nectar secretion rate. Temperature affects the rate of photosynthesis that contributes, directly or indirectly, to nectar production (Burquéz & Corbet, 1991, 1998). Nectar secretion decreases at low temperature in most species but decreasing nectar production is also reported with increasing temperature in *Ipomopsis longiflora* (Polemoniaceae; Freeman & Head, 1990) and *Trifolium repens* (Fabaceae; Jakobsen & Kristjansson, 1994). The optimum range of temperature for nectar secretion is known in only a few species (Jakobsen & Kristjansson, 1994 and references therein; Nicolson, 1995; Petanidou & Smets, 1996 and references therein). In Mediterranean plants, nectar secretion is adapted to higher temperatures (Petanidou & Smets, 1996; Petanidou, 2007); e.g., optimal nectar secretion in *Thymus capitatus* Lamiaceae) is at 32.5°C. When this plant is grown at lower temperatures, nectar production is a function of light intensity (Petanidou & Smets, 1996).

In natural conditions, the best nectar yields may occur in years with high precipitation (Petanidou & Smets, 1996). Water availability has long been invoked as a major factor in the regulation of nectar secretion rate (Wyatt

et al., 1992; O'Brien et al., 1996); in *Impatiens glandulifera*, however, it is apparently without any major effects (Burquéz & Corbet, 1998).

Elevated CO_2 levels significantly stimulate nectar secretion rates, increasing nectar volume rather than sugar content in *Tropaeolum majus* (Tropaeolaceae; Lake & Hughes 1999) and *Cucumis melo* (Cucurbitaceae; Dag & Eisikowitch, 2000). However, Davis (2003) and Erhardt et al. (2005) found that the effect of elevated CO_2 levels and increased UV-B radiation on nectar production varied from species to species, making it difficult to generalize. The relationship between these two global changes (increased CO_2 and UV-B levels) and nectar characteristics is intriguing from the ecological point of view because it may modify the behaviour of foraging animals.

Soil nutrients may also affect nectar production. Shuel (1955) demonstrated that nectar yield per flower in *Antirrhinum majus* (Scrophulariaceae) was comparatively good under conditions of low nitrogen supply and moderate growth. Gardner and Gillman (2001) found that fertilizer treatments increased the concentration of proline and glutamine in nectar of *Agrostemma githago* (Caryophyllaceae) but had no effect on other amino acids.

8.2 Intraspecies variability

Broad intraspecies variability of nectar characteristics is reported by numerous authors. Intraspecies variability of nectar features may be revealed at different levels: in individual flowers, between flowers of the same plant, between plants of a population, and between populations.

Variability within individual flowers occurs in several species of Brassicaceae that have a compound heterogeneous nectary—the nectarium (Davis et al., 1998)—composed of two lateral and two median nectaries. The lateral nectaries have relatively rich quantities of phloem bundles that penetrate the secretory tissue, and produce much more nectar sugars than the median nectaries (Davis et al., 1998). Intraflower variability is also observed in *Helleborus foetidus* (Ranunculaceae), whose flowers generally have four nectaries that produce nectar with different relative abundances of the three main sugars (sucrose, glucose, and fructose) (Herrera et al., 2006).

Nectar variability among flowers of an individual plant can be expressed in many different ways:

- **Different position in the inflorescence.** Demonstrated in *Gaura mutabilis* (Onagraceae), where upper flowers contained three times as much nectar as lower flowers (Cruden et al., 1983).

- **Differences in inflorescence size.** In *Asclepias quadrifolia* (Asclepiadaceae) larger inflorescences produce less nectar per flower than smaller ones (Pleasants & Chaplin, 1983).

- **Differences in microenvironment around the flower.** *A. castanea* flowers directly exposed to sun have a lower volume and higher concentration than flowers in the shade (Nicolson & Nepi, 2005).

- **Differences in flower age.** This may cause differences in nectar secretion rate (nectar volume) and sugar concentration. These differences are documented in several species and it is common for older flowers to have a lower volume of nectar. They may have higher or lower solute concentrations than younger flowers (Wyatt & Shannon, 1986; Petanidou et al., 1996; Navarro, 2001; Nicolson & Nepi, 2005).

- **Differences in the sexual phase of dichogamous flowers.** In *Delphinium* sp. (Ranunculaceae; Cruden et al., 1983), *Lobelia cardinalis* (Campanulaceae; Devlin & Stephenson, 1985), *Echium vulgare* (Boraginaceae; Klinkhamer & de Jong, 1990), *Alstroemeria aurea* (Alstroemeriaceae; Aizen & Basilio, 1998), and *Euphorbia boetica* (Euphorbiaceae; Narbona et al., 2005) the total sugar content was higher in the male phase. On the other hand, in *Metrosideros collina* (Myrtaceae; Cruden et al., 1983), *Polyscias sambucifolia* (Araliaceae; Gillespie & Henwood, 1994) and *C. carvi* (Apiaceae; Langenberger & Davis, 2002), nectar with a higher total sugar content was produced during the female phase. Carlson and Harms (2006) formulated two sets of adaptive hypotheses about the evolution of patterns of gender-biased nectar production: sexual selection hypotheses and inbreeding avoidance hypotheses.

- **Different flower morphs in individual plants.** *Linaria genistifolia* (Scrophulariaceae; Davis & Vogel, 2005) may have unspurred zygomorphic flowers, single-spurred zygomorphic flowers, and 3–5-spurred actinomorphic flowers on the same individual. On average, multi-spurred flowers produce three times more nectar sugar than single-spurred flowers, and 400 times more than spurless flowers.

- **Differences in visitation by pollinators.** This is important when pollination induces changes in nectar secretion; e.g., in *P. sambucifolia*,

pollinated flowers show attenuated nectar secretion and reduced sugar content compared to unpollinated ones (Gillespie & Henwood, 1994).

In species with a long flowering season, such as *L. vulgaris*, which flowers from June to November, nectar volume and sugar concentration vary sharply between early and late flowers in response to variations in environmental parameters, while the relative abundance of sugars (glucose, fructose, and sucrose) remains almost constant (Nepi et al., 2003). In *H. foetidus*, flowering from January to March, a sharp variation in the relative abundance of sugars was found between the early and late flowers (Herrera et al., 2006). In the same species within-plant variation was responsible for 86% of all the variance of nectar characteristics in a population (Herrera et al., 2006). At the opposite extreme there is the case of *Impatiens capensis* (Balsaminaceae), the nectar properties of which were not found to vary significantly within individuals (Lanza et al., 1995).

At population level, differences in floral nectar production among plants may be genetically determined and may interact with environmental conditions (differences in soil moisture, exposure, type of substrate, etc.) (Leiss & Klinkhamer, 2005b). Because nectary traits are often very responsive to environmental variation, even substantial amounts of genetic variation may be swamped out in the field (Mitchell, 2004). Most genetic studies on floral nectar variability concern production rate and concentration, whilst we know very little about the heritability of other major traits—such as sugar ratios, amino acid composition, taste, and scent—that are probably less plastic in response to environmental variation than are production and concentration (Cruden et al., 1983; Leiss et al., 2004; Mitchell, 2004 and references therein). These few studies indicate that there is abundant genetic variation in nectar traits. According to Mitchell (2004), no studies concerning heritability of extrafloral nectar traits have been published. The genetic control of nectar traits has also been the subject of very little research. In *Mimulus* (Scrophulariaceae) and *Petunia* (Solanaceae), a minimum of two quantitative trait loci (QTLs) are involved in controlling the amount of nectar produced while the hexose:sucrose ratio in *Petunia* is under the control of a major QTL which might code for an invertase (Galliot et al., 2006).

Dioecious plants often show differences in nectar features between flowers of the two sexes, as demonstrated for *Silene latifolia* and *S. dioica* (Caryophyllaceae) (Shykoff, 1997; Hemborg, 1998). In a review of 19 dioecious species, Eckart (1998) reported that ten exhibited higher nectar production in pistillate flowers, the remainder yielding more nectar in staminates. Although 19 species is not a very representative sample, it seems

plausible to hypothesize that there is no correlation between nectar production and this kind of sexual expression.

Differences are also found in species with dimorphic flowers, e.g., in the distylous plant *Palicourea padifolia* (Rubiaceae), long-styled flowers produce a higher nectar volume than short-styled flowers (Ornelas et al., 2004). On the other hand, in the distylous species *Turnera subulata* (Turneraceae), no differences in volume or concentration were revealed in the two flower forms (Schlindwein & Medeiros, 2006). A strong and fixed dimorphic system of nectar production, probably with a genetic basis, was observed in populations of *Prosopis glandulosa* (Fabaceae) in the Chihuahuan desert, in which half the individuals produce nectar and the other half are nectarless (Golubov et al., 1999). In this situation nectarless individuals would have an advantage if they received floral visitors attracted by the individuals having nectar, while avoiding the cost of nectar production.

8.3 Interpopulation differences

Interpopulation differences in nectar characteristics also exist. Galetto and Bernardello (1995) report differences in sugar composition in two Argentinian populations of *Lycium cestroides* (Solanaceae), while Lanza et al. (1995) describe differences in the amino acid profile of three populations of *I. capensis* (Balsaminaceae). Large variability in amino acid concentration—greater than in amino acid composition—was found among plants of the same population by Gardener and Gillman (2001).

Differences in habitat may contribute to nectar variability. Early studies by Andrejeff (1932), Hocking (1968), and Heinrich and Raven (1972) showed that bee flowers from high elevations and latitudes produce nectars with a greater energy content than conspecifics at lower elevations and latitudes. On the other hand, both hawkmoth- and hummingbird-pollinated flowers in high-elevation habitats have lower sugar concentrations than those from lower elevations (Cruden et al., 1983).

Differences in the dynamics of nectar production, related to different pollination systems, are demonstrated between populations of the columnar cactus *Pachycereus pecten-aborigenum* growing in the tropics and at northern latitudes. In the tropics, nectar is only produced at night and pollination is exclusively by bats, while in northern regions nectar is produced by day and night and pollination is by bats and diurnal pollinators (Valiente-Banuet et al., 2004).

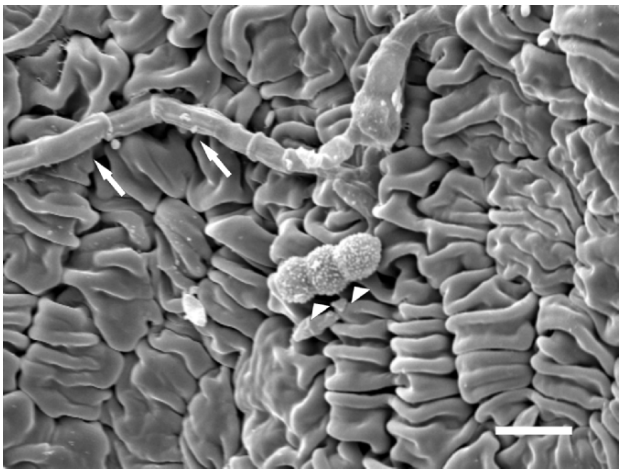

Figure 5. Fungal hyphae (*arrows*) and conidial spores (*arrowheads*) on *Hedera helix* (Araliaceae) floral nectary surface. The epidermal cells are covered by ridged cuticle. Bar = 10 μm.

8.4 Variability and experimental design

All these sources of intraplant and intraspecies variability of nectar properties must be considered before planning experiments on nectar production. Galetto and Bernardello (2005) provided some recommendations to neutralize this variability:

- To decrease intraplant variability it is necessary to include flowers of the same age but different sizes, from different flower stems, or from different positions in inflorescences, and from different locations within the plant in each flower set.
- To decrease within- and between-population variability it is necessary to include flowers of the same age from different plants and, when possible, from different populations in each flower set.
- It is also recommended to concentrate measurements in the same period and to measure the microclimate (temperature and RH) around or within the flowers.

We can speculate about the significance of such high potential variability of nectar traits. Many researchers argue that nectar variability may itself be adaptive because pollinators encountering variable rewards are more likely to move from plant to plant, promoting outcrossing. Potts et al. (2004) report that nectar resource diversity alone explains the majority of variation in bee species richness in complex communities. It therefore seems that variability in nectar features is favourable for both plants and visiting animals.

Mitchell (2004) postulates that floral nectaries should have more variable nectar traits than extrafloral nectaries on the same plant. Indeed, variability in rewards from extrafloral nectaries could reduce movements of ants within plants, reducing defence against predators which is the commonly recognized function of the association between ants and extrafloral nectaries.

A further source of variability in nectar composition is associated with accidental pollution of nectar by foragers. Visitors may alter nectar composition, adding amino acids by direct contact, by salivation, by damaging neighbouring tissues and causing cell leakage, and by dislodging pollen into the nectar (Willmer, 1980; Gottsberger et al., 1990). Gottsberger et al. (1990) found that contamination of nectar by pollen caused an increase in amino acid content, especially of proline. As few as five pollen grains of *H. rosa-sinensis*, with a mean diameter of about 200 μm, distinctly increase the amino acid content of 20 μl of nectar. The increase in amino acid content after nectary puncturing was caused mainly by asparagine. The authors advised that particular care must be taken during nectar sampling for experimental purposes: damage to nectary tissue may occur especially when using glass micropipettes or capillaries. On the other hand, addition of *Aloe marlothii* pollen to the nectar of this species has no significant effect on nectar amino acid concentrations (Nicolson, unpublished).

Nectar, especially when it is fully exposed to the environment and presented for a long period, may also be contaminated by airborne bacteria, fungi (Fig. 5), or algal spores, as demonstrated by Clair-Maczulajtys and Bory (1983) and Davis (1997). These micro-organisms may alter nectar composition, though to what extent is not known experimentally and probably depends on nectar concentration and composition.

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Chapter 5

NECTAR CHEMISTRY

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1 INTRODUCTION

Nectar properties tend to be similar for plants visited by the same kinds of pollinators, and much of the available information on nectar chemistry has been collected in the context of pollination syndromes. These are defined as broad associations between floral features and types of animal pollinators (Faegri & van der Pijl, 1979; Proctor et al., 1996) and are discussed further by Nicolson (2007, Chapter 7 in this volume). Faegri and van der Pijl included nectar volume in their classic descriptions of the various syndromes. The concept was extended to include nectar chemistry (specifically sugar and amino acid content and composition) in the influential reviews of Baker and Baker (1982a 1983b). Herbert and Irene Baker analysed many different substances in nectar and were largely responsible for drawing attention to its chemical complexity. However, the adaptive significance of nectar components has perhaps been overemphasized and is now being examined more critically.

The techniques used in nectar analysis have always been constrained by small fluid volumes, but advances over the years have enabled quantification of different substances rather than mere identification. This particularly applies to chromatographic techniques for sugars and amino acids—formerly measured with paper and thin-layer chromatography, now with gas chromatography (GC) and high-performance liquid chromatography (HPLC) (Kearns & Inouye, 1993). The significance of the sugar and amino acid composition

S.W. Nicolson, M. Nepi, and E. Pacini (eds.), Nectaries and Nectar, 215–264.

of nectar is often less clear than that of its volume and concentration, which jointly determine the energetic reward available to foragers. However, there is an encouraging trend towards a more holistic approach, such as investigations of the nectar of related species with different pollinators, combining measurements of nectar production and chemistry, and their variation in time and space. Examples are recent studies of *Lycium*, *Ipomoea,* and *Nicotiana* (Galetto et al., 1998; Galetto & Bernardello, 2004; Kaczorowski et al., 2005). The importance of plant phylogeny in nectar studies is becoming more apparent and the geographical focus is becoming wider.

2 WATER

Little is known about the carbon costs of nectar production (Pacini & Nepi, 2007), and even less about the impact on plant water budgets. High water costs of flowering have been measured in the extreme case of the huge inflorescence of *Agave deserti* (Nobel, 1977) and in *Polemonium viscosum* (Galen et al., 1999). In general the water losses due to nectar itself are probably minor compared to transpiration from floral structures, especially in species with large, showy corollas. In other words, more water is needed to produce and maintain the advertisement than the reward. Transpiration from both may have a cooling effect: Patiño and Grace (2002) have shown that high evaporation from corollas and sepals cools the flowers of Convolvulaceae, and evaporation from the dilute nectar of *Fritillaria imperialis* is enough to lower the nectar temperature by almost 1^{o}C (Corbet et al., 1979).

The water investment in nectar may be more significant in plants that are subject to water stress (Chapotin et al., 2003; de la Barrera & Nobel, 2004). Drier conditions are generally reflected in fewer flowers produced and also in smaller-sized flowers. This saves water, because smaller flowers contain less water and produce less nectar (Plowright, 1981; Cresswell & Galen, 1991). In Mediterranean shrub communities, these changes can be seen in perennial species of Lamiaceae as the flowering season advances, and are thought to conserve water (Petanidou et al., 2000). Devoto et al. (2006) studied the pollination ecology of *Embothrium coccineum* (Proteaceae) trees along a steep east–west rainfall gradient caused by the Andes in Patagonia, and they found a significant increase in nectar concentration towards the drier end of the gradient, but no consistent changes in volume. In *Epilobium angustifolium* (Onagraceae), drought treatment leads to a substantial reduction in flower size with a concomitant reduction in nectar volume relative to controls, although the concentration of nectar is largely unaffected (Carroll et al., 2001). Water stress also affects the volume of nectar in *Ipomopsis*

longiflora (Polemoniaceae) without changing the concentration (Villarreal & Freeman, 1990). Conversely, supplemental watering increases nectar production in *Delphinium nelsonii* (Ranunculaceae) (Zimmerman, 1983) and *Echium vulgare* (Boraginaceae), particularly in *E. vulgare* plants that have been selected as low nectar producers (Leiss & Klinkhamer, 2005). After nectar removal, plants appear to be more conservative in replacing the sugar component of nectar than the water, although a meta-analysis of removal effects showed considerable variability (Ordano & Ornelas, 2004).

2.1 Nectar concentration

Corbet (2003) gives a useful account of techniques for field measurements of nectar volume and concentration, necessary for collecting data on both standing crop and rate of production. Concentrations are usually measured using refractometers, although alternative procedures are needed for flowers that produce very small nectar volumes (Kearns & Inouye, 1993; Dafni et al., 2005). The use of filter paper wicks to remove small nectar volumes was recommended by McKenna and Thomson (1988), and is especially valuable in the study of butterfly nectar resources, although the method yields total sugar rather than volume and concentration (Holl, 1995). Refractometers give the sugar concentration on a % weight/weight basis (sucrose equivalents; g sucrose per 100 g solution), so these units are commonly used in the literature concerning nectar and nectar consumers (and are used throughout this chapter). There are, however, several sources of error that could be avoided by expressing sugar concentrations on a molar basis instead:

- Some authors report concentrations without distinguishing between % w/w and % w/v.
- Others calculate energy values without first converting sugar concentrations to units of % w/v or molarity, and this error is greater at higher concentrations (Bolten et al., 1979).
- Sugars mixed on a % w/w basis have been assumed to be equicaloric, but hexose solutions prepared in this way have only 95% of the energy value of the corresponding sucrose solutions (Fleming et al., 2004).
- An additional source of error comes from ignoring non-sugar solutes such as amino acids and inorganic ions (see discussion on page 219).

Nectar sugar concentrations vary significantly, both within and between species. Extreme concentrations are found in the nectars of *Aloe castanea* (Asphodelaceae), in which sugar concentration is less than 10% (0.3 M) (Nicolson & Nepi, 2005), and caraway *Carum carvi* (Apiaceae), in which the average sugar concentration is 66.5% (2.5 M) (Langenberger & Davis,

2002). These two species show little variance in their nectar sugar concentrations, while other species show a significant variance: for example, 4–72% in *Clintonia borealis* (Liliaceae) (Plowright, 1981), and 2–62% in *Echium plantagineum* (Boraginaceae) (Corbet & Delfosse, 1984). Temporal variation in nectar sugar can be caused by nectary activity (secretion or reabsorption) and/or removal by foragers. Variation in the water component of nectar occurs through the same mechanisms, but nectar water is also affected by equilibration with ambient humidity (Corbet, 2003). Because of these sources of variation, it is clear that the nectar of a plant species should not be characterized by single measurements of its volume and concentration. In disregard of this variability, erroneous ecological significance is sometimes attributed to nectar concentrations (especially when values for many species are averaged), because plants with similar pollination syndromes tend to have similar nectar concentrations. In broad terms, insect-pollinated flowers produce relatively concentrated nectars, whereas flowers pollinated by birds and bats generally produce dilute nectars (Pyke & Waser, 1981; Baker & Baker, 1982a, 1983b).

2.2 Chemical and microclimatic influences on nectar concentration

Percival (1961) carried out a semi-quantitative study of nectar sugars in 900 angiosperm species. Her study noted that plant families with deep or tubular flowers tend to produce nectar rich in sucrose, whereas shallow flowers tend to produce nectar rich in monosaccharides. Hexose nectars would be expected to evaporate more slowly than sucrose nectars of the same concentration on a w/w basis, because more solute particles are present to lower the effective concentration of the solvent (water) (Corbet, 1978). For the same sugar concentration, hexose nectars have much higher osmolalities than sucrose nectars (Corbet, 1978; Corbet et al., 1979; Nicolson, 1994), and this leads to slower evaporation and lower final concentrations that are in equilibrium with the ambient relative humidity. Physical relationships are therefore involved in the hexose dominance observed by Percival (1961) in shallow nectaries, although phylogenetic effects will also be important.

The correlation between sugar composition and nectar concentration may arise very early in floral development. Nectar originates from sucrose-rich phloem sap or from sucrose synthesized in the nectary tissue, and the proportion of monosaccharides depends on the presence and activity of various nectary enzyme systems, including invertase. Hydrolysis of sucrose increases nectar osmolality, thus drawing water into the nectar. This water influx can potentially convert a 30% sucrose nectar into a 20% hexose nectar,

with a 1.56 times increase in volume (Nicolson, 2002). Sucrose hydrolysis in the nectary also serves to maintain a favourable concentration gradient for sucrose transport across the nectary tissue: thus total sugar production may be significantly greater in flowers producing hexose nectars. This may explain differences in composition and concentration between the nectars of flowers visited by hummingbirds and passerine birds (Nicolson & Fleming, 2003).

In addition to sugars, many nectars contain other solutes in sufficient amounts to mask the true concentration of sugar in nectar. Corbet et al. (1979) plotted the refractive index of various nectars against either ambient relative humidity or the nectar osmolality: microclimatic and chemical effects, respectively, were shown by deviations from the corresponding curves for pure sugar solutions. The effects of non-sugar solutes will depend on their refractive index (which is highly correlated with molecular size) and their concentration (Inouye et al., 1980). The latter authors calculated potential refractive index contributions from various non-sugar solutes, obtaining values ranging from 1.9% to 3.6% as sucrose w/w. Such solutes may account for a significant proportion of the apparent sugar content estimated by refractometry, resulting in overestimates of energy content (Inouye et al., 1980).

Regardless of chemical effects on concentration, microclimatic effects tend to predominate, and the usual post-secretory change in nectar is an increase in concentration due to evaporation, especially in open flowers. This results in the commonly observed inverse relationship between volume and concentration (Corbet et al., 1979; Plowright, 1981; Nicolson, 2002). To illustrate the power of evaporation, a 20% sucrose solution will lose water to air at all relative humidities below 98% (Corbet et al., 1979). Fortunately for many nectar consumers, humidity gradients inside flowers are modified by long corollas that slow the exchange of water between nectar and air (Plowright, 1987), and large volumes of nectar evaporate more slowly because of the reduced surface/volume ratio. Evaporation can be rapid when small drops of sugar solution are placed inside the corolla but not in contact with the nectaries (Castellanos et al., 2002). Other features of floral morphology may reduce evaporation, such as the massed stamens in brush-type flowers, e.g., in *Eucalyptus*. A dense barrier of stamen filaments in the red flowers of the cactus *Echinocereus coccineus* protects the abundant nectar for hummingbird visitors (Scobell & Scott, 2002). Microclimatic effects must also be taken into consideration when flowers are protected from visitors in order to compare nectar production rates with standing crops (Wyatt et al., 1992; Corbet, 2003).

Two orchid genera (*Aerangis* and *Rangaeris*) in Kenya are pollinated by sphingid moths, which drink from floral spurs that can exceed 10 cm in length (Martins & Johnson, 2007). Sampling of the nectar at intervals along the spurs showed steep concentration gradients, from about 1% at the mouth of the spur to 20% at the tip. It is not clear how the gradients in the nectar columns are generated or maintained, but perhaps they encourage moths to probe more deeply.

As an example of the complex factors influencing nectar rewards, Búrquez and Corbet (1998) carried out a detailed study of nectar dynamics in the annual herb *Impatiens glandulifera* (Balsaminaceae), a Himalayan species that is now invasive in Europe. They examined the interacting effects of nectary activity, microclimatic modification, and animal visitors. Most importantly, the variables affecting nectar secretion are different from those affecting its solute concentration. Nectar secretion depends on air temperature and plant age. It declines in older plants where fruits constitute an additional carbohydrate sink. Nectar concentration depends on short-term microclimatic factors, especially relative humidity, which has an immediate effect (slowed by high secretion rates, large volumes, and long corollas). In that study, a wide spectrum of visitors ensured that the nectar-standing crop was too small to measure for most of the day. Incidentally, the successful invasion of *I. glandulifera* may, in part, be due to the fact that it offers bumblebees higher rewards than native plants (Chittka & Schürkens, 2001). In considering associations of nectar concentration with pollinator type, Búrquez and Corbet (1998) stressed that selection does not act on the nectar concentration itself, but on the factors that determine it—corolla structure, the rate of secretion, and the chemical composition, which alters concentration/humidity relationships.

Table 1. Effect of rain on nectar concentration (% w/w) in *Protea* species with and without furred inflorescences. Values are means ± SE (n). (Unpublished data of S.W. Nicolson and C.A. Beuchat, collected in Kirstenbosch Botanic Gardens, Cape Town.)

***Protea* species**	**Inflorescence type**	**Concentration in dry weather**	**Concentration after rain**
P. coronata	Furred	25.0 ± 0.5 (6)	23.7 ± 1.1 (8)
P. longifolia	Furred	27.6 ± 1.5 (14)	21.8 ± 2.4 (12)
P. neriifolia	Furred	27.5 ± 0.9 (12)	22.1 ± 3.2 (8)
P. aurea	Smooth	16.6 ± 0.6 (4)	3.0 ± 1.7 (4)
P. compacta	Smooth	23.6 ± 1.2 (12)	0.8 ± 0.2 (6)
P. eximia	Smooth	29.9 ± 2.9 (7)	3.3 ± 2.2 (7)
P. obtusifolia	Smooth	22.1 ± 0.6 (6)	6.9 ± 2.0 (10)
P. repens	Smooth	21.6 ± 1.0 (13)	1.9 ± 0.7 (11)

Exposed nectars may sometimes be diluted by high humidity, and both nectar and pollen may need protection from rain (Corbet, 1990). When bee-pollinated *Pulsatilla cernua* (Ranunculaceae) flowers become pendulous during anthesis, rain damage to pollen is avoided (Huang et al., 2002), but the nectar of the relatively open flowers is also protected. Downward flower orientation is common in bird-pollinated flowers, and it is suggested that the corolla serves as an umbrella (Aizen, 2003). However, this has seldom been tested. Tadey and Aizen (2001) found that the narrow tubular shape and surface properties of the petals were more important than orientation in preventing flooding in the mistletoe *Tristerix corymbosus* (Loranthaceae). Unwettable floral surfaces, constrictions, and hairs all serve to prevent contact between nectar and rainwater, but nectar may still gain water in the vapour phase from rain droplets inside flowers (Corbet & Delfosse, 1984). Pubescent hairs protect nectar from dilution by rain as well as from evaporation, and a good example is seen in *Protea* species (Proteaceae), which flower during winter in the southwestern Cape of South Africa and are a major nectar source for the endemic Cape sugarbird, *Promerops cafer*. In those *Protea* species which have heavily furred involucral bracts surrounding the tightly packed florets, the abundant nectar which pools at the base of the inflorescence is little affected even by heavy rain (Table 1).

Variation in nectar concentration, and the low concentration of many nectars, means that nectar feeders must often ingest and process excess water in order to meet their energy requirements (Nicolson, 1998; Martínez del Rio et al., 2001). The consequences for animal physiology, such as chronic diuresis, food warming costs for endotherms consuming large volumes of cold dilute nectar, additional metabolic costs for bees carrying larger nectar loads, are discussed by Nicolson (2007, Chapter 7 in this volume). The most immediate effect of varying concentration is on ingestion rates, and here viscosity is an important property of sugar solutions.

2.3 Viscosity and feeding rates

Both the temperature and solute concentration of nectar have substantial effects on its viscosity (Fig. 1). Viscosity is inversely proportional to temperature, which suggests advantages to feeding on warm nectar (Heyneman, 1983). In the case of insect nectarivores, both the decreased viscosity and the increased body temperature at higher ambient temperatures contribute to the volume ingested (Pivnick & McNeil, 1985). While energy content increases linearly with sugar concentration, viscosity increases exponentially (Fig. 1), with the important consequence for nectar feeders that the most efficient energy intake occurs at intermediate sugar concentrations. It was Baker (1975)

who first drew attention to these opposing effects of two important fluid properties of nectar. At low concentrations, energy intake is limited by the low energy content, while at high concentrations it is limited by the high viscosity of the solution. The balance between costs and benefits determines the optimal nectar concentrations for different nectar feeders. Concentrations predicted by modelling studies are supported to varying extents by empirical work (see Nicolson, 2007, Chapter 7 in this volume).

Mouthpart structures and the type of fluid feeding must be considered along with the physical properties of the solution being ingested. Two principal mechanisms are involved in nectar uptake by insects: either the mouthparts use capillary action in licking or lapping nectar, or they are modified to form long tubes for sucking nectar (Kingsolver & Daniel, 1995; Krenn et al., 2005). Biophysical models (Kingsolver & Daniel, 1995) predict that lower solute concentrations are necessary for efficient injestion through narrow tubes, and this is illustrated by the relatively low concentrations of butterfly nectars compared to those utilized by bees. Although most bees are capillary feeders, suction feeding has evolved in the Neotropical euglossine bees, which collect more dilute nectars than other sympatric bees (Borrell, 2004). For bumblebees, Harder (1986) explained the depressive effect of viscosity on ingestion rates as follows: the licking rate is constant, and provided the tongue becomes saturated at each lick, the volume ingested will be constant. However, when concentrations reach 35–40% or higher, the increased viscosity begins to reduce the volume taken up during each immersion of the tongue. This explains the finding that energy uptake rates are maximal at 50–65% for many bees (Roubik & Buchmann, 1984; Harder, 1986), but lower for the euglossine bees (Borrell, 2004). Similarly, energy intake rates are higher in the more primitive ponerine ants, which lick sugary food, than in formicine ants, which are suction feeders (Paul & Roces, 2003).

Nectarivorous birds also lick nectar from flowers, and the nectar flows by capillarity onto their grooved tongues. The biophysical model developed for hummingbird feeding by Kingsolver and Daniel (1983) suggested that the optimum nectar concentration for hummingbirds is 20–25% for small volumes (involving single licks of the tongue), but higher for larger volumes. For nectar feeders in general, regardless of the feeding mechanism used, Heyneman (1983) predicted 22–26% as an optimum concentration for large volumes, with the proviso that this would increase if travel costs were high in relation to total foraging costs; (see also Harder, 1986). The temporal scale is crucial in modelling hummingbird feeding: Gass and Roberts (1992) demonstrated upward shifts in optimal concentration as they considered in turn the tongue loading phase of the licking cycle, the whole licking cycle, and

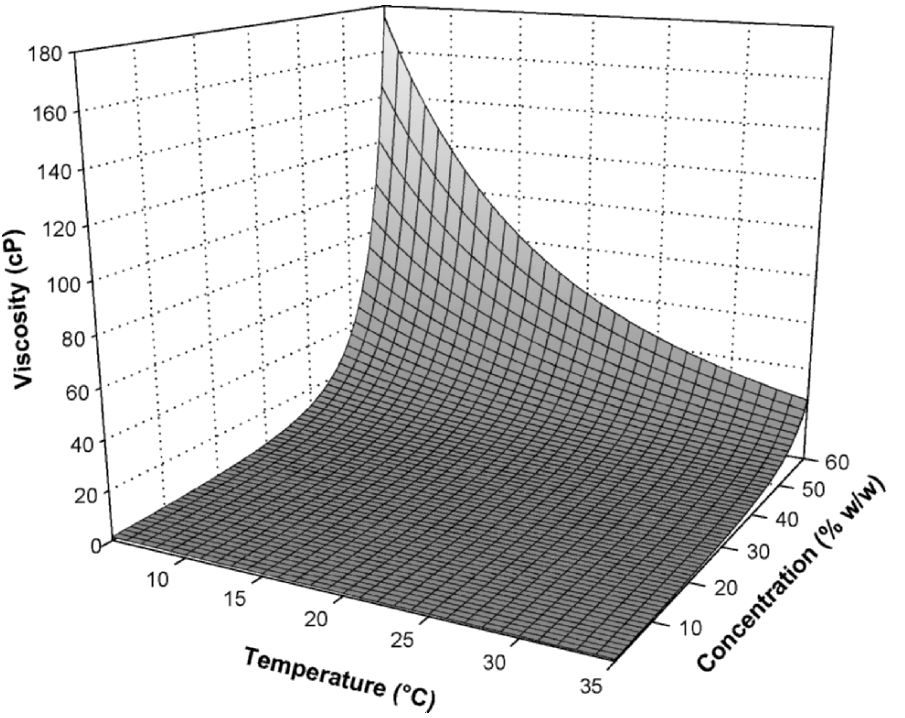

Figure 1. Viscosity of sucrose solutions plotted against temperature and concentration, using equation from Mathlouthi and Génotelle (1995).

flower handling. They also questioned the applicability of models to the low nectar volumes generally available in hummingbird flowers.

Viscosity relationships in nectar may not be as simple as in pure solutions. Sucrose solutions are more viscous than hexose solutions containing the same weight of sugar, and the difference increases with concentration (Weast, 1980). Values for mixtures are likely to be intermediate between those for the pure sugars (Heyneman, 1983). Using capillary descent times, Heyneman (1983) measured viscosities of various hummingbird nectars, obtaining higher values than for equivalent sugar solutions, and she attributed the discrepancy to the presence of non-sugar solutes. These effects are not easily predicted and empirical data are not available. It is possible that variations in nectar composition, including the presence of oligosaccharides, may lead to unexpected viscosities. Occasionally, high molecular weight polymers result in a jelly-like consistency in the nectar of vertebrate-pollinated flowers (Johnson et al., 2001; Sazima et al., 2001).

3 SUGARS

Nectar chemistry is dominated by three simple sugars: the disaccharide sucrose and its component monosaccharides—fructose and glucose. All are derived from sucrose translocated in phloem sap or synthesized in the nectary. The relative amounts of each are determined by nectary invertase which hydrolyses sucrose to glucose and fructose, before or during nectar secretion (Pate et al., 1985). The sharp dichotomy between sucrose and hexose nectars seen within genera such as *Erica* and *Leucospermum* (Barnes et al., 1995; Nicolson & van Wyk, 1998), with nectars at both extremes of the sucrose–hexose continuum, is indicative of the absence or presence of invertase activity. Partial hydrolysis is apparently responsible for the mixed sugar composition seen in the majority of nectars (Baker & Baker, 1983b).

Other minor sugars are present in trace amounts in nectar (Baker & Baker, 1982a, 1983b). These may be monosaccharides, (e.g., mannose, arabinose, xylose), disaccharides (maltose, melibiose) or, more rarely, oligosaccharides (raffinose, melezitose, stachyose). Sorbitol is also a frequent constituent of Mediterranean nectars (Petanidou, 2005). Tests of a pollinator's preference among nectar sugars are often restricted to sucrose, glucose, and fructose (Chapter 7); where the less abundant nectar sugars have been included, they are generally less attractive and less useful nutritionally (Barker & Lehner, 1974). Trisaccharides are more common in honeydew, another carbohydrate-rich fluid that is produced by homopteran insects, its sugar composition depending on both the insect and the host plant (Völkl et al., 1999).

An exception to the dominance of the three main sugars is seen in two sister genera of the Proteaceae, *Protea* and *Faurea*, in which the pentose sugar xylose comprises up to 39% of total nectar sugars (Nicolson & van Wyk, 1998). Xylose is, however, absent from the nectar of other genera of Proteaceae in South Africa and Australia. Studies of sugar preference and sugar absorption efficiencies among various pollinators of Proteaceae are reviewed by Jackson and Nicolson (2002)—insect and bird pollinators are strongly averse to xylose and show very poor intestinal absorption of this sugar. In contrast, the Namaqua rock mouse, *Aethomys namaquensis*, feeds on *Protea* nectar with a relatively high xylose concentration compared to that of bird-pollinated species (Nicolson & van Wyk, 1998) and is able to utilize xylose via microbial fermentation in the hindgut (Johnson et al., 2006a). Xylose has also been reported in the gelatinous nectar of a rodent-pollinated African lily, *Massonia depressa* (Johnson et al., 2001).

The presence of unusual sugars in nectar may be a consequence of phloem sap composition, with sugars passing untransformed through the nectaries (Jackson & Nicolson, 2002). Unfortunately, studies that measure both nectar and phloem composition of the same species are rare (Adler, 2000; but see Pate et al., 1985). Xylose is present in the phloem sap of *Tanacetum vulgare* (Asteraceae), where it comprises 33% of phloem sugar and 14–89% of sugar in the honeydew of four aphid species feeding on the plant (Völkl et al., 1999). Raffinose is a characteristic phloem sugar of Myrtaceae, among other plant families (Ziegler, 1975), and the mean molar ratio of raffinose to sucrose is 0.29 in the phloem sap of *Eucalyptus globulus* (Pate et al., 1998). The fact that this trisaccharide (also occurring in sugar beet) was not identified in the nectar of several *Eucalyptus* species (Nicolson, 1994) may merely reflect the conditions used in HPLC analyses (B.-E. van Wyk, personal communication). In orchid nectars analysed by thin-layer chromatography, raffinose was a common constituent (Jeffrey et al., 1970). High mannose levels in the nectar of lime trees (*Tilia*) during drought conditions may be due to unusual phloem sap composition, and are toxic to honeybees (Crane, 1977), owing to low activity in these insects of the enzyme mannosephosphate isomerase (Sols et al., 1960).

3.1 Constancy of sugar composition within species

Although many older data were single analyses, the percentages of sucrose, fructose, and glucose are often relatively constant when nectar samples from different individuals of a species are analysed. Assuming that partial hydrolysis leads to the mixed sugar composition of most nectars, it is difficult to see how the proportions of the three main sugars remain consistent in multiple nectar samples from a given species. Consistency in sugar composition is also surprising in view of the fact that microbes in nectar are carried from one flower to the next by contaminating visitors (Willmer, 1980; Sandhu & Waraich, 1985; Ehlers & Olesen, 1997; Antonovics, 2005). Such inoculation is inevitable during pollinator visits, especially when they occur at high frequency. As an example, Williams and Thomson (1998) observed a single plant of *Penstemon strictus*, with fewer than 30 open flowers, and recorded an average of 4.55 bumblebee flower visits per minute over the whole day. Nectar is a nutritious fluid and an excellent medium for growth of bacteria such as *Erwinia amylovora*, the causative agent of fire blight in apple and pear orchards (Bubán et al., 2003). Colonization by yeasts may produce fermentation volatiles in nectar that are important in flower–pollinator relationships (Raguso, 2004). The likely result of microbial contamination is a decreased proportion of sucrose as flowers age, as happens in *Citrus* flowers (Loper et al., 1976). It has been suggested that exposure of extrafloral

nectars to the drying and contaminating effects of the environment may lead to greater prominence of hexose sugars, as well as higher sugar concentrations (Koptur, 1994). Conversely, protection from microbial contamination may be a factor in the tendency noted by Percival (1961) for tubular flowers to contain nectar rich in sucrose. The antimicrobial properties of nectar (see "Nectar redox cycle" on page 241) no doubt play a major role in the remarkably consistent proportions of the main nectar sugars in many plant species.

Changes in sugar proportions with age (as well as variation among florets, inflorescences, and plants) tend to be minor compared to the distinct differences observed between species (Nicolson & van Wyk, 1998). Nectar sugar composition remains constant in the long-lived flowers of three species of *Eucalyptus*, in spite of continual exposure to visitors (Davis, 1997). In *Combretum fruticosum* (Combretaceae), there is no decrease in sucrose with flower age (Bernardello et al., 1994). Progressive hydrolysis, if it occurs, will be more obvious in species with nectars that are initially high in sucrose. The proportion of sucrose, although higher in female flowers of *Cucurbita pepo* (Cucurbitaceae), does not vary with age in either male or female flowers (Nepi et al., 2001), but these flowers were screened to exclude pollinators. *Ipomopsis longiflora* (Polemoniaceae) has high nectar sucrose, and in flowers exposed to visitors the percentage of sucrose declined significantly with flower age (Freeman & Wilken, 1987). Further investigation of the nectar sugar composition of this species showed that the percentage of sucrose also declined with increasing temperature, under both field and laboratory conditions, but was unaffected by water stress (Freeman & Head, 1990; Villarreal & Freeman, 1990). Since sealing the flowers prevented this decline, it seems to be due to external factors such as animal visitation, rather than to secretory changes with age. For two bat-pollinated species of columnar cacti, nectar sampling throughout the night (not commonly done) showed that sucrose levels were lowest after midnight (Petit & Freeman, 1997).

The idea that nectar sugars are relatively constant within species has been challenged by Herrera et al. (2006), who measured nectar sugars of *Helleborus foetidus* (Ranunculaceae) in southern Spain, comparing variation on different levels, including variation between the five separate nectaries within flowers. Differences among plants accounted for 14% of total variance, and differences among flowers of the same plant were the most important at 56%. Differences among nectaries of the same flower (the level at which bumblebees forage) were responsible for the remaining 30%. The common practice of pooling nectar from different flowers on the same plant

will naturally obscure the fine-scale variation between nectaries and flowers. On a broader scale, Herrera et al. (2006) point out that much higher nectar sucrose levels have been recorded in populations of *H. foetidus* from other parts of Europe.

In six species of Brassicaceae, including *Arabidopsis thaliana*, the glucose/fructose ratio differs between lateral and median nectaries in the same flowers (Davis et al., 1998), but pooling between flowers was necessary to show this because of the very small nectar volumes. Unbalanced glucose/fructose ratios are an indication that more than simple hydrolysis in the nectary is involved in the determination of nectar sugar composition. Low glucose in relation to fructose was apparent in late season nectar samples of *H. foetidus* (Herrera et al., 2006), and seems to be characteristic of the Gesneriaceae (Stiles & Freeman, 1993; Baker et al., 1998; Perret et al., 2001, 2003). Higher glucose than fructose (10 times higher for some species) is characteristic of the genus *Haworthia* (Asphodelaceae) (van Wyk et al., 1993; Smith et al., 2001), and has also been recorded in nectar of *Lycium* species (Solanaceae) (Galetto et al., 1998). Glucose also dominates the hexose fraction in *Combretum fruticosum* (Combretaceae), the difference increasing with flower age (Bernardello et al., 1994). However, other populations of this widely distributed species were shown to have more balanced hexose sugars (Gryj et al., 1990).

3.2 The use of sugar ratios can be misleading

Abundant data on nectar sugar composition (and the assumption that nectar chemistry of a species is constant) have led to the suggestion that there are co-evolutionary relationships between the sugar proportions in nectar and the types of floral visitors. Based on extensive but largely unpublished analyses of nectar sugar composition, Baker and Baker (1982a, 1983b) grouped unrelated plant species according to pollinator type and demonstrated convergence in sugar composition between plants with the same visitors. To summarize their findings briefly, high sucrose in nectar was broadly correlated with pollination by moths and butterflies, long-tongued bees, and hummingbirds. In contrast, high proportions of glucose and fructose were characteristic of species pollinated by flies, short-tongued bees, passerine birds, and Neotropical bats.

This categorization was based on nectar/sugar ratios, defined as the ratio by weight of sucrose to the combined hexose sugars, S/(G + F) (Baker & Baker, 1982a, 1983b). Four classes of nectar were recognized (Table 2). This terminology, although widely adopted by later authors, places undue emphasis

Table 2. Sugar ratios in nectar. (From Baker & Baker, 1982a.)

Class	S/G + F	% sucrose
Sucrose-dominant	>1.0	51–100
Sucrose-rich	0.5–1.0	34–50
Hexose-rich	0.1–0.5	10–33
Hexose-dominant	<0.1	0–9

on the sucrose content of nectar. The transition from "hexose-rich" to "sucrose-rich" occurs at 33% sucrose, when it should occur at 50% sucrose. The overemphasis on sucrose may have arisen from considering a "balanced" nectar as one with equal weights of the three sugars and thus a sugar ratio of 0.33/0.67 = 0.5 (Baker & Baker, 1983b), when in fact a sugar ratio of 1.0 is more appropriate to describe a balanced nectar (containing equal weights of sucrose and hexoses). The statement, firmly entrenched in the literature, that hummingbird flowers are prevailingly sucrose-rich or sucrose-dominant (Baker & Baker, 1983b) merely indicates that most of their nectars have a sugar ratio above 0.5, i.e., sucrose concentrations greater than 33% of total sugar. However, the idea of sucrose dominance is supported by the mean value of 64.4% sucrose in the nectars of 278 plant species pollinated by hummingbirds (Nicolson & Fleming, 2003).

A further reason to avoid the use of sugar ratios is that percentages of the three sugars are not independent; acknowledging this, Baker and colleagues omitted ratios in a later review of nectar and fruit sugars (Baker et al., 1998). Sugar ratios are still in widespread use but fortunately many authors present the percentage sugar composition as well (e.g., Perret et al., 2001; Torres & Galetto, 2002; Galetto & Bernardello, 2003; Petanidou, 2005; Wolff, 2006).

The use of sugar ratios will be avoided in the following discussion, together with the terms "sucrose-dominant", etc. The alternative is to express the proportions of sucrose, glucose, and fructose as percentages of total sugar; usually the percentage of sucrose is sufficient information (unless there is particular interest in the relative proportions of glucose and fructose). Data presented as sugar ratios can be converted as follows: where the ratio R = S/(F + G), the percentage of sucrose is % sucrose = 100R/(1 + R) (Freeman et al., 1984).

3.3 Is sugar composition determined by floral visitors or common ancestry?

The sugar composition data used by Baker and Baker (1982a, 1983b) to calculate sugar ratios remain largely unpublished today, so it is not possible to compare the average percentages of sucrose in nectars consumed by the various pollinator classes. Evidence is available from other publications, however, and there has been considerable interest in the dichotomy between nectars of hummingbird and passerine bird flowers. Stiles and Freeman (1993) analysed the nectars of 112 species of bird-visited plants in Costa Rica, and found high sucrose in all the putatively hummingbird-pollinated species (mean 73% of total sugars). The sugar chemistry of both nectar and fruit is correlated with bird and bat consumers (Baker et al., 1998): in this large data set, species pollinated by hummingbirds had a mean nectar sucrose concentration of 58%, while passerine-pollinated species in the Old and New Worlds averaged only 8% and 3% sucrose respectively. Baker et al. (1998) also examined their data on the basis of genera and families, because species are not independent units and more intensive sampling of some genera than others may distort the findings. Also with this in mind, Stiles and Freeman (1993) verified that 23 species of the major hummingbird-pollinated genus *Heliconia* had the same mean sucrose concentration as the rest of their sample. In a recent survey, 278 hummingbird-visited species were found to have a mean 64.4% of their nectar sugar as sucrose, while 259 species of sunbird-visited plants showed a bimodal pattern, some having high sucrose levels but nearly half producing nectar with less than 10% sucrose (Nicolson & Fleming, 2003). A similar dichotomy in sucrose proportions occurs in honeyeater flowers, although fewer species have been examined (Nicolson & Fleming, 2003).

The distinctive ornithophilous genus *Erythrina* (Fabaceae) has a pantropical distribution and is unusual in including both hummingbird and passerine pollinators. Hummingbirds are pollinators of *Erythrina* species with concentrated nectar high in sucrose, and passerine birds pollinate species with dilute nectar high in hexoses (Baker & Baker, 1982b). The sucrose concentration averages 54.6 ± 1.9% (mean ± SE) in the nectars of 25 hummingbird-pollinated species, in sharp contrast to 4.0 ± 0.4% in the nectars of 23 passerine-pollinated species from both the Old and New World (Baker et al., 1998). Amino acid concentrations also differ, being much lower in the hummingbird nectars than in the passerine nectars (Baker & Baker, 1982b); they are exceptionally high in some southern African species such as *Erythrina lysistemon* (S.W. Nicolson, unpublished data). Bruneau's (1996) hypothesis of phylogenetic relationships within *Erythrina* is based on both

morphological and chloroplast DNA characters, and among the morphological (and chemical) characters that she used were nectar sucrose and amino acid concentrations. Similar results were obtained from these two independent data sets, supporting the inclusion of the morphological data (for discussion see Weller & Sakai, 1999). The phylogeny suggests that transitions from passerine to hummingbird pollination have occurred at least four times in the genus, and these transitions involve different floral modifications in each case and are not homologous (Bruneau, 1997). The pollinator shifts are accompanied by major changes in nectar concentration, sugar composition, and amino acid content (Baker & Baker, 1982b)—and probably nectar volume—indicating the absence of phylogenetic constraint on nectar chemistry in this genus (Bruneau, 1997). *Erythrina crista-galli* is basal in the cladogram, and field studies have shown that it is mainly visited by honeybees and carpenter bees, in spite of negligible sucrose in its dilute nectar, suggesting a transition from insect pollination, which is typical of the Fabaceae, to bird pollination (Galetto et al., 2000).

In relating nectar sugar composition to pollinator type, Baker and Baker (1983b) were aware of phylogenetic constraints on the adaptation of nectar sugars to pollinators: "In characterizing long-tongued bee-flowers as offering a sucrose-rich reward, we could fall into the trap set for us by phylogenetic constraint. Many of the … flowers in our sample come from the Lamiaceae …" It is increasingly apparent that some plant families show characteristic nectar sugars, and the Lamiaceae is an example of a family with high nectar sucrose (see also Petanidou, 2005). The taxonomic value of nectar sugars in angiosperms can be demonstrated by extensive sampling of closely related species, as for example within Scrophulariaceae, Proteaceae, and Gesneriaceae (Elisens & Freeman, 1988; Nicolson & van Wyk, 1998; Perret et al., 2001). As a clear demonstration, sugar composition in the sub-family Alooideae (Asphodelaceae) is highly conservative within genera and reflects taxonomic affinities rather than pollinator types (van Wyk et al., 1993). In this study, 47 *Aloe* species averaged 1.0% sucrose, while 12 *Gasteria* species averaged 88.5% sucrose; both genera are bird-pollinated, but *Aloe* flowers are much larger and produce higher volumes.

The sugar composition of nectars in the most advanced dicotyledon subclass, the Asteridae, has been analysed by Schwerdtfeger (1996). His 900 nectar samples were collected mainly from botanic gardens in Germany. In agreement with Baker and Baker (1983b), the data show strong correlations with pollinator classes, so that high sucrose nectars are found in flowers pollinated by bees, butterflies, moths, and hummingbirds, and high-hexose nectars in flowers pollinated by small, unspecialized insects, passerine birds

and Neotropical bats. However, when these data are analysed at the generic level, families such as Lamiaceae, Rubiaceae, and Gesneriaceae are characterized by high mean sucrose percentages of 65.5%, 84.9%, and 75.7%, respectively, and coefficients of variation (CV) of less than 30%. In contrast, the large, specialized and diverse family Asteraceae gave a mean value of 40.0% sucrose, with a CV of 71% indicating great variation among the 60 genera analysed. Torres and Galetto (2002) demonstrated an evolutionary trend towards generalist pollination systems in Argentinian Asteraceae, with shorter corollas, higher hexose proportions, and more diverse visitors. Also in Argentina, Galetto and Bernardello (2003) looked for convergence of nectar sugars in plants pollinated by bees, moths, and butterflies in two widely separated regions, and found plant phylogeny to be a stronger determinant of nectar sugars than visitor guilds. Hexose nectars were characteristic of Asteraceae, Fabaceae, Solanaceae, and Verbenaceae, whereas sucrose nectars prevailed in Bromeliaceae and Onagraceae. In general, nectars from Patagonia demonstrated very high hexose contents, regardless of floral syndrome or systematic relatedness.

Wolff (2006) examined the nectar of 47 species of Gentianales in a montane forest in Ecuador in relation to observed floral visitors. Nectar concentrations did not differ significantly between pollination systems, and sugar composition was different only for fly- and bat-pollinated flowers, which had higher proportions of hexose. The main difference among pollination systems was in the volume of nectar offered. This rather conservative sugar composition seems to be a common finding in recent studies of South American floral nectars (see also Chalcoff et al., 2006).

The dichotomy between high-sucrose nectars in hummingbird-pollinated plants and predominantly high hexose nectars in sunbird-pollinated plants was formerly attributed to the preferences of hummingbirds for sucrose and the fact that some passerine birds are unable to digest sucrose and therefore obliged to utilize hexose nectars (Martínez del Rio et al., 1992). This is, however, not true of more specialized passerine nectar feeders (Lotz & Nicolson, 1996). More recent work shows that the dichotomy between sucrose and hexose nectars is not related to bird preferences, because both hummingbirds and sunbirds show a lack of sugar type preference when the solutions offered are equicaloric (Fleming et al., 2004). A more important factor may be the general lack of overlap in the major plant families visited by hummingbirds and by passerine birds (the genus *Erythrina* is a notable exception). Geographical consideration of bird–flower associations shows that they vary greatly on different continents (Stiles, 1981).

For the well-researched North American hummingbird flora, it is generally accepted that hummingbird pollination is derived from insect pollination (Stebbins, 1989; Grant, 1994). Hummingbird-pollinated plants north of Mexico belong to different genera and families but are convergent in floral colour, size, shape, and nectar rewards (Brown & Kodric-Brown, 1979). In addition, their pollinators are a small group of closely related species, among the smallest hummingbirds in the family Trochilidae (Bleiweiss, 1998). The hummingbird plants consist of isolated species in otherwise insect-pollinated genera such as *Aquilegia* (Ranunculaceae), *Ipomopsis* (Polemoniaceae), *Penstemon* (Scrophulariaceae), and *Salvia* (Lamiaceae), and phylogenetic inertia can explain the predominance of sucrose in the hummingbird nectars. Recently, Perret et al. (2001, 2003) investigated nectar sugar composition in relation to pollination syndromes in the Neotropical tribe Sinningieae of the Gesneriaceae, and found similar high-sucrose nectar (58–89%) in both hummingbird and bee flowers, with composition changes occurring only in bat flowers. Bee pollination is also the primitive condition in tribe Antirrhineae of the Scrophulariaceae, where the sugar composition of 45 species was also relatively constant, despite a variety of pollinators (Elisens & Freeman, 1988). Similarly, in *Nicotiana* species pollinated by hummingbirds and hawkmoths, increased floral size is accompanied by higher volumes and lower concentrations, but the sucrose proportion and total energy change much less (Kaczorowski et al., 2005). This is discussed further by Nicolson (2007, Chapter 7 in this volume).

In conclusion, phylogenetic history appears to be the primary determinant of nectar chemistry, but pollinators have a secondary effect. Interestingly, Roulston et al. (2000) found no evidence of any association between pollen protein content and the dietary demands of pollinators; rather, protein concentrations were highly conserved within plant genera and families (but pollen, unlike nectar, is not solely a reward).

4 INORGANIC IONS

Published data on ion concentrations in nectar are scarce. Hiebert and Calder (1983) measured K^+ and Na^+ concentrations in 19 species visited by hummingbirds. Their findings suggested a phylogenetic component to patterns of nectar ion composition, in particular high K^+ concentrations in the nectar of Ranunculaceae. Heinrich (1989) found K^+ to be dominant in the nectars of 20 plant species. Higher K^+ than Na^+ concentrations in nectar are in agreement with the relative concentrations of these ions in phloem sap (Ziegler, 1975; Pate et al., 1985, 1998), but Robards and Oates (1986), using X-ray

Table 3. Nectar cation concentrations in southern African species of *Protea, Leucospermum, Erica,* and *Aloe*. Values are means ± SD*.

Genus	No. species	Sugar (% w/w)	K^+ (mM)	Na^+ (mM)
Protea	9	25.9 ± 2.8	17.3 ± 9.3	18.0 ± 3.7
Leucospermum	5	26.4 ± 12.8	9.7 ± 3.6	15.4 ± 5.2
Erica	5	21.5 ± 7.3	4.9 ± 3.4	3.5 ± 0.5
Aloe	7	12.4 ± 3.7	4.2 ± 3.1	3.3 ± 1.8

*S.W. Nicolson unpublished data, except for *Leucospermum* (Nicolson & W.-Worswick, 1990)

microanalysis of *Abutilon* nectary hairs, found that K^+ in the phloem appeared to be excluded from the nectar. Table 3 presents cation concentrations for four genera of southern African plants, showing that Na^+ levels may exceed those of K^+. *Protea* and *Leucospermum* are in the Proteaceae, and high nectar Na^+ concentrations have also been recorded in two other genera in this family, *Banksia* and *Adenanthos* (Bradshaw & Bradshaw, 1999).

Exceptionally high K^+ concentrations have been recorded in the nectar of onion (*Allium cepa,* Alliaceae) flowers: up to 13,000 ppm, equivalent to 333 mM K^+, when the nectar is concentrated by evaporation (Waller et al., 1972). These authors suggested that these high K^+ concentrations may be responsible for the reduced attractiveness of the nectar to honeybees and resulting poor pollination of onion crops. Onion flowers have concentrated nectar (52–65%) with high hexose levels (Hagler et al., 1990), which may also influence their attractiveness to honey bees. The concentrations of Ca^{++} and Mg^{++} have been measured in a few studies (Waller et al., 1972; Heinrich, 1989; Kronestedt-Robards et al., 1989; Barclay, 2002).

Four species of Fabaceae visited by carpenter bees have nectar with high sugar concentrations but extremely low ion levels (Na^+ 0.3 to 3.8 mM, K^+ 1.4 to 6.4 mM). This suggests problems of ion conservation, especially when high metabolic water production in these large bees contributes to water excess (Nicolson, 1990).

5 AMINO ACIDS

The presence of amino acids in nectar has been known since the mid-1950s, when Ziegler (1956) compared sieve element fluid to other plant fluids, including floral nectar, and demonstrated ninhydrin-reactive material in nectar. Subsequently, Lüttge, using descending paper chromatography, identified

glutamine, asparagine, methionine, serine, tyrosine, cysteine, proline, and alanine in the nectar of plantain bananas and later in the nectars of five additional species (Lüttge, 1961, 1962).

It was not until the early 1970s, however, that the presence of amino acids in nectar became apparent on a large scale. In those studies Baker and Baker (1973) used a simple method to screen large numbers of nectars for ninhydrin-reactive material. Nectar spotted onto filter paper was reacted in a ninhydrin assay, and the intensity of the coloured spot was compared to a linear standard curve of histidine spots to give semi-quantitative information on the level of amino acids present (but not their chemical nature). Of 266 species tested, only six failed to show amino acids in this assay. The Bakers compared the nectars from flowers pollinated by different guilds (i.e., bee, butterfly, moth, fly, and bird). For the most part the coefficients of variation for the presence of amino acids in nectar were so large that little information could be obtained; the authors were, however, able to conclude that specialized flowers which attract carrion and dung flies were especially rich in ninhydrin-reactive material, as were butterfly-pollinated flowers. They suggested that plant nectars cannot be overlooked as a potential source of amino acids in the nutrition of butterflies and flies. Based upon the phylogeny of the plant species examined, they also suggested that amino acids have been a constituent of nectars since the earliest stages of angiosperm evolution. All 20 of the normal amino acids found in protein have been identified in various plant nectars, and the essential amino acids may be an important nitrogen source for nectarivorous pollinators (Nicolson, 2007, Chapter 7 in this volume).

Although all ten essential amino acids are commonly present in floral nectars, some non-essential amino acids such as asparagine and glutamine can occur in much higher concentrations. In *Erythrina* species pollinated by passerine birds, the total amino acid concentrations are far higher than in hummingbird-pollinated species (Baker & Baker, 1982b), and it is non-essential amino acids that are largely responsible for the difference (S.W. Nicolson, unpublished data). The passerine-pollinated *Erythrina* species produce relatively dilute nectars (Baker & Baker, 1982b), so the warning of Inouye et al. (1980) that non-sugar components may lead to overestimation of nectar sugar concentrations is a valid one in the case of *Erythrina*.

Gottsberger et al. (1984, 1990) cautioned against attributing too much significance to measured amino acid concentrations, because of the likelihood of amino acids leaching from dislodged pollen grains. However, this was disputed by Baker and Baker (1986). Pollen addition to nectar of *Aloe*

marlothii has no effect on the already high amino acid concentrations (around 50 mM; S.W. Nicolson, unpublished data).

5.1 Non-protein amino acids

In addition to the normal 20 amino acids found in proteins, plants also make a large number of non-protein amino acids (Fowden et al., 1979). Many of these compounds are toxic to protein biosynthesis and they frequently accumulate in seeds where they serve as deterrents to insect feeding (Swain, 1977). Because of the function of nectar as an attractant, most of these non-protein amino acids are thought to be excluded from nectar. However, a few of the non-toxic non-protein amino acids, including β-alanine, ornithine, homoserine, and γ-aminobutyric acid (GABA) are known to accumulate in nectar. Baker (1978) found non-protein amino acids in the nectars of 36% of 283 California species examined and in the nectars of 55% of 69 tropical trees and vines. In extrafloral nectars, Baker et al. (1978) reported the presence of non-protein amino acids in 22 of 33 species of tropical and temperate-zone angiosperms. Consistent with this is the recent finding that GABA is present in each of eight *Nicotiana* species examined and is even the predominant amino acid in the nectars of *N. plumbaginifolia* and *N. alata* (Kaczorowski et al., 2005). In *Impatiens capensis*, Lanza et al. (1995) found especially high nectar concentrations of glutamine and hydroxyproline, a component of plant cell walls. It is apparent that non-protein amino acids are consistent and sizable components of certain floral nectars, but whether they have any role in attraction of pollinators must await further studies.

5.2 Nectar amino acids are under the control of environmental factors

In an early study of the role of environmental factors in the expression of nectar amino acids, Baker and Baker (1977) showed semiquantitatively that the amino acid complement of nectar from six different species was fairly constant from sample to sample even though environmental conditions and growth locations were widely divergent. Recent studies using improved methods of quantitation such as HPLC have demonstrated that the amino acid composition of *Impatiens capensis* nectar can vary significantly within a single plant, within a population, and also between populations (Lanza et al., 1995). Other investigators have found that amino acid concentrations may vary with the age of the flower (Gottsberger et al., 1990; Petanidou et al., 1996). Although the concentrations of amino acids in nectar from any given species can be quite variable, Gardener and Gillman (2001a) found

that the overall amino acid composition of nectar is generally more highly conserved than the individual amino acid concentrations.

Other factors known to affect nectar composition include the response to elevated CO_2 levels. A series of studies showed that doubling CO_2 levels in the greenhouse for 60 to 80 days resulted in reduced levels of amino acids in the nectar of four of five species tested (Rusterholz & Erhardt, 1998; Erhardt et al., 2005). These authors conclude that elevated CO_2 levels could have detrimental effects on the interactions between flowers and visiting pollinators (such as butterflies) that utilize nectar as the primary source of amino acids in their diet.

The amino acid composition of nectar is also known to be affected by growth conditions. Gardener and Gillman (2001b) demonstrated that increasing fertilizer availability to *Agrostemma githago* significantly altered the amino acid composition of floral nectar, with specific increases in glutamine, proline, and asparagine. One non-protein amino acid, GABA, showed a decrease with increased fertilization. Local differences in nectar composition may lead to a greater variety of visiting pollinators and higher cross-fertilization among local populations, with important consequences for plant genetic diversity. Modern farming practices cause the drift of applied fertilizers at cultivated field margins, and an unexpected consequence may be nectars with significantly improved nutritional qualities, as postulated by Kleijn and Snoeijing (1997).

Extrafloral nectar production is also known to be under environmental control. Smith et al. (1990) examined the production and composition of extrafloral nectar following simulated herbivory of *Impatiens sultani*. The volume and carbohydrate concentration of nectar were unchanged between intact and defoliated plants, but HPLC analysis showed a dramatic increase in the amino acid concentrations in extrafloral nectars 24 h after experimental defoliation. This may be a plant response to attract additional defensive insects. The plants apparently return to homeostasis rather rapidly—72 h after defoliation, the amino acids of extrafloral nectar were restored to normal levels. Cowpea (*Vigna unguiculata*) has two types of extrafloral nectaries, and those on the inflorescence have much higher levels of amino acids than those on the leaf stalks (Pate et al., 1985). Pitcher plants produce extrafloral nectar to attract insect prey, and Dress et al. (1997) suggest that variation in its amino acid composition may be related to previous capture success.

5.3 Contribution of amino acids to the taste of nectar

One unique aspect of the presence of amino acids in nectar is the potential contribution of these compounds to its taste (Gardener & Gillman, 2002). Amino acids have much more diverse chemical strucures than sugars and their taste also varies with concentration (Birch & Kemp, 1989). It has been well established that insects have several different classes of labellar chemosensory receptors responding to water, sugar, and salts (Shiraishi & Kuwabara, 1970; Hansen et al., 1998). The water cell is believed to control drinking behaviour (Dethier, 1976). The sugar cell recognizes the sugars in nectar and is believed to mediate the attraction of flies to sucrose-rich solutions (Omand & Dethier, 1969). Stimulation of the salt cell in conjunction with the water cell results in enhanced elicitation of feeding behaviour (Hansen et al., 1998).

Examination of the effects of amino acids on insect chemoreceptors (Shiraishi & Kuwabara, 1970; Hansen et al., 1998) has permitted the description of four taste classes of amino acids. Those in Class I (asparagine, glutamine, alanine, cysteine, glycine, serine, threonine, tyrosine) have no effect on the chemoreceptors of two species of fly. Those in Class II (arginine, aspartic acid, glutamic acid, histidine, lysine) are generally inhibitory to fly chemoreceptors. Two amino acids in Class III (proline and hydroxyproline) have the unique ability to stimulate the salt cell (Hansen et al., 1998; Wacht et al., 2000). Class IV (isoleucine, leucine, methionine, phenylalanine, tryptophan, valine) includes amino acids with the ability to stimulate the sugar cell. Thus, amino acids in nectar have the potential to modify insect behaviour by stimulating insect chemosensory receptors. Gardener and Gillman (2002) have devised a graphical method to represent the composition of amino acids in nectar, and Figure 2 uses this to show the possible “taste” of a number of plant nectars (Carter et al., 2006). It should be stressed that this is based on the chemosensory responses of flies and amino acids may taste differently to vertebrate pollinators (see Birch & Kemp, 1989).

Among the amino acids found in nectar, proline is unique because it can stimulate the salt cell, resulting in increased feeding behavior (Hansen et al., 1998). Proline has also been identified at high levels in some nectars (Gardener & Gillman, 2001a; Kaczorowski et al., 2005; Carter et al., 2006). In choice tests, both cabbage white butterflies and honeybees have shown preferecnes for sugar solutions enriched by amino acids, including proline (Inouye & Waller, 1984; Alm et al., 1990; Carter et al., 2006). Proline is an especially important amino acid for insects. It is by far the most abundant amino acid in honeybee haemolymph, and is required for egg laying (Crailsheim &

Leonhard, 1997; Hrassnigg et al., 2003). Proline obtained from plant nectars by honeybees may also regulate the secretion of invertase that is required for conversion of nectar to honey (Davies, 1978). Haemolymph proline is selectively degraded during the initial stages or lift phase of flight (Brosemer & Veerabhadrappa, 1965; Micheu et al., 2000). The metabolism of proline produces 71% of the levels of ATP that are produced by glucose. However, the initial steps of glucose metabolism require the consumption of ATP, while metabolism of proline does not. Thus, proline is a more efficient fuel in the short run, while glucose is a far superior fuel in the long run. The accumulation of both glucose and nectar presents insects with a dual action fuel: proline for rapid, short-term bursts of energy production and a large amount of glucose for extended flight (Carter et al., 2006).

Also deserving special mention is phenylalanine, the most abundant amino acid in the nectars of 73 mainly bee-pollinated Mediterranean plant species sampled by Petanidou et al. (2006). (Unfortunately, proline could not

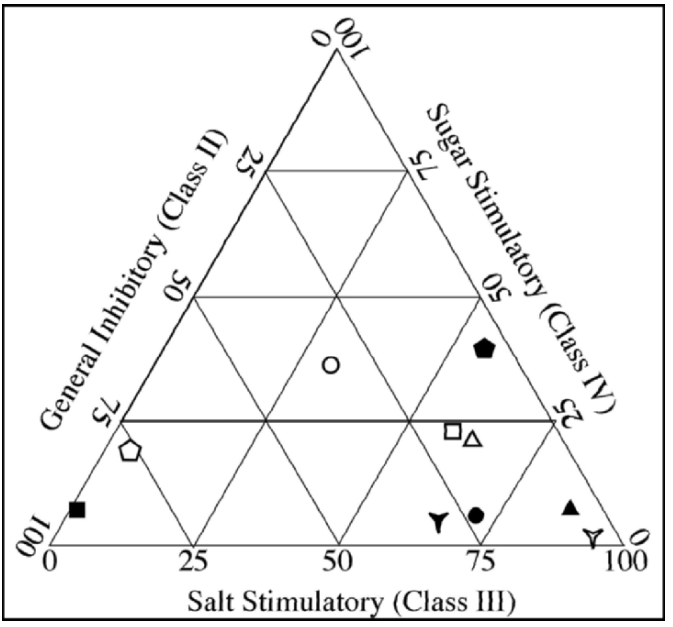

Figure 2. Possible stimulation of insect chemoreceptors by amino acids in various plant nectars: ■ *Gossypium hirsutum* (Hanny & Elmore, 1974); ▲ *Impatiens sultani* extrafloral nectar (Smith et al., 1990); ⬠ *Sarracenia purpurea* pitcher extrafloral nectar (Dress et al., 1997); ⬟ *Lotus corniculatus*, ⅄ *Trifolium pratense,* ⏷ *Scabiosa columbaria* (Rusterholz & Erhardt, 1998); ○ *Agrostemma githago* (Gardener & Gillman, 2001b); □ *Glycine tomentella*, ● *Glycine canescens,* and △ *Nicotiana langsdorffii* × *N. sanderae* var. LxS8 (Carter et al., 2006).

be identified by the HPLC methods used in their study.) Phenylalanine was highest in the Lamiaceae, where it comprised on average 47% of the total amino acids, and high proportions of phenylalanine have also been recorded among the nectar amino acids of *Salvia fruticosa* and *Satureja thymbra* in Israel (Dafni et al., 1988). Phenylalanine is known to be a phagostimulant for bees (Inouye & Waller, 1984). However, the fact that it stimulates the sugar cell in flies (Shiraishi & Kuwabara, 1970) is unlikely to be important in the responses of bees to the concentrated nectars in Mediterranean systems (see also Petanidou, 2007, Chapter 8 in this volume).

6 PROTEINS

The existence of proteins in nectar was reported long ago (Pryce-Jones, 1944; Lüttge, 1961). The first enzymatic activity to be identified in nectar was invertase, found in the floral nectar of *Tilia* sp. (Beutler, 1935). However, for the most part, these earliest studies did not characterize nectar proteins. Baker and Baker (1975) studied 129 species of plants using a bromophenol blue assay and found that 17% showed a positive test for the presence of protein.

Table 4 lists proteins that are reported to be secreted into various plant nectars. The term *nectarin* refers to any protein that is secreted into the nectar of plants. Lüttge (1961) reported the presence of a tyrosinase in the nectar of *Lathraea clandestina*, but its function in the nectar of plants is still not clear 45 years later. Tyrosinase catalyses the hydroxylation of phenolic compounds (Metzler, 2003) and consequently could function to modify phenolic compounds secreted into nectar (see "Phenolics" on page 247). Transglucosidase and transfructosidase identified in nectar (Zimmerman, 1953, 1954) are involved in the production of glucosyl or fructose oligosaccharides. The function of oligosaccharides in nectar is not clear, but they could provide additional nutritional benefits.

Phosphatases, enzymes that catalyse the removal of phosphate from organic molecules, have been reported in the nectar of a number of species (Cotti, 1962; Zalewski, 1966). Phosphatases are known to have multiple functions in eukaryotes, e.g., recovery of phosphate during times of phosphate starvation (Bozzo et al., 2002), signal transduction (Li et al., 2001), metabolism of nucleic acids (del Pozo et al., 1999), and defensive functions (Chandra & Low, 1995). Each of these functions is important in plants; none, however, has been examined for nectar phosphatases. Zauralov (1969) reported the presence of oxidizing enzymes in the nectar of milkweed. The enzymes identified were polyphenol oxidase, cytochrome oxidase, and

Table 4. Proteins identified in various plant nectars.

Protein/enzyme	Species	Function	Reference
Invertase	*Tilia* sp. *Acacia* spp.	Hydrolysis of sucrose	Beutler, 1935 Heil et al., 2005
Transglucosidase	*Robinia pseudacacia*	Polymerization of glucose molecules	Zimmerman, 1953
Transfructosidase	*Impatiens holstii*	Polymerization of fructose molecules	Zimmerman, 1954
Phosphatase	several	Breakdown of phosphate-rich molecules—possibly defence	Cotti, 1962
Tyrosinase	*Lathraea clandestina*	Production of melanin	Lüttge, 1961
Mannose-binding lectin	*Allium porrum*	Possibly defence	Peumans et al., 1997
Alliinase	*Allium porrum*	Possibly allelochemical defence	Peumans et al., 1997
Nectarin I—Superoxide dismutase	*Nicotiana* sp.	Generates hydrogen peroxide	Carter & Thornburg, 2000
Nectarin II—Breakdown product of *Nec3*	*Nicotiana* sp.	Probably non-functional	Carter & Thornburg, 2004b
Nectarin III—Bifunctional carbonic anhydrase and MDH reductase	*Nicotiana* sp.	pH-balanced nectar and recycles ascorbate to protect from hydroxyl free radicals	
Nectarin IV—Xyloglucan-specific Endoglucanase Inhibitor Protein (XEGIP)	*Nicotiana* sp.	Prevents fungal invasion of the gynoecium	Naqvi et al., 2005
Nectarin V—Glucose oxidase and possible dehydroascorbate reductase	*Nicotiana* sp.	Generates hydrogen peroxide; may recycle ascorbate to protect from hydroxyl free radicals	Carter & Thornburg, 2004c; unpublished results

ascorbate oxidase. Scogin (1979) identified the presence of esterase and malate dehydrogenase in the nectar of *Fremontia californica* and *F. mexicana*. These two species differed in their protein profiles, and hybrid plants showed a composite nectar protein profile. In addition to esterase and malate dehydrogenase, other proteins were also observed, but were not fully characterized. These proteins accumulated to 87 μg/ml of nectar.

6.1 Proteins in leek nectar

Subsequently there is a rather lengthy gap in the literature until the next identification of nectar proteins. In examining the nectar of *Allium porrum*,

Peumans et al. (1997) were the first investigators to perform any molecular characterization on nectar proteins. They identified two defence-related proteins in the nectar of leeks. The mannose-specific lectin from nectar is a 13 kDa protein that was present at about 150 μg/ml of nectar. It belongs to the structurally and evolutionarily conserved protein family of monocotyledon mannose-binding lectins. Other proteins from this family have antibiotic properties, particularly against nematodes and insects with piercing–sucking mouthparts (Hilder et al., 1995; Powell et al., 1995; Rabhé et al., 1995). Because the monocotyledon mannose-binding lectins occur in the families Amaryllidaceae, Alliaceae, Orchidaceae, Araceae, and Liliaceae, Peumans et al. (1997) also tested nectar from these families. Using a mannose agglutination assay, these authors found that several *Allium* species (onion, shallot) along with snowdrop (*Galanthus nivalus*) contained mannose agglutination positive material. However, no mannose agglutination activity was found in the nectars of other Amaryllidaceae, orchids and lilies. By feeding leek nectar to honeybee colonies, they demonstrated that the mannose-binding lectin is apparently not toxic to honeybees, even though it is toxic to aphids and nematodes (Hilder et al., 1995; Powell et al., 1995; Rabhé et al., 1995).

Alliinase (alliin lyase) is a pyridoxal-phosphate-containing enzyme involved in the production of the organosulphur compounds responsible for both the odour and pungency of *Allium* plants, as well as for their antimicrobial properties (Ankri et al., 1997; Perez-Giraldo et al., 2003). Alliinase acts on the raw sustrate alliin to produce allicin, an active abiotic agent against a wide variety of organisms, including both Gram-positive and Gram-negative bacteria, fungi such as *Candida albicans*, parasites such as *Entamoeba histolytica* and *Giardia lamblia*, and viruses (Ankri & Mirelman, 1999).

Both of these proteins, mannose-binding lectin and alliinase, are found at relatively high levels in phloem stalk exudates. In addition, Peumans et al. (1997) also examined the levels of chitinase in leek nectar. Although present at significant levels in leek stalk exudates, this enzyme is not significantly transferred into the nectar of leeks. Mannose-binding lectin and alliinase are also found in honey produced by bees working leek umbels; the amounts are, however, reduced by 10-fold and 25-fold, respectively, despite the fact that honey is concentrated nectar (Peumans et al., 1997).

6.2 Nectar redox cycle

The composition of nectar makes it an excellent microbial growth medium (Sugiyama et al., 1991; Bubán et al., 2003; Raguso, 2004). Because visiting

pollinators are not aseptic and carry microbes into the reproductive tract of flowers, plants must have developed specific defences against these microbes. An interspecific cross of ornamental tobacco, *Nicotiana langsdorffii* × *N. sanderae* (LxS8), has been used to investigate the defence properties of nectar. This cross was chosen because it has extremely large nectaries and it produces significantly more nectar than either of the parent lines. Using this line, it is relatively easy to collect several hundreds of milliliters of nectar. Because the ability to collect large volumes of nectar is a major limitation of most nectar studies, the LxS8 hybrid makes an excellent system for the study of nectar biochemistry as well as for studying the physiology of nectar production (also see Chapter 6 in this volume).

The nectar of LxS8 ornamental tobacco contains a limited array of only five proteins (Carter et al., 1999). The analysis of these novel proteins has permitted researchers to identify several unique biochemical functions of nectar (Fig. 3), and to hypothesize that these enzymes function in a novel biochemical pathway, termed the *Nectar Redox Cycle* (Carter & Thornburg, 2000, 2004a). The pathway functions to maintain nectar in a microbe-free state, thereby protecting the gynoecium and developing embryos contained therein from microbial invasion (Thornburg et al., 2003; Carter & Thornburg, 2004; Carter et al., 2007).

The analysis and characterization of Nectarin I (*Nec1*) revealed that it was a novel manganese-containing superoxide dismutase, functioning to generate high levels (4 mM) of hydrogen peroxide (Carter & Thornburg, 2000). This level is 40-fold higher than that produced by human neutrophils when they engulf and destroy invading micro-organisms (Prince & Gunson, 1987). Because nectar also contains cations, high levels of hydrogen peroxide create a problem by generating hydroxyl free radicals (Rowley & Halliwell, 1983; Halliwell & Gutteridge, 1999). Fortunately, nectar also contains an antioxidant, ascorbate, which is capable of detoxifying these dangerous hydroxyl free radicals. In this detoxification process, ascorbate is reduced to monodehydroascorbate. Two other nectar proteins serve to regenerate the ascorbate in nectar. Nectarin III (*Nec3*) is a bifunctional protein having both monodehydroascorbate reductase activity and also carbonic anhydrase activity (Carter & Thornburg, 2004a). Nectarin II is a proteolytic breakdown product of *Nec3*. The carbonic anhydrase activity is thought to function to buffer nectar in order to provide a pH-balanced meal for pollinators. Further, carbonic anhydrase may have direct antimicrobial activity, as was recently shown for potato interactions with *Phytophthora infestans* (Restrepo et al., 2005). Nectarin V (*Nec5*) is a berberine-bridge enzyme-like glucose oxidase that functions to provide additional levels of hydrogen perox-

ide (Carter & Thornburg, 2004a). In addition, there is preliminary evidence that dehydroascorbate can serve as a terminal electron acceptor to regenerate ascorbate.

The high levels of hydrogen peroxide that accumulate in the nectar of ornamental tobacco are thought to serve as a defence that protects the metabolite-rich nectar from invading micro-organisms carried to the flower by wind or by non-sterile pollinators. This biochemical pathway contained wholly within soluble nectar serves to maintain nectar in a microbe-free state. Thus in ornamental tobacco, nectar functions not only to attract insect pollinators, but also to protect the gynoecium from microbial invasion of the metabolite-rich fluid (Thornburg et al., 2003, Carter et al., 2007).

To determine how widespread the nectar redox cycle is throughout the angiosperms, Carter and Thornburg (2000) have examined the nectars of 15 plant species for *Nec1* homologues and identified nine species that showed immunoreactive material in their nectars. Based upon this, they concluded that *Nec I* and perhaps the Nectar Redox Cycle are widespread throughout the angiosperms. In addition, insects may be well equipped to handle oxidative nectar, because many insect guts are antioxidative (Ahmad et al., 1991; Barbehenn et al., 2001) and enzymes such as catalase and peroxidase are known to be localized in the insect gut and Malpighian tubules (Ahmad et al., 1988; Felton & Summers, 1995).

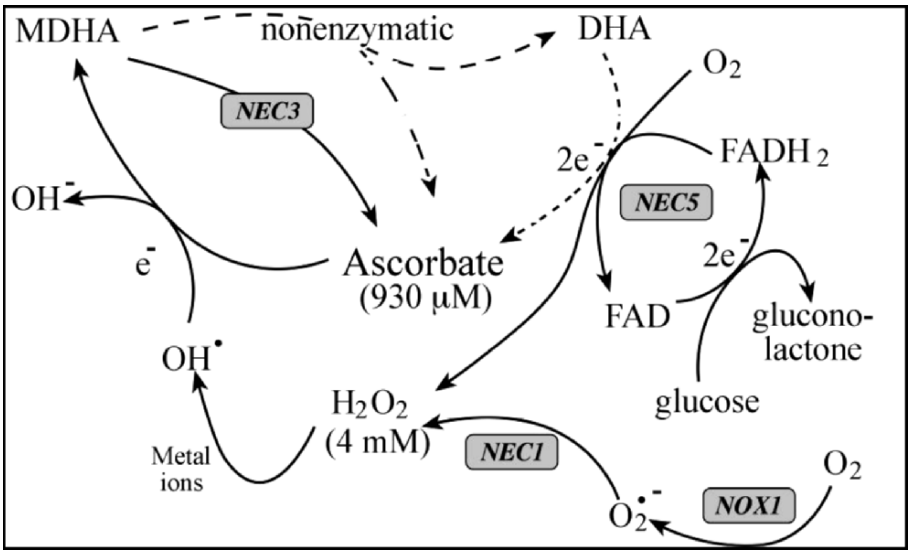

Figure 3. Nectar Redox Cycle.

Recently, the last of the five ornamental tobacco nectarins (*Nec4*) was characterized as a xyloglucan-specific endoglucanase-inhibiting protein (XEGIP), which serves to protect the gynoecium from invading fungi (Naqvi et al., 2005). Xyloglucan-specific endoglucanases (XEGs) are fungal enzymes that hydrolyse the plant cell wall hemicellulases, thereby weakening plant cell walls and offering fungi opportunities for invasion. The XEGIPs function to inhibit these fungal pathogenesis factors much in the same way that polygalacturonase inhibitor proteins inhibit fungal polygalacturonases (Pressey, 1996). The expression pattern of *Nec4* mRNA implies that the nectary continues this protective function even after pollination has occurred. The identification of *Nec4* as a potent inhibitor of fungal endoglucanases provides strong support for the hypothesis that nectar also functions to protect the floral base from invading microorganisms.

7 OTHER NECTAR CONSTITUENTS

A wide variety of other biochemicals also accumulates in plant nectars. Some of these may enrich the nectar, providing a better diet for visiting pollinators, while others are thought to decrease the palatability of nectar to unwanted floral visitors. Alkaloids, coumarins, saponins, and non-protein amino acids in nectar may render it toxic or repellent to some animals (Guerrant & Fiedler, 1981; Detzel & Wink, 1993; Adler, 2000). However, Rhoades and Bergdahl (1981) argued that the ability of specific pollinators to tolerate toxic compounds would serve as a co-evolutionary mechanism to manipulate animal behaviour to the plant's advantage and to exclude nectar thieves. Furthermore, if insects co-evolved to prefer toxic compounds, this would focus pollinators on a single plant species or group of closely related species, thereby maximizing the effectiveness of pollinator visitation. Pyrrolizidine alkaloids in nectar are inhibitory to generalist-feeding butterflies but attractive to specialist feeders (Masters, 1991). Iridoid glycosides in *Catalpa speciosa* (Bignoniaceae) are feeding attractants for caterpillars, and also occur in the nectar of this species. They were shown to deter nectar thieves but not the legitimate pollinators (Stephenson, 1982).

A nectar property rarely considered is pH, which ranges from 3 in *Silene alba* (Caryophyllaceae) to 10 in *Viburnum costaricanum* (Caprifoliaceae), but is generally slightly acidic (Baker & Baker, 1983a). A more extreme example is the parasitic *Lathraea clandestina* (Scrophulariaceae), which flowers at ground level and produces pungent nectar-containing ammonia, which is tolerated by pollinating bumblebees but deters ants. The freshly secreted nectar is slightly acidic (about pH 6.5), becoming alkaline (pH 11.5)

in aged flowers: this exceptionally high pH is due to dissolved ammonia produced after the primary secretion of nectar (Prys-Jones & Willmer, 1992).

In addition, some plant species produce mildly toxic or narcotic levels of chemical constituents in their nectars. The nectar of *Epipactis helleborine* (Orchidaceae) was recently found to contain a number of narcotic substances, including oxycodone, 3-{2-{3-{3-(benzyloxy)propyl}-3-indol, and 7,8-dehydro-4,5-epoxy-3,6-D-morphinan (Jakubska et al., 2005). The authors suggest that after imbibing these narcotic substances, pollinators become naturally intoxicated, and their more "sluggish" behaviour increases the time spent within the flower and the chances of successful pollination. A similar role was previously suggested for ethanol in the flowers of these orchids (Ehlers & Olesen, 1997). However, this intoxication of pollinators could lead to undesirable levels of geitonogamous self-pollination, i.e., between flowers on the same plant (Klinkhamer & De Jong, 1993).

Before we deal with several structural categories of nectar constituents, it should be mentioned that pesticides can accumulate in floral and extrafloral nectars, so care must be taken in the chemical control of insect or microbial pests (Jaycox, 1964; Lord et al., 1968; Barker et al., 1980).

7.1 Lipids

The presence of lipids has been reported in numerous plant nectars (Vogel, 1971; Baker & Baker, 1975; Bernardello et al., 1999; Vesprini et al., 1999). Flowers offering fatty oils instead of or in addition to nectar are found in ten different plant families, and oil flowers are visited and pollinated by specialized bees (Buchmann, 1987). Secretion of lipids is from highly specialized epithelial cells termed elaiophores or glandular trichomes, and the progression from lipid-containing nectars to pure oils is not surprising in view of similarities in the cells involved (Fahn, 2000). Elaiophores are metabolically active secretory epidermal cells that generate large quantities of lipids under a thin protective cuticle, forming lipid-filled blisters. These lipids have been well studied in *Calceolaria* species (Scrophulariaceae) and in the rhattanys (*Krameria* species, Zygophyllaceae). The major lipids to accumulate in these nectars appear to be β-acetoxy fatty acids of varying chain length between C_{16} and C_{20} (Vogel, 1971; Seigler et al., 1978). These are present as the modified free fatty acids as well as in diglycerides. In addition to β-acetoxy fatty acids, the oil nectars of *Mouriri myrtilloides* and *M. nervosa* contain unmodified free fatty acids of C_{14} to C_{20} (Buchmann, 1987). Nectar of other species such as *Jacaranda ovalifolia* (Bignoniaceae) and *Trichocereus*

andalgalensis (Cactaceae) contains so much lipid that it has a milky consistency to the naked eye (Baker & Baker, 1975). The nectar of bat-pollinated *Dactylanthus taylorii* (Balanophoraceae) has been especially well studied (Ecroyd et al., 1995). These investigators have identified the ethyl and benzyl esters of hexadecanoic acid and C_{18} to C_{23} polyunsaturated fatty acids. In addition, the nectar contained C_{21} to C_{31} hydrocarbons.

Because lipids are a highly reduced form of carbon, on a molar basis they are among the highest-energy compounds available in nature, and may provide pollinators with a rich energy source. However, oil nectars are also more expensive for plants to produce. Among Patagonian species, lipids are relatively common nectar constituents, about 30–50% of these species accumulating nectar lipids, especially among the Fabaceae (Forcone et al., 1997; Bernardello et al., 1999). These authors argue that the extreme conditions found in Patagonia may necessitate a high-energy food resource for effective pollination.

Finally, a wide variety of lipids are known to accumulate in extrafloral nectar of cotton (*Gossypium hirsutum*, Malvaceae). Using gas chromatography, Stone et al. (1985) identified free fatty acids including palmitic, stearic, palmitolenic, oleic, linoleic, and linolenic acids. They also identified a number of phospholipids. The fatty acid concentration was greatest in the extrafloral nectar from young plants and decreased as the plants matured.

7.2 Organic acids

Phloem sap and nectar both contain primary metabolites of the plant that are involved in fundamental plant physiological processes (Pate et al., 1985). These include amino acids, especially some non-essential amino acids such as asparagine and glutamine, and organic acids that are Krebs cycle intermediates (fumarate, malate, oxaloacetate, and succinate). Apart from early studies demonstrating the presence of organic acids in various nectars (Baker & Baker, 1975, 1983a), little further attention has been paid to these substances in nectar. Pate et al. (1985) compared the composition of phloem sap and two types of extrafloral nectar in *Vigna unguiculata*, and found that succinate and malate were the main organic acids of both phloem and nectar, with malonate also significant in the nectar. The energy value of organic acids to insects has seldom been considered, but leafhoppers (Cicadellidae) metabolize xylem organic acids with high efficiency, as assessed by comparing the chemical profiles of xylem fluid and honeydew (Andersen et al., 1989).

Ascorbic acid (vitamin C) is well known as an antioxidant in floral nectar (Baker & Baker, 1975). It was identified early on in nectar at moderately high concentrations (>2 mg/ml) (Griebel & Hess, 1940). Ascorbate has subsequently been identified in the nectars of many plant species (Bukatsch & Wildner, 1956; Baker & Baker, 1975; Carter & Thornburg, 2004a; Naef et al., 2004; Horner et al., 2007); it is, however, not known how widespread ascorbate accumulation is in nectar. Most of the ascorbate is lost when honeybees convert nectar into honey. Ascorbate is an essential nutrient for many insects and also an antioxidant (see "Nectar redox cycle" on page 241) that minimizes the negative effects of phenols in ingested plant tissue (Barbehenn et al., 2001)—this might help pollinators to cope with phenolic compounds in nectar.

7.3 Phenolics

Phenolic substances are quite widespread in nectars (Radzevenchuk et al., 1976; Baker & Baker, 1982a; Ferreres et al., 1996). Their accumulation may render the nectar toxic, so that it then becomes repellent to some visitors (Frankie et al., 1982; Hagler & Buchmann, 1993). Recently, Johnson et al. (2006b) examined the responses of potential pollinators to the dark nectar of *Aloe vryheidensis*, which contains phenolics—honeybees and sunbirds were deterred by the bitter taste, but larger passerine birds that are likely to be more effective pollinators were not.

Phenolic substances are also relatively common scent products of flowers (Knudsen et al., 1993; Sroka et al., 2001; del Bano et al., 2003; Deachathai et al., 2006). As well as attracting pollinators or repelling nectar thieves, these scent compounds may have a defensive function, either due to antimicrobial activity or because they serve as signalling molecules to predators and parasitoids (Pichersky & Gershenzon, 2002). Because of their solubility in aqueous solutions and their production in the vicinity of the floral nectar these phenolic compounds may also dissolve in nectar (Raguso, 2004).

Phenolic compounds in honey can serve as markers for its botanical origin (Bogdanov et al., 2004). Ferreres et al. (1996) have shown that Portuguese heather nectar (*Erica* sp.) collected from the crop of bees contained at least four phenolic compounds. The phenolic aglycones were identified as quercetin, kaempferol, myricetin, and isorhamnetin. Gil et al. (1995) identified a phenolic profile in rosemary nectar that showed 15 different flavonoids, the most prominent being kaemferol-3-sophoroside and quercetin-3-sophoroside. In some cases, nectar phenolics appear to be metabolized by honeybees during the manufacture of honey (Liu et al., 2005).

Phenolics have fluorescent properties, and it was suggested that fluorescent compounds accumulated in nectar might serve as a guide for pollinators, especially honeybees, which can see in the UV, although this has been disputed (Thorp et al., 1975; Kevan, 1976). Two bright blue fluorescent compounds in the nectar of *Fremontia* sp. were identified as 5,7-dimethoxygenistein-4′glucoside and its aglycone, 5,7-dimethoxygenestein (Scogin, 1979). The chemical causes of coloured nectar in some plant genera such as *Melianthus*, *Aloe*, and *Schiedea* are not well known (Hansen et al., 2007), although aurones (flavonoid pigments) were identified in the red nectar of *Nesocodon mauritanicus* (Campanulaceae) (Olesen et al., 1998).

7.4 Alkaloids

Alkaloids have been detected in the nectar of a large number of plants (Hazslinsky, 1956; Baker & Baker, 1975; Galetto & Bernardello, 1992; Adler & Wink, 2001). Alkaloids are generally thought to have a detrimental effect on pollinator visitation (Baker & Baker, 1982a; Adler, 2000); however, a direct test showed that the pyrrolizidine alkaloid, monocrotaline, had no significant effect on insect feeding (Landolt & Lenczewski, 1993). Recently, Singaravelan et al. (2005) tested the responses of honeybees to four secondary compounds found naturally in floral nectar: nicotine, anabasine, caffeine, and amygdalin. Except for anabasine, naturally occurring concentrations did not have a deterrent effect, and low concentrations of nicotine and caffeine elicited a significant feeding preference in the bees. This is interesting in view of the common observation of bees drinking from Coca-Cola cans!

The relationship between nectar alkaloids and plant fitness has been examined for gelsemine, the main alkaloid of *Gelsemium sempervirens* (Loganiaceae). When Adler and Irwin (2005) increased the gelsamine content in nectar of this plant, both nectar robbers and pollinators visited fewer flowers and for shorter times, and pollen transfer decreased. The presence of this alkaloid in nectar does not appear to benefit the plant, and may be a pleiotropic consequence of its production in other plant tissues.

7.5 Terpenoids

Diverse and odiferous terpenoid compounds are produced by almost all flowers. They are primary constituents of essential oils, and have been identified as accumulating in nectars of a number of plants (Juergens, 2004; Naef et al., 2004; Raguso, 2004). Terpenoids are generally thought to be insect attractants (Plepys et al., 2002; Andersson, 2003; Tholl et al., 2004). However, many terpenoids have antifeedant activity, reflected in their interactions with

GABA receptors in the insect herbivore, resulting in feeding satiation (Mullin et al., 1991; Ozoe et al., 1999). The finding that this can be overcome in some species by co-administration of GABA (Passreiter & Isman, 1997) suggests a possible interaction between these compounds and the high level of accumulation of GABA, a non-protein amino acid, in some plant nectars (section 5.1, above).

While terpenoids do occur in plant nectars, most are produced by cells with specialized metabolic potential that are dispersed throughout the flower (Bergström et al., 1995; Dudareva et al., 1998; McTavish et al., 2000). These specialized cells produce the volatile compounds that serve as pollinator attractants (Plepys et al., 2002; Andersson, 2003). Such attractants require the nectar reward to be effective (Hammer & Menzel, 1995). Insect-learning studies have demonstrated that associative learning requires both a conditioned stimulus and an unconditioned stimulus. If insects are presented with an attractive odour without a sucrose reward, the response to the odour soon disappears or is extinguished (Bitterman et al., 1983). Many insects clearly show a preference for one attractant over another (Honda et al., 1998; Natale et al., 2003), driven by electrophysiological responses (Raguso et al., 1996), and the presence of the feeding stimulus results in the reinforcement of lesser attractants over dominant ones (Cunningham et al., 2004). Thus, long-range factors such as terpenoids generally serve as attractants for visiting pollinators, but are of little use if there is not an associated nectar reward to reinforce the conditioned stimulus. As stated earlier for the phenolic compounds, some volatile terpenoids are soluble in the aqueous nectar and their presence in nectar may be due to passive absorption by the nectar. In a direct test of this hypothesis, Raguso demonstrated that several floral scent compounds, including geraniol, linalool, and jasmone, were taken up by artificial nectars and subsequently volatilized (Raguso, 2004).

8 CONCLUSION

The rich composition of nectar suggests that this metabolic offering is a major consideration both for the plant and for many animal visitors. Using nectar as an energy source, pollinators move pollen from flower to flower, but at the same time they carry a host of microbial contaminants. There must exist mechanisms to maintain nectar in a microbe-free state, and indeed the nectary expresses a number of protein-defence factors that function to protect the gynoecium and the nectar itself. Phenolic, terpenoid, and other ingredients of essential oils are produced in flowers and may accumulate in nectar, probably with additional antimicrobial benefits (Raguso, 2004).

The ecological significance of many biochemicals in plant nectars is not known. It has been suggested that nectar rewards should not be too great because of the risk of increased levels of geitonogamous self-pollination (Klinkhamer & De Jong, 1993). These authors were referring to limits on nectar volume, but the same function of controlled attractiveness can be ascribed to certain secondary compounds occurring in nectar. However, it is not necessary to postulate an adaptive function if secondary compounds involved in herbivore resistance are present in nectar as a passive consequence of their presence in phloem (Adler, 2000), and this is an area that deserves special attention. Information is accumulating rapidly on the chemical diversity of nectar, the phylogenetic background to this diversity, and the nutritional and behavioural implications for nectar consumers. We look forward to future studies that will elucidate the patterns and processes involved.

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Chapter 6

MOLECULAR BIOLOGY OF THE *NICOTIANA* FLORAL NECTARY

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1 INTRODUCTION

The secretion of floral nectar in most angiosperms is under very specific developmental control. Secretion begins when the flowers open and continues while the flowers remain receptive to pollination. Nectar flow is often increased by pollinator visitation. However, after pollination, nectar secretion ceases and the remaining nectar may be reabsorbed (Búrquez & Corbet, 1991; Nepi et al., 1996b, 2001; Stpiczyńska, 2003).

To take advantage of the very powerful recombinant DNA methodology that is available to today's molecular biologists, it is almost imperative that one focuses attention on a single species, or even a single plant line that becomes the model plant for any given trait. *Arabidopsis thaliana* has served as an excellent model plant for many traits and physiological measurements. The genetic, biochemical, and molecular biological resources available for *Arabidopsis* researchers far surpass those available for any other species. Unfortunately, while *Arabidopsis* is excellent for many traits, it is not a good model for nectar and nectary analysis, because the nectaries are small and inconspicuous and are lost when the flower falls. Therefore, to evaluate the biochemistry and molecular biology of the floral nectary, we have investigated many species of plants and have focused on a diploid ornamental tobacco line. As a genetic tool, tobacco is not as desirable as *Arabidopsis*. Still, several advantages of ornamental tobacco have led us to choose this line as our model organism.

S.W. Nicolson, M. Nepi, and E. Pacini (eds.), Nectaries and Nectar, 265–288.

First, *Arabidopsis* has multiple types of nectaries (Davis et al., 1998), while tobacco has a single, large nectary at the base of the gynoecium (Carter et al., 1999). Second, the tobacco nectary is 500-fold larger than the nectaries of *Arabidopsis*, which means that we can readily isolate sufficient quantities of material for cloning, microscopy, and biochemical analyses. Third, a single *Arabidopsis* bolt typically produces less than 20 flowers at a time. Our tobacco line is indeterminate, continuously producing large numbers (up to 150) of flowers. Consequently, we can isolate large quantities of materials for bioanalysis, including hundreds of millilitres of tobacco nectar and tens of grams of nectary tissue at any desired developmental stage. Finally, Mysore et al. (2001) have proposed that there is much to be learned regarding general angiosperm genetics by a more broad-based approach investigating a number of plant species, rather than a strict reliance on the *Arabidopsis* genome. Thus, we believe that analysis of nectary function in ornamental tobacco nectaries can serve as a general model for nectary function among a large proportion of the angiosperms and can enlarge our global understanding of plant genomics, particularly with regard to this unique floral organ.

We have therefore worked exclusively with a diploid ornamental tobacco line (LxS8). This line was derived from an interspecific cross of *Nicotiana langsdorffii* × *N. sanderae* (Kornaga, 1993; Kornaga et al., 1997). This line produces extraordinary levels of nectar, much higher than either of its parents. It is both male and female fertile, but is largely self-incompatible. It does, however, set high levels of seed when cross-pollinated. We routinely propagate it clonally to produce large numbers of identical plants. We have also developed methods for transformation of this line of tobacco.

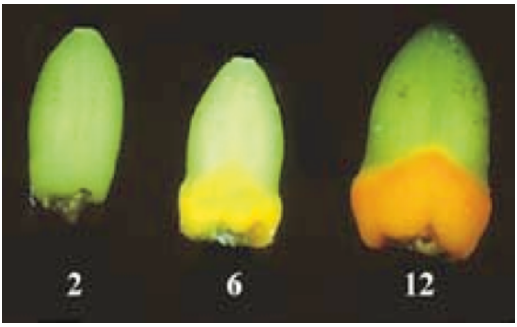

Figure 1. Tobacco nectary developmental stages. The nectary is located at the base of the gynoecium. Stage 2 (early filling), stage 6 (filling/beginning ripening), stage 12 (mature, at anthesis)

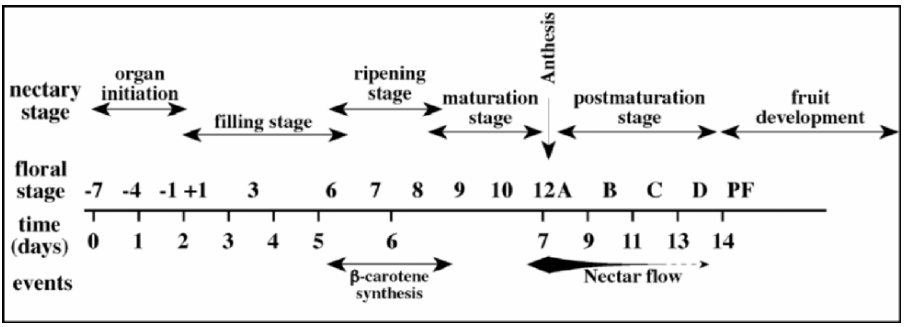

Figure 2. Correlation of floral maturation with nectary development. The floral development in tobacco extends from stage +1 through stage 12. The minus stages refer to the development of the bud, prior to floral development. After stage 12, the maturation of the flower continues with little change for several days; these stages are referred to as 12A to 12D. After fertilization (PF), fruit development begins.

2 THE ORNAMENTAL TOBACCO NECTARY

In ornamental tobacco, the nectary consists of a single large torus-shaped structure at the base of the ovary. As it matures, the nectary changes from lime green to yellow to bright orange due to accumulation of high levels of β-carotene (Fig. 1). Nectar is secreted only from a limited region on each lateral face of the nectary. These regions have an abundance of open stomata that together make up the nectary pore (Thornburg et al., 2003). Outside of this region, stomata do not occur on the nectary surface.

Flower development in tobacco has previously been divided into 12 stages based upon morphological floral features (Koltunow et al., 1990). We have correlated the development of the floral nectary in ornamental tobacco with the overall floral developmental patterns, and a profile demonstrating this correlation is shown in Fig. 2.

The development of the floral nectary consists of a number of discrete stages. **Organ initiation** of the floral nectary occurs at a primordial floral stage while the flower bud is still quite small. By floral stage 1, the nectary is a well-differentiated ring of cells surrounding the developing gynoecium. The **filling stage** of nectary development is characterized by an engorgement of the nectary. During this stage, the nectary enlarges two- to three-fold. This begins at early stages and continues through floral stage 7 (approximately 72–96 h). **Ripening** begins about floral stage 6 and lasts until about floral stage 10. It is characterized by the synthesis and accumulation of β-carotene. During this time frame, the nectary turns from lime green to bright orange

Table 1. Composition of LxS8 nectar. Nectar from LxS8 plants was analysed by a variety of methods and these components have been identified as significant nectar constituents.

Volume (μl)		24
Protein (μg/ml)		240
Sugars (M)	Sucrose	0.55
	Glucose	0.47
	Fructose	0.43
Amino acids (μM)	Proline	2,020
	Tyrosine	547
	Serine	319
	Asparagine	114
H_2O_2 (mM)		4
Ascorbate (μM)		920

due to the accumulation of β-carotene. The duration of the ripening stage is approximately 12–24 h. **Nectary maturation** occurs from floral stage 9 through floral stage 12, during which nectar proteins begin to be synthesized. Nectar flow begins at late stage 10, approximately 18 h prior to floral opening. **Anthesis** occurs at stage 12. The duration of the maturation stage is about 12 h. The **post-maturation stage** encompasses all stages that occur after anthesis. It lasts as long as the flower is receptive to pollination. During this time, nectar flow continues; the flow is strongest at the beginning and weakens the longer the flower remains open. This stage can sometimes last 6–8 days if the flower is not pollinated.

The nectaries of LxS8 ornamental tobacco plants produce a nectar composed of specific compounds (Table 1). Chief among these are the sugars glucose, fructose, and sucrose, although specific amino acids, vitamin organic acids, proteins, and reactive oxygen species are all significant components of this nectar (Carter et al., 1999, 2006; Carter & Thornburg, 2000, 2004a, b, c).

3 DEVELOPMENTAL PROCESSES

When examining the development of nectaries in ornamental tobacco, it is clear that there are a number of changes that occur as the nectary matures. The two most obvious changes relate to the size of the nectary and its change in colour (Fig. 1). Although this is difficult to see in the figure, by stage 2

the nectary, located at the base of the gynoecium, is a distinct organ from the remainder of the gynoecium. It is small and does not extend out from the gynoecium. However, by stage 6, the nectary has enlarged significantly, and by stage 12, the nectary is considerably larger still.

It is also apparent that the colour of the nectary of ornamental tobacco changes significantly during the process of development. At stage 2, the colour is indistinguishable from the remainder of the gynoecium. By stage 6, it is a pale yellow colour and by stage 12, a deep pumpkin-orange. Therefore, it is clear that the development of the ornamental tobacco nectary is a complex process that results in both obvious and non-obvious changes in the organ. Defining these changes in the floral nectary as it develops will lead to an increased understanding of this unusual floral organ, and may permit long-term manipulation of nectary function to achieve improved pollinator attraction and increased yields among a variety of angiosperms.

3.1 Origin of the floral nectary

Very little is known about the origins of the floral nectary in ornamental tobacco. To investigate this, one must turn to other species. *Arabidopsis thaliana*, in particular, has proven instrumental in understanding the origins of the floral nectary.

In attempts to understand the origin of the carpel, a unique gene termed *CRABS CLAW* was identified that affected not only the carpel but also the floral nectaries. To date, this is the only known gene that uniquely affects nectary function and development. *CRABS CLAW* belongs to the small YABBY family of transcription factors. The *CRABS CLAW* protein contains a zinc finger and a helix-loop-helix domain that are thought to mediate DNA binding (Bowman & Smyth, 1999). Mutants of *CRABS CLAW* cause the gynoecium to develop into a wide, short structure in which the apical ends of the two carpels remain unfused. In addition, the *CRABS CLAW* mutants also lack floral nectaries (Bowman & Smyth, 1999).

Most of the subsequent work in this arena has been aimed at characterizing the other YABBY family members (*FILAMENTOUS FLOWER/YABBY3, INNER NO OUTER*, *YABBY2, YABBY5*) and the YABBY interaction partners that function in plants to promote an adaxial/abaxial asymmetry in the lateral organs (Siegfried et al., 1999; Eshed et al., 1999; Golz et al., 2002). However, *CRABS CLAW* has been shown to suppress the radial growth of the developing gynoecium and to promote its longitudinal growth (Alvarez

& Smyth, 1999). Further, *CRABS CLAW* functions independently of the ABC floral identity and its ectopic expression is not sufficient for nectary development (Baum et al., 2001).

Recent studies indicate that *CRABS CLAW* is regulated by a number of positive and negative regulators expressed in the nectary Anlagen (Lee et al., 2005a). A phylogenetic footprinting of the *CRABS CLAW* promoter identified a number of regulatory elements in the promoter that included putative binding sites for *LEAFY* and MADS-box transcription factors. The authors propose that B-class and C-class genes act redundantly with each other and in combination with the *SEPALLATA* genes to activate *CRABS CLAW* in the nectary Anlagen. The transcription factors *SHATTERPROOF1/2* may also participate in the transcriptional regulation of *CRABS CLAW* in appropriate backgrounds (Lee et al., 2005a). Additional studies demonstrate that both Rosids and Asterids (Brassicaceae, Solanaceae, and Malvaceae) utilize *CRABS CLAW* as a general regulator of nectary development (Lee et al., 2005b). Thus, *CRABS CLAW* is widely expressed among the angiosperms and appears to function early in nectary gland formation. Further studies will be required to define the biochemical mechanisms and downstream events mediated by *CRABS CLAW* expression that (along with other transcription factors) result in nectary formation.

3.2 Conversion of chloroplasts into chromoplasts

Perhaps the most dramatic change in the nectary of ornamental tobacco, as it develops, is its change in colour. At early stages of development, the nectary is lime green due to the differentiation of chloroplasts in the nectary cells. About the middle of the developmental profile, the nectary begins to take on a pale yellow hue and with further development, the nectary becomes bright orange. This bright orange pigment, which accumulates to tremendous levels in the nectaries, has been isolated and characterized. It matches both the retention time and the spectral properties of pure β-carotene (Horner et al., 2007).

The reason that the nectary would accumulate such a high concentration of β-carotene is not entirely clear. However, it must be noted that the nectary undergoes a huge oxidative stress as a result of the nectar redox cycle (see Nicolson & Thornburg 2007, Chapter 5 in this volume) and the β-carotene very likely serves as an intracellular antioxidant to inhibit damage to the nectary caused by the highly oxidative environment in the nectar.

Because β-carotene is synthesized and accumulates in plastids, we began by examining the nature of nectary plastids during development. The plastids that accumulate in the nectary can be morphologically divided into at least

two different categories. Initially, they appear as chloroplasts with thylakoid membranes. With time, however, the plastids morph into forms that accumulate first starch (see below), then high levels of carotenoids. These unusual plastids can best be described as amylochromoplasts (Horner et al., 2007). The mechanisms responsible for β-carotene accumulation or for the conversion of chloroplasts into amylochromoplasts have not yet been elucidated. Further studies along these lines are in progress.

3.3 Filling of the nectary

As can be seen in Fig. 1, one of the most significant changes that the ornamental tobacco nectary undergoes during development is its dramatic increase in size. This swelling is a prelude to the active secretion of floral nectar that occurs just a few days later. Our early light and electron microscopic studies of developing ornamental tobacco nectaries revealed that neither phloem nor xylem vessels innervate the tobacco nectary. However, the composition of ornamental tobacco floral nectar is 35% (w/v) sugar. The source of this sugar was not clear.

Developmental studies of the nectaries of other species suggest that the accumulation of starch is a prominent feature of many nectaries (Zauralov & Pavlinova, 1975; Nepi et al., 1996a; Peng et al., 2004; Stpiczyńska et al., 2005). To evaluate whether ornamental tobacco nectaries accumulated starch during their development, we stained nectary tissue from two stages of development with I_2/KI. Nectaries from stage 9 (late ripening stage/early maturation stage) and stage 12 (anthesis, secretory stage) both stained a deep purple, a positive indication for starch. Subsequently, total glycans were analysed from nectaries at these same developmental stages. The data in Table 2 show that stage 9 nectaries contain four to five times more starch than mature nectaries.

In recent studies, we have evaluated the production of starch throughout nectary development. This was demonstrated using three different methods (PAS staining and transmission electron microscopy (TEM) of nectaries, as well as direct starch isolation from nectaries). All three methods gave good agreement and provide experimental evidence that permits us to conclude that the nectary stores starch during its development and that the starch is degraded immediately prior to anthesis. We also monitored the production of nectar sugars. These studies demonstrated that the time frame of nectar production correlated with that of starch degradation. Thus, the degradation of starch that occurs immediately prior to anthesis is likely the source of sugars flowing into nectar.

Table 2. Glycans in developing and mature nectaries. Nectaries from stage 9 and stage 12 flowers were isolated and compared for the presence of both free sugars and polysaccharides.

	Concentration found (mg/g fresh weight)	
	Stage 9	**Stage 12**
Free glucose	0	0.5
Total glucose	2.7	3.8
Free fructose	>0.01	>0.01
Total fructose	2.4	4.6
Sucrose	1.0	0.18
Starch	20.8	4.7

In addition, the chemical nature of starch was characterized at different stages of nectary development with respect to both composition and structure. The amylose/amylopectin ratio did not change dramatically throughout nectary development, although there was a general trend towards the more complex starch forms at later stages of floral development. We also evaluated the structure of starch from various nectary stages using FACE (fluorescent-assisted carbohydrate electrophoresis). These analyses demonstrated that there was an increase in the overall chain length of both short and long side chains as the nectary develops up to stage 9, when the maximum chain lengths are attained. In the later stages of floral nectary development, starch is significantly reduced in complexity as would be expected during starch degradation.

Using starch metabolism in *Arabidopsis* foliage, maize kernels, and potato tubers as a model, we identified a list of 26 target genes that participate in starch anabolism and catabolism. We used a variety of strategies to isolate cDNAs that encode 18 of the 26 targeted starch metabolic genes (Ren et al., unpublished). We have analysed the timing of expression of these genes within the nectary using real-time RT-PCR. These analyses define three distinct patterns of expression: one characteristic of the anabolic starch metabolism genes, a second pattern characteristic of the catabolic starch metabolism genes, and a third in which there is general expression throughout nectary development. In cases where clones encoding multiple isoforms of starch metabolic enzymes were identified (e.g., Starch Synthase), most of the isoforms showed little expression at the mRNA level; however, single unique isoforms were found to be dramatically upregulated at the transcript level during nectary development.

As the flower nears anthesis, the nectary starch is dramatically degraded to liberate large quantities of sugar for nectar production. Starch is mobilized by a combination of phosphorolytic and hydrolytic mechanisms (Beck &

Ziegler, 1989). No single enzyme has been shown to completely convert starch to simple sugars, so multiple enzymes most likely are involved. Phosphorolytic enzymes such as starch phosphorylase and R1 enzyme (starch water dikinase) are expressed throughout nectary development. Genes that are specifically turned on during the time just prior to anthesis include α-amylase and β-amylase (Ren and Thornburg, unpublished observations). Thus, starch metabolism plays a central role in plant life, allowing for efficient storage and utilization of carbohydrates, but also functions in floral biology by storing carbohydrate equivalents for nectar production.

4 PROTECTION OF THE GYNOECIUM

Nectar is a metabolite-rich fluid freely offered to visiting pollinators to maximize rates of pollen transfer. These visiting pollinators often harbour micro-organisms that have the potential to infect the gynoecium. Because the composition of nectar compares favourably with many bacterial growth media, the potential for deleterious infection of the gynoecial environment is high. Indeed, in spite of the fact that insects are non-sterile and are often promiscuous, floral infections are rare in plants.

We have hypothesized that this lack of infection is due to the presence in ornamental tobacco nectar of a series of enzymes that contsitute a novel biochemical pathway, the Carter–Thornburg nectar redox cycle (for details see Nicolson & Thornburg, 2007, Chapter 5 in this volume; Carter et al., 1999; Carter & Thornburg, 2000, 2004a, b, c). Briefly, this pathway functions to generate very high levels of hydrogen peroxide, up to 4 mM (Carter & Thornburg, 2000). This is 40 times the level produced by human neutrophils in response to microbial attack (Prince & Gunson, 1987) and these levels are indeed toxic to micro-organisms (Thornburg et al., 2003). We propose that the enzymes in the nectar redox cycle, together with other factors (Naqvi et al., 2005), function to maintain nectar in an axenic state, which then protects the gynoecial environment from infection by pollinator-borne microbes (Thornburg et al., 2003). Our laboratory is currently testing this hypothesis directly by knocking out each of the components of the nectar redox cycle.

5 GENE EXPRESSION

Changes in phenotype are modulated by changes in gene expression. To evaluate the changes in gene expression in the nectary we have used several

methods, including macroarray analysis and EST (expressed sequence tagged) gene analysis. Current ongoing studies include microarray analysis.

5.1 Macroarray analysis identifies defence genes

We have previously utilized a tomato macroarray system to evaluate the expression of a limited number (~500) of genes (Thornburg et al., 2003). These studies have led to a number of unique observations. Not only are the nectarins (nectar-expressed proteins) expressed during nectary development but, in addition, we have previously identified a number of defence-related proteins that are upregulated during nectary development. Some of these, shown in Table 3, include antimicrobial, antifungal, and antiherbivore proteins.

This broad spectrum of defence gene expression points to a unique and previously unrecognized feature of the nectary gland—that the nectary has a major defence function in addition to its function of secreting nectar. Because of the metabolically rich complexity of nectar and the non-sterile nature of visiting pollinators, it is, in retrospect, not surprising that the nectary should have an active function in defence.

The mechanisms that induce defence gene activation are receiving significant attention in today's plant literature. There are a number of factors known to affect defence gene function in plants. Two of the most important may be hydrogen peroxide and ascorbate. Both of these small molecular weight compounds have significant effects on plant defence gene expression. Both compounds also have important roles in nectary biology. Because of their presence in the nectary, it is not surprising that they may also affect defence gene expression in the nectary.

5.1.1 Role of hydrogen peroxide in plant stress and defence

Reactive oxygen intermediates, including hydrogen peroxide, are triggered by an oxidative burst in plant cells that occurs in response to a variety of biotic and abiotic stresses (Levine et al., 1994; Desikan et al., 2000). These reactive oxygen intermediates act as signalling molecules to initiate defence gene expression. The production of the reactive oxygen species that proceed hydrogen peroxide production appears to be mediated by a membrane-bound NADPH oxidase that is responsible for the induction of a number of defence genes (Orozco-Cardenas et al., 2001; Torres et al., 2002). One major response to the production of these reactive oxygen species, especially hydrogen peroxide, is the activation of a number of defence genes (Alvarez et al., 1998;

Table 3. Defence genes upregulated during nectary development.

Protein/cDNA	Function	Reference
Snakin 1	antimicrobial peptide	Segura et al., 1999
γ-thionin	antifungal peptides	Pelegrini & Franco, 2005
PR1	antifungal protein	Stintzi et al., 1993
PR5	antifungal protein	Stintzi et al., 1993
Chalcone synthase	flavonoid biochemistry	Bell et al., 1986
Wound-induced win1	antifungal protein	Ponstein et al., 1994
Wound-induced pin1	antiherbivore protein	Green & Ryan, 1972
Wound-induced pin2	antiherbivore protein	Bryant et al., 1976

Chamnongpol et al., 1998; Sasabe et al., 2000). Other downstream signalling events mediated by hydrogen peroxide include calcium mobilization and protein phosphorylation (Lecourieux et al., 2002; Neill et al., 2002).

The nectar of ornamental tobacco contains very high levels of hydrogen peroxide (up to 4 mM); it is therefore not surprising that a large number of defence genes are expressed in the nectary. We are currently working to knock out the components of the nectar redox cycle in an attempt to produce plants that lack these high levels of reactive oxygen species. It will be of great interest to evaluate the expression of defence genes in such plants. Nitric oxide also appears to function in this process (de Pinto et al., 2002) but, to date, the presence of nitric oxide in the nectary has not been examined.

5.1.2 Role of ascorbate in plant stress and defence

Another biological compound that has potential to affect the defence pathways in the nectary is the antioxidant ascorbate. While it is not clear what mechanisms are responsible for gene regulation by ascorbate, ascorbate knockout plants do indeed show significant levels of altered gene expression (Kiddle et al., 2003; Pastori et al., 2003). In the absence of ascorbate, many defence genes are activated. These include the two pathogenesis-related proteins glucanase and chitinase. These genes appear to be responsive to ascorbate because they are downregulated in response to added ascorbate (Pastori et al., 2003). Genes that increase following application of ascorbate to ascorbate-depleted plants include metallothionein and superoxide dismutase.

If ascorbate is affecting gene expression in the nectary, then we might expect to observe differences in ascorbate-regulated gene expression at stages of nectary development when ascorbate levels differ significantly. Ascorbate is generally low in the nectary until late in development (Horner et al., 2007), when ascorbate is rapidly synthesized to serve as the extracellular antioxidant functioning in the nectar redox cycle. Analysis of our macroarrays (Table 4, Thornburg et al., 2003) reveals that a number of genes appear to show such ascorbate-mediated regulation. In presecretory nectaries (stage 6) ascorbate is relatively low. At this time, a number of stress and defence genes are highly expressed. Later, at anthesis (stage 12), when ascorbate production is very high, these same genes are now downregulated. This group of genes includes the same genes identified by Pastori et al. (2003). Further, Kiddle et al. (2003) showed that metallothionein was upregulated in response to ascorbate application. Our analysis (Table 4) also shows this upregulation for metallothionein. Thus, ascorbate also appears to have a role in gene regulation during nectary development.

Table 4. Gene expression levels from macroarray analysis.

	Percentage of leaf*	
Gene	**Stage 6**	**Stage 12**
PR-1	419 ± 40	192 ± 101
PR-5	2,873 ± 735	214 ± 118
Chitinase	752 ± 410	387 ± 125
CHS	452 ± 128	211 ± 100
Subtilisin-like	634 ± 78	465 ± 84
Metallothionein	952 ± 248	1,831 ± 489

*mRNAs from stage 6 or stage 12 nectaries were labelled with [^{32}P] and the labelled cDNAs were hybridized to a macroarray containing approximately 500 tomato defense genes. Results are average amounts of hybridization as a percentage of that in the control leaf ± SE. (Thornburg et al., 2003.)

5.2 EST analysis

We have sequenced 12,534 nectary-expressed ESTs from three different stages of nectary development: stage 6 (presecretory developing nectaries), stage 12 (mature secretory nectaries at anthesis) and stage PF (nectaries 44 h post-fertilization). These have been clustered using the TIGR clustering program (tigrcl) to identify groups of overlapping sequences that are referred to as tentative contigs (TCs) or unigenes. This clustering permitted the identification of 6,158 nectary-expressed unigenes. Following annotation of

these sequences, they have been deposited in a mySQL database. The sequences of these individual ESTs and the clustered unigenes are freely available to the scientific community at the Nectary Gene Expression (NecGEx) website (http://www.bb.iastate.edu/necgex/ests/db). These sequences have also been deposited in the GenBank as Accession # EB688597 to EB701044.

Preliminary analysis of these unigenes has revealed several novel features of nectary development and function. These features include:

- The identification of specific transcription factors (TFs) that may regulate nectary development and function as well as downstream target genes that are driven by these TFs.
- The identification of cDNA clones encoding the entire metabolic pathway for β-carotene, a compound that accumulates to very high levels in the nectary (see "Conversion of chloroplasts into chromoplasts" on page 270).
- The discovery that the nectary is preprogrammed for the ethylene response that occurs in flowers following fertilization.
- The discovery that many of the most highly expressed ESTs function in in stress responses, suggesting novel functions for the nectary (see "Macroarray analysis identifies defence genes" on page 274).

Additional in-depth analysis will certainly yield increased information about the nectary. One goal is to produce nectary-specific microarrays that will permit us to probe deeply into the nectary transcriptome to identify the factors that affect nectary development and function.

5.3 Nectary-specific gene expression

The expression of genes in the nectary has also received recent attention. Nectarin I, the major protein secreted into the nectar of tobacco plants, is a novel manganese-containing superoxide dismutase (Carter et al., 1999; Carter & Thornburg, 2000). We have isolated the *Nec1* gene and analysed the expression of the *Nec1* promoter in transgenic plants (Carter & Thornburg, 2003). The tissue specificity of marker gene expression demonstrated that this promoter was expressed uniquely in the floral nectary. Other floral or additional plant organs did not express the *Nec1* promoter. Further, the *Nec1* promoter was expressed only when nectar was actively being secreted from flowers. The promoter is not active in presecretory nectaries, and it is also not active following fertilization of the flower, when nectar secretion ceases. This analysis suggests that anthesis and protein secretion into nectar are coordinated events.

Sequential deletion analysis of the *Nec1* promoter revealed that there are multiple regulatory elements within the promoter. One of these elements, which contains a *MYB* binding site, is responsible for the temporal pattern of *Nec1* expression. The other element, which is upstream from the temporal element, is apparently involved in tissue specificity of *Nec1* expression. When this element is in place, the *Nec1* promoter shows tight nectary-specific expression. When this element is deleted, however, the *Nec1* promoter suddenly gains activity in the floral petals. We have interpreted these analyses to mean that this second element contains a binding site for a petal-expressed repressor protein that normally functions in the flower to limit expression of the *Nec1* promoter to the nectaries (Carter & Thornburg, 2003). It is not clear whether the *Nec1* promoter contains additional elements that are important in *Nec1* expression or whether there are additional tissue-specific repressor proteins that further control *Nec1* expression by limiting the expression of this promoter.

6 NECTARY MOLECULAR BIOLOGY IN OTHER SPECIES

In addition to the nectar-specific proteins (*nectarins*; see Nicolson & Thornburg 2007, Chapter 5 in this volume), there are a number of other nectary-specific genes that have been identified from various species. Leek (*Allium porrum*) has been shown to express two nectarins, a 50 kDa alliinase and a 13 kDa mannose-specific lectin (Peumans et al., 1997). Both of these proteins have defence-related functions (see Nicolson & Thornburg 2007, Chapter 5 in this volume) and probably serve to maintain nectar in an axenic state, much as does the nectar redox cycle.

6.1 Other nectary-expressed genes

A list of genes known to be specifically expressed in plant nectaries is presented in Table 5. These genes correspond to a diverse group of proteins that include defence-related proteins, transcription factors, signal transduction factors, and biosynthetic enzymes. Most of these proteins are not specific, but are also expressed in other plant organs. ESTs encoding many of these genes have been identified among our EST studies, thereby confirming that these genes are indeed nectary-expressed.

Important exceptions from Table 5 include the *CRABS CLAW* (*CRC*) transcription factor from *Arabidopsis thaliana* (discussed in “Origin of the floral

Table 5. Genes expressed in nectary tissues, compiled from published studies that specifically indicated the expression of these genes in nectary tissues.

Gene	Species	Function	Reference
Nec1	*Nicotiana* sp.	Superoxide dismutase	Carter & Thornburg, 2000
Nec2/3	*Nicotiana* sp.	MDHR and carbonic anhydrase	Carter & Thornburg, 2004b
Nec4	*Nicotiana* sp.	XEGIP	Naqvi et al., 2005
Nec5	*Nicotiana* sp.	Glucose oxidase and DHA reductase	Carter & Thornburg, 2004c
ALL	*Allium porrum*	Alliinase	Peumans et al., 1997
MBL	*A. porrum*	Mannose binding lectin	Peumans et al., 1997
CRC	*Arabidopsis thaliana*	*CRABS CLAW* transcription factor	Bowman & Smyth, 1999
LTP1	*A. thaliana*	Lipid Transfer Protein	Thoma et al., 1994
CRT	*A. thaliana*	Calcium-binding protein	Nelson et al., 1997
GPa1	*A. thaliana*	G protein	Weiss et al., 1993
UGD	*A. thaliana*	UDPG dehydrogenase	Seitz et al., 2000
myb305	*Antirrhinum majus*	Transcription factor	Jackson et al., 1991
NTR1	*Brassica napus*	SAM salicylic acid methyl transferase	Song et al., 2000
MSG	*Glycine max*	Latex protein	Stromvik et al., 1999
ACP3	*Cucumis sativus*	ACC oxidase	Kahana et al., 1999
ACO3/4	*Petunia hybrida*	ACC oxidase	Tang et al., 1994
NEC1	*P. hybrida*	Unknown	Ge et al., 2000
ADH	*P. hybrida*	Alcohol dehydrogenase	Garabagi et al., 2005
MTA	*Pisum sativum*	Metallothionien	Fordham-Skelton et al., 1997
MEN9	*Silene latifolia*	Sex-specific expression	Robertson et al., 1997

nectary" on page 269) and the *Nec1* gene from *Petunia*. The *Nec1* protein is a transmembrane protein that localizes to petunia nectaries. It is highly expressed in nectaries and much less so in stamens. Its expression is highest in open flowers that are actively secreting nectar (Ge et al., 2000). Antisense co-suppression and transposon inactivation of the *Nec1* function yielded petunia plants with an "early open anther" phenotype. *Nec1* may function in the anthers in the development of stomium cells, which are ruptured in normal anther development to release pollen at maturity (Ge et al., 2001).

6.2 Metabolism and nectar production

Since nectar contains such high levels of sugars, carbohydrate metabolism in the nectary is extremely important in understanding nectary function. The earliest studies involved the secretion of nectar from excised nectaries of a number of species; the nectaries were fed with a number of sugars (Frey-Wyssling et al., 1954; Matile, 1956; Heinrich, 1975). A few studies have examined the role of glycolysis in the secretion of nectar (Zauralov, 1969a; Bargoni, 1972a, b; Bosia & Pescarmona, 1972; Nichol & Hall, 1988). These studies generally indicate that glycolytic enzyme activity as well as glycolytic intermediates are high in nectaries. As the nectary matures, glycolytic enzyme activity decreased (Zauralov, 1969b), as did nectar secretion. Anaerobic conditions significantly affected the secretion from nectaries incubated on glucose and fructose, but had less effect when nectaries were incubated on sucrose, implying that respiration may be required for nectar secretion. The role of respiration in nectar secretion was confirmed through the use of respiration inhibitors. Inclusion of respiration inhibitors (azide, KCN, 2,4-dinitrophenol, NaF, and arsenite) in the incubation medium also inhibited secretion of nectar in a variety of species (Matile, 1956; Zauralov, 1969b; Zauralov & Zauralova, 1970).

Of special interest is the examination of the role of sorbitol among the woody Rosaceae. Sorbitol is a major soluble carbohydrate in the phloem of the woody Rosaceae (Watari et al., 2004); floral nectars, however, contain very little sorbitol (Bieleski & Redgwell, 1980). Conversion of sorbitol to other sugars occurs within the nectary primarily during phloem unloading. Recently a number of sorbitol transporters have been isolated from sink organs of several species (Gao et al., 2003; Watari et al., 2004; Zhang et al., 2004). Understanding the involvement of these transporters in nectary function will be very interesting.

Only a few other studies have investigated the role of nectary metabolic enzymes in nectary function. Nichol and Hall (1988) examined a number of metabolic enzymes in the nectaries of *Ricinus communis*. They localized acid phosphatase in the parenchyma of the nectar, while ATPase was localized in the nectary epidermis. In other studies, Zauralov and Pavlinova (1975) found that high levels of acid invertase in gourd nectaries were responsible for the high-hexose content found in the nectaries of that species.

There is clearly a lack of recent work on the role of general nectary metabolism in nectar secretion. We hope that the studies outlined in “EST

analysis" on page 276 will help in a more global understanding of the role of general metabolism in nectary function.

6.3 Hormones and nectar production

The involvement of plant hormones in flowering is a complex problem marred by myriad studies with contradictory results. Similarly, the role of plant hormones in nectar production is not entirely clear-cut. Two plant hormones have been studied repeatedly in relation to nectar production. The first of these is auxin, which appears to inhibit nectar secretion. In 1956, Philippe Matile examined the effect of IAA on nectaries from two plant species. These studies showed that IAA strongly inhibited nectar secretion in *Euphorbia pulcherrima* nectaries; this inhibition was much weaker in *Abutilon striatum* (Matile, 1956). In later studies, both pretreatment and constant exposure of *Antirrhinum majus* nectaries to 500 μM IAA inhibited nectar secretion by 25–50%. Interestingly, Ca^{++} ions nullified the inhibitory effects of IAA on sugar secretion (Shuel, 1964, 1967). Later, using radio-isotopes, Shuel (1978) demonstrated that incubation with IAA resulted in an inhibition of nectar secretion within hours, and the decrease in radioactivity incorporated into nectar was accompanied by increased incorporation of radioactivity into nectary protein. Furthermore, these investigators demonstrated that such levels of IAA initiated a reorganization of the nectary, which led to a cessation of secretion, an increased incorporation of labelled uracil into RNA, and a stimulation of growth that resulted in a considerable enlargement of the nectary (Shuel & Tsao, 1978). Finally, in very recent work, Aloni et al. (2005) demonstrated that one of the functions of IAA in *Arabidopsis thaliana* flowers was to retard nectar secretion up to the time of anthesis.

The second plant hormone whose role in nectar production has been examined is gibberellic acid. Flowers from plants treated with GA_3 had the maximum volume of nectar per flower and the highest levels of sugar concentration in nectar. As might be expected, the GA_3-treated plants that showed increased sugar and nectar levels also showed increased pollinator visitation (Mishra & Sharma, 1988). By contrast, GA_3 treatments inhibited the initiation of nectaries in normal buds of *Nigella damascena* (Raman & Greyson, 1978). The contradictory nature of these studies suggests that additional work is needed to examine the role of these and other plant hormones in the process of nectar secretion. Although many of these studies are dated and were performed prior to the "omics" scientific era, they suggest directions of nectary research that should be renewed with the modern tools that are available today.

6.4 CO_2 and nectar

Datura wrightii flowers open at dusk and wilt by the following noon. These flowers are pollinated by the hawkmoth *Manduca sexta* (Lepidoptera: Sphingidae). Newly opened flowers secrete large amounts of nectar along with high levels of CO_2 (Guerenstein et al., 2004). It is thought that this CO_2 release signals the availability of nectar to visiting pollinators. In studies using artificial flowers, emitting either high (double the current atmospheric concentration) or low (equivalent to current atmospheric concentration) CO_2 levels, moths overwhelmingly preferred the flowers emitting higher levels of CO_2 (Thom et al., 2004). This suggests that plants use CO_2 emission to signal the presence of an adequate nectar supply to visiting pollinators. The mechanisms that result in CO_2 release have not yet been elucidated, but it is clear that at the time of anthesis, flowers have very high metabolic activity and there are clearly biochemical pathways that could result in the emission of CO_2. The role of these pathways in floral development has, however, not yet been investigated.

Recently, other studies have examined the influence of elevated CO_2 on floral nectar production in a number of dicotyledon species (Lake & Hughes, 1999; Davis, 2003). Both studies concluded that the quantity of nectar changed, but the quality of nectar (defined as the solute concentration) was not significantly affected by elevated CO_2.

In recent studies, the effect of elevated CO_2 on nectar production in *Epilobium angustifolium* was investigated (Erhardt et al., 2005). These studies showed that elevated CO_2 significantly increased nectar production in this species; however, the authors also noted that these results are not consistent with earlier observations, and are therefore likely to be species-specific.

In a world in which atmospheric levels of CO_2 are predicted to double in the 21st century (Watson et al., 1996), it is clear that flowers and their pollinators will have to learn how to deal with elevated levels of CO_2, if they are to be as successful in the future as they have been in the past. Mechanisms that can sense double the level of atmospheric CO_2 as an indicator of nectar presence may no longer function a century from now. Whether plants and pollinators can compensate for the doubling of atmospheric CO_2 is not clear. If they cannot, this could result in a significant reduction in fecundity for both the plants and their pollinators.

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Chapter 7

NECTAR CONSUMERS

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1 INTRODUCTION

The emphasis in this chapter is on the enormous diversity of nectar consumers, and the energetic and nutritional implications of a nectar diet for such different animals. They include the following groups:

- Pollinators, for whom the most important reward is floral nectar (Simpson & Neff, 1983).
- Predators or parasitoids that defend plants against herbivores and are rewarded with extrafloral nectar (Koptur, 1992; Wäckers & Bonifay, 2004).
- Nectar robbers (Maloof & Inouye, 2000).

All of them, legitimate or otherwise, benefit from a rich and easily utilized food that comes in attractive packaging, and sugar in the nectar fuels the flight that enables them to be pollen vectors. Table 1 gives an indication of the variety of animals involved. They range from the many holometabolous insects and some birds and bats that feed primarily on nectar and are major pollinators, to various arthropods and mammals that feed opportunistically or occasionally on nectar. Most are flying animals and nectar sugars are used to power their flight. The diversity of nectar consumers is emphasized by Proctor et al. (1996), also for Australian flowering plants by Armstrong (1979), and for a tropical dry forest in Costa Rica by Opler (1983). Nectar is apparently one of the most ubiquitous foods on earth.

Plant products designed to attract mutualistic animals should be adapted to the energetic and nutritional requirements of the animals. This is reflected

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in differences in nectar volume, concentration, chemistry, and time of secretion, in addition to the constraints on availability to different pollinators owing to flower structure. Strong correlations have been found between nectar volume and floral biomass, because nectar production increases with nectary size (Opler, 1983; Szabo, 1984; Galetto & Bernardello, 2004), although photosynthetic input from other parts of the plant is also an important factor in nectar production (Pacini & Nepi, 2007, Chapter 4 in this volume) Similarly, daily sugar production per flower is correlated with the body size and energetic requirements of pollinators (Brown et al., 1978). Flowers must meet high energy demands if their pollinators are endothermic, and if they forage expensively by hovering. Figure 1 gives an indication of the huge differences in nectar volumes offered to different consumers, using the data collected in Costa Rica by Opler (1983), but does not take into account differences in concentration, which also determine energy rewards.

The energy that animals gain from nectar must exceed the cost of acquiring it, which includes the time to handle flowers and the time to travel between them. The relationship between the energy reward provided and the energy requirements of the visitor determines the extent of movement between flowers and plants (Heinrich, 1975). It is generally assumed that competition

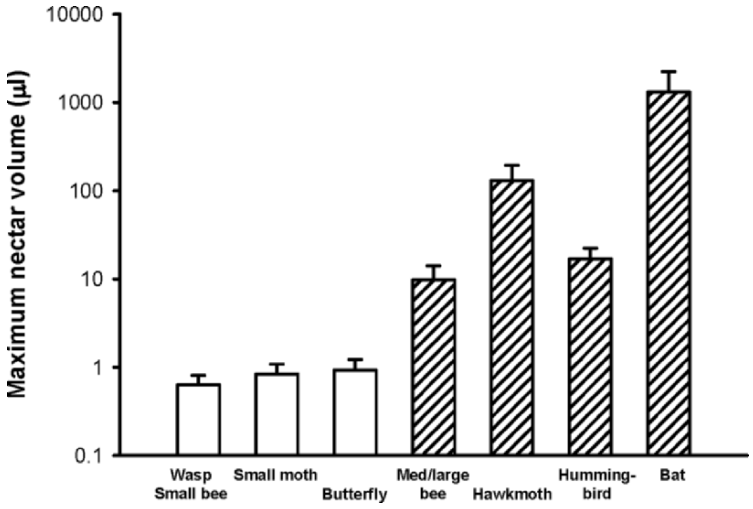

Figure 1. Maximum nectar production (µl) for various pollinator classes, roughly in order of increasing body size. Values are means ± SE (*note log scales*). *Hatched bars* indicate endothermic animals. No data for flies. (From Opler, 1983.)

Table 1. The diversity of nectar-consuming animals. The list is not intended to be comprehensive, but rather to show the zoological variety of nectar consumers. Their nectar use ranges from obligatory to occasional. Many other animals are occasional nectar feeders. (Armstrong, 1979; Proctor et al., 1996.)

Class	Order	Family	Common names
Arachnida	Araneae	Salticidae, Thomisidae	jumping spiders, crab spiders
	Acari	Phytoseiidae, Ascidae	predatory and flower mites
Insecta	Thysanoptera	Thripidae	thrips
	Coleoptera	Scarabaeidae	scarab beetles: flower chafers
		Lycidae	net-winged beetles
	Diptera	Culicidae	mosquitoes
		Bombyliidae	bee flies
		Syrphidae	hover flies
		Muscidae, Calliphoridae	house flies, blowflies, etc.
		Tabanidae, Nemestrinidae	long-tongued flies
	Lepidoptera	Noctuidae	army worms, etc.
		Sphingidae	hawkmoths
		Pieridae, Nymphalidae, Papilionidae, Lycaenidae, etc.	butterflies
	Hymenoptera	Ichneumonidae, Braconidae, Encyrtidae, and many others	parasitic wasps
		Sphecidae, Pompilidae	non-social wasps
		Vespidae	social wasps
		Andrenidae, Halictidae, Colletidae, Melittidae	short-tongued bees
		Megachilidae, Apidae	long-tongued bees
		Formicidae	ants
Reptilia	Squamata	Gekkonidae	geckos
Aves	Psittaciformes	Psittacidae	lorikeets
	Apodiformes	Trochilidae	hummingbirds
	Passeriformes	Meliphagidae	honeyeaters
		Fringillidae	honeycreepers, flower-piercers
		Nectariniidae	sunbirds, sugarbirds, flowerpeckers
Mammalia	Marsupialia	Tarsipedidae	honey possum
	Chiroptera	Pteropodidae	fruit bats
		Phyllostomidae	leaf-nosed bats
	Rodentia	Muridae, Cricetidae	rats and mice
	Primates	Lemuridae	lemurs
		Cercopithecidae	monkeys and baboons

for pollinators acts as a selective agent in nectar production, the result being a compromise between producing enough to attract pollinators but not so much that they are unwilling to move to other plants (Klinkhamer & De Jong, 1993); in other words, keeping them "hungry but faithful" (Willmer & Stone, 2004). This strategy is taken to the extreme in nectarless orchids, where the absence of nectar is thought to promote cross-pollination and nectar supplementation has been shown to reduce it (e.g., Jersakova & Johnson 2006). Pollinators vary their foraging behaviour in several ways in response to variation in nectar: visit frequency to plants, probing time per flower, number of flowers probed, and flight distance after departure. These responses to nectar are well known for foraging bees and hummingbirds, but have now been recorded in flies as well (Jersáková & Johnson, 2006). The foraging behaviour of pollinators is beyond the scope of this chapter, but see Rathcke (1992) for a broad review of pollinator responses to both average nectar volume per flower and within-plant variation in nectar per flower, and the consequences for pollination success.

The standing crop is the amount of nectar that pollinators actually encounter in flowers, and its correlation with nectar production will be strong if pollinator visits are rare, but weak when pollinator pressure is high and standing crops are low and variable (Zimmerman, 1988; Cresswell & Galen, 1991). In studying the nectar dynamics of Neotropical hummingbird-pollinated plants, McDade and Weeks (2004a, b) found high variation in nectar volume even in protected flowers, while in open flowers continuous harvesting of nectar by the birds meant that the remaining volumes were usually insufficient for refractometer readings.

2 NOT ALL FLORAL NECTAR DRINKERS ARE POLLINATORS

2.1 Generalization and specialization in pollination systems

In the classic syndromes of pollination biology, pollinators are inferred from a suite of floral characters, including rewards (Faegri & van der Pijl, 1979; Ollerton & Watts, 2000). The match of flower and pollinator is particularly clear in ornithophilous flowers (those with traits characteristic of bird pollination), which tend to be large, tubular, and red, with no detectable odour and copious amounts of dilute nectar. Another well-defined syndrome is melittophily (bee pollination), characterized by scented zygomorphic flowers of varied colours (often blue or purple), with moderate amounts of concentrated

nectar. Unrelated plants show this phenotypic convergence because they share a functional group of animals as pollinators (Fenster et al., 2004).

Implicit here is the assumption of floral adaptation to pollinators and hence specialization in plant–pollinator interactions (Herrera, 1996; Waser et al., 1996). These topics have been the subject of considerable recent debate, much of it focussed on geographic and phylogenetic differences in levels of specialization (Waser et al., 1996; Johnson & Steiner, 2000; Fenster et al., 2004; Ollerton et al., 2006). The paradox is that visitors to flowers are much more diverse than the syndrome classification suggests. Many plants are pollinated by several animal species, sometimes taxonomically distant from one another (Herrera, 1996). Not surprisingly, the number of plant–pollinator interactions observed increases with sampling effort in both time and space. Meticulous field observations are necessary, and complete sampling requires nocturnal observations, should not ignore vertebrates, and should be carried out over the whole flowering season and over many years (Ollerton et al., 2006). Herrera (1988) found that *Lavandula latifolia* (Lamiaceae) in southeastern Spain was visited by nearly 85 bee, fly, and butterfly species over its extended flowering period. On a finer time scale, temporal changes in volume, concentration, viscosity, and composition ensure that attractiveness to a succession of different pollinators changes throughout the day, which favours generalization in pollination systems (Corbet et al., 1979). A plant species growing in different habitats may be exposed to quite different pollinator populations (Bernardello et al., 1994; Hingston & McQuillan, 2000). In addition, only a few of the many visitors may be pollinators, and measurements of pollination effectiveness (e.g., Fishbein & Venable, 1996) are much more time-consuming than rough categorization. However, for present purposes, we are interested in all nectar consumers, regardless of whether they are effective pollinators, opportunistic visitors, ant guards, or robbers that deplete the nectar rewards.

2.2 Nectar robbing and nectar theft

Robbing causes further blurring of the distinctions between pollination systems, and may be an added influence on the rates of nectar production by flowers. The protection of nectar in long narrow corolla tubes is designed to exclude unwanted visitors, but such animals may puncture the base of the corolla and steal nectar. Following the terminology of Inouye (1980), these nectar robbers can be contrasted with nectar thieves, which access the nectar by the normal route, but are morphologically unsuited (usually too small, e.g., flower mites or ants) to effect pollination. Primary nectar robbers include carpenter bees and bumblebees, and birds such as the flowerpiercers

(*Diglossa*; Thraupidae). Secondary robbers (commonly honeybees) use the holes already made by others for easy access to nectar. Robbing levels can be high and it is generally assumed that nectar robbers are not pollinators, but in fact reviews of the literature have shown that the consequences for fruit set may be harmful, neutral, or even beneficial (Maloof & Inouye, 2000; Irwin et al., 2001). Certainly it is not uncommon for robbing bees to pollinate the plants that they visit. For example, carpenter bees (*Xylocopa californica arizonensis*) pierce the flowers of ocotillo (*Fouquieria splendens*) for nectar while gathering pollen from the exserted stamens, and these bees are effective pollinators (Scott et al., 1993). Classification of avian visitors as either "pollinators" or "robbers" of particular plant species may also be simplistic (Arizmendi et al., 1996).

The relatively large tubular flowers of hummingbirds are particularly prone to robbing by a variety of taxa. Those of *Justicia aurea* (Acanthaceae) and *Macleania bullata* (Ericaceae) are each visited legitimately by two hummingbird species, and robbed by other smaller hummingbirds and by bees, ants, and butterflies (Willmer & Corbet, 1981; Navarro, 1999). Temporal and microclimatic differences help to divide the nectar resources among these animals, with ectothermic and endothermic visitors foraging at different times of day and on inflorescences in shade or in sun (Willmer & Corbet, 1981).

Robbing changes the quality of nectar as well as its quantity (Maloof & Inouye, 2000; Irwin et al., 2001), thus increasing the variance in nectar rewards and influencing the foraging behaviour of pollinators. The concentration of the residual nectar is likely to increase through exposure to the atmosphere: this confirms the value of a long corolla in reducing evaporation. In hummingbird-pollinated *Ipomopsis aggregata*, flowers punctured by bumblebees at the base of the corolla had a nectar concentration of 30%, compared to 20% in unrobbed flowers (Pleasants, 1983). (Nectar concentrations are expressed as % w/w throughout this chapter.) Apart from the evaporation effect, robbed flowers may also have elevated amino acid concentrations, due to both mechanical transmission (as on the tongues of bumblebees) and damage to floral tissue (Willmer, 1980).

Do flowers adapt to robbing by producing extra nectar? Replenishment after legitimate removal serves to maintain a constant standing crop in flowers that receive multiple visits (Castellanos et al., 2002). The flowers of *Tillandsia* species (Bromeliaceae) replenish their nectar after hummingbird visits have been simulated by repeated nectar removal, although this response is by no means universal (Ordano & Ornelas, 2004). These authors

suggest that positive responses to nectar removal, as in *Tillandsia*, may be attributed to a lack of resource limitation and compensation for high levels of robbery and theft. These flowers are infested with flower mites, which are common in some hummingbird flowers and collectively consume large amounts of nectar (Lara & Ornelas, 2002). Colwell (1995) used exclusion experiments in Costa Rica to show that mites (*Proctolaelaps kirmsei*) consumed on average 40% of the nectar produced by *Hamelia patens* (Rubiaceae). This casts some doubt on published estimates of nectar production, which usually ignore these "cryptic nectarivores" (McDade & Weeks, 2004b).

3 INSECT NECTAR CONSUMERS

Insects and angiosperms are both major components of global biodiversity, connected by two conspicuous interactions: herbivory and pollination. It is estimated that biotic pollination links a quarter of a million angiosperm species with a similar number of animal species, most of which are insects (Waser, 2006). Most of the pollinators are included in three of the four largest insect orders: the holometabolous Diptera, Lepidoptera, and Hymenoptera. Complete metamorphosis allows radical differences between the larval and adult diets and the mouthparts used to ingest them, and often the adult mouthparts show convergent evolution of the proboscis for nectar drinking (Krenn et al., 2005).

Many insects without extensible mouthparts (and this includes most Coleoptera) also feed occasionally or regularly on nectar. Several hemimetabolous orders have been recorded as flower visitors (Armstrong, 1979; Proctor et al., 1996); the Thysanoptera, in particular, are closely associated with flowers and probably imbibe nectar as well as eating pollen (Kevan & Baker, 1983; Proctor et al., 1996; Ananthakrishnan & Gopinathan, 1998). Many predatory arthropods supplement their diet with plant material (for a review see Coll & Guershon, 2002), and nectar feeding is important in a variety of arthropods with otherwise carnivorous habits, including blood-sucking Diptera. Nectar is a water and energy source for various spiders (Pollard et al., 1995; Jackson et al., 2001) extrafloral nectar is an alternative food for predatory mites (Choh et al., 2006), and ambush bugs (Heteroptera: Phymatidae) hunt on flowers but also drink their nectar (Yong, 2003).

Nectar is an uncommon diet for immature insects, except for bee larvae, which are usually fed a mixture of pollen and nectar. Some species of flower-breeding *Drosophila* have larvae that feed on floral tissue, nectar, or

pollen (Brncic, 1966). In South Africa, larvae of *D. flavohirta* live bathed in nectar in the flower cups of *Eucalyptus* sp. and are thought to have an adverse effect on honey production (Tribe, 1991; Nicolson, 1994). For laboratory-reared larvae of the green lacewing *Chrysoperla carnea* (Neuroptera: Chrysopidae), *Drosophila* larvae are a suboptimal food, but the inclusion of pollen and sucrose in their diet greatly enhances growth (Patt et al., 2003). This is another example of a predator that supplements its diet with nectar.

Of the four holometabolous orders mentioned above, the Coleoptera are least important as nectar feeders, although according to Armstrong (1979), a quarter of beetle families and half of fly families in Australia are anthophilous (flower-frequenting). In the Diptera, Lepidoptera, and Hymenoptera, the evolution of a crop has been a key factor in carbohydrate feeding (Stoffolano, 1995). All three of these advanced orders depend on liquid carbohydrate resources to provide immediate energy for the flight between flowers, which enables cross-pollination. They have independently evolved an expandable and impermeable crop—diverticular in structure in the Diptera and Lepidoptera, and linear in the Hymenoptera—located in the abdomen. The crop is crucial because of the unpredictability of nectar resources: it is used for storage, transport back to the nests of social insects, and in addition, it prevents the osmotic shock that would result from sudden exposure of the haemolymph to high sugar concentrations. Nectar is released from the crop to the midgut for digestion and absorption as required, or transferred to the nestmates of social insects by trophallaxis. All nectar-feeding insects show highly efficient assimilation of the common nectar sugars, regardless of the concentration ingested (Hainsworth et al., 1990). For a review of the implications of a nectar diet for the energy and water balance of insects, see Nicolson (1998).

In the following sections, I discuss the major nectar-consuming insect orders. However, Corbet (2006) has recently provided a broad functional classification of flower types and their insect visitors (Table 2) that cuts across the insect orders. This classification emphasizes that gradients of nectar quantity and accessibility in flowers are matched by gradients of body size, tongue length, and endothermy in their insect visitors. This contrasts with the concept of pollination syndromes, where plants are supposedly pollinated by a single taxonomic group of pollinators. The importance of nectar accessibility was also highlighted by a multivariate analysis of classical pollination syndromes (Ollerton & Watts, 2000), which showed that beetle, fly, and wasp syndromes clustered together—all characterized by exposed nectar presentation.

Table 2. A functional classification of flower types and their insect visitors. (From Corbet, 2006.)

Flower types	Attributes of visitors	Taxa involved
Fully exposed nectar; little or no intrafloral temperature elevation; includes Apiaceae, some Asteraceae	Low flight threshold temperature; little or no endothermy; <30 mg body mass if hairy, <100 mg if not	Most flies, small beetles, short-tongued Hymenoptera
Moderate amounts of partly concealed nectar; often elevated intrafloral temperatures in sunshine; includes some Asteraceae, cup-shaped flowers as in Rosaceae	Larger insects with moderate flight threshold temperature	(a) Robust, hairy, endothermic insects (some syrphids, short-tongued bees, larger beetles) (b) Slender, poorly insulated basking insects (some syrphids, bombyliids, and butterflies with low wing loading)
Flowers with abundant, deeply concealed nectar, e.g., Fabaceae, Lamiaceae	Long-tongued, robust, endothermic insects with good insulation and high energy requirements	Long-tongued bees, sphingid and noctuid moths, butterflies with high wing loading, a few syrphids and bombyliids

3.1 Coleoptera

Although beetles are the oldest pollinators (Grimaldi, 1999), they tend to be clumsy and destructive in flowers. They visit flowers for multiple reasons: feeding on nectar, pollen, and floral tissue, sheltering for prolonged periods, or congregating for mating purposes. Nectar feeding by beetles is thus difficult to observe directly. The chewing mouthparts of beetles limit access to exposed floral rewards (Proctor et al., 1996), although there are exceptions to this, as in the large genus *Lycus* (Lycidae) (Figs. 2 and 4A). In beetle–flower associations, nectar feeding has been a late development and pollen is generally more important, as in the bowl-shaped "poppy guild" flowers of the Mediterranean (Dafni et al., 1990). Similarly, nectar production is suppressed in the African Iridaceae that are specialized for pollination by hopliine monkey beetles (Goldblatt & Manning, 2006).

Nectar feeding in beetles is probably best known in the subfamily Cetoniinae of the Scarabaeidae, partly due to their relatively large size. Cetoniid beetles are specialized for a diet of nectar and pollen and sometimes petals, and may even show flower constancy (Woodell, 1979; Englund, 1993), but are sluggish in their movements from flower to flower (Heinrich & McClain, 1986). Mouthparts of the cetoniid *Trichostetha fascicularis* lack cutting edges but bear numerous setae, and dense brushes on the maxillae are used for both

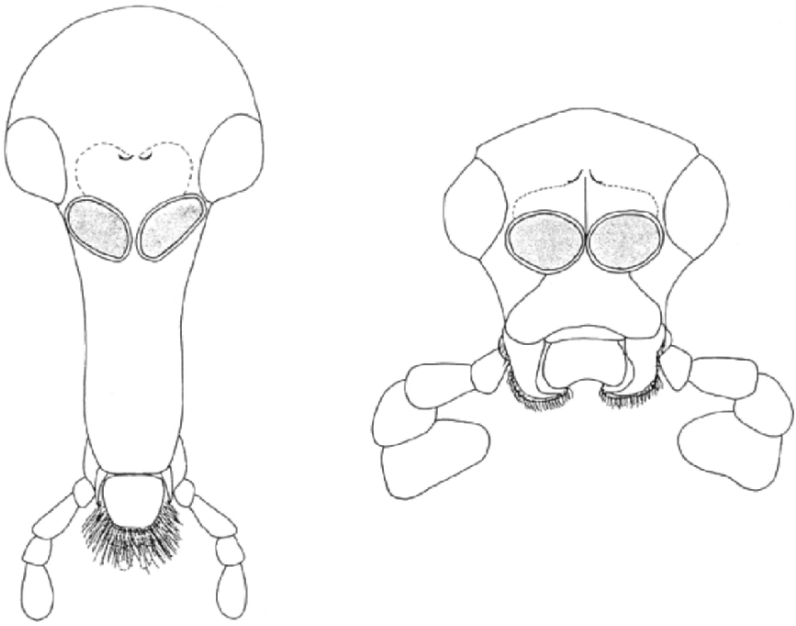

Figure 2. Head and mouthparts of two genera of Lycidae: *Lycus* (*left*) feeds on nectar and *Calopteron* (*right*) is a predator. In *Lycus*, the rostrate head permits access to deep nectaries, the mandibles are rudimentary, and there are tufts of long hair on the maxillae and labium. (From Stamhuis, 1992.)

mopping up nectar and gathering pollen (Johnson & Nicolson, 2001). Another cetoniid beetle pollinates a South African orchid by sweeping dilute nectar from "lollipop hairs": this presentation of nectar as droplets on floral hairs makes it easily accessible to wasps and beetles with short mouthparts (Johnson et al., 2007). Extreme lack of specialization is demonstrated by *Mausoleopsis aldabrensis*, which is highly abundant on the Indian Ocean island of Aldabra and appears to act as a universal pollinator for the flora of the island (Woodell, 1979).

3.2 Diptera

The evolution of flower feeding in Diptera is discussed by Grimaldi and Engel (2005). The Diptera appeared long before the flowering plants, and Downes and Dahlem (1987) suggested that honeydew use is likely to have preceded nectar feeding, the pseudotracheate labellum of flies being ideal for dissolving and imbibing dried films of honeydew on leaves. Within the suborder Nematocera, the mosquitoes are well known as nectar feeders. Males

feed exclusively on nectar, while in females, nectar meals are directed to the crop and blood meals to the midgut. The suborder Brachycera is more important in nectar feeding. Adult fruit flies (Tephritidae) commonly feed on nectar, as well as honeydew and the juices from damaged fruit. Major dipteran pollinators occur in the families Syrphidae and Bombyliidae (bee flies, which possess a long but non-retractable proboscis), and in the muscoid families with lapping labellae (Kearns, 1992). The Syrphidae (hover flies) are important pollinators, but eat mainly pollen (Gilbert, 1981; Haslett, 1989). The most specialized dipteran nectar feeders are long-tongued flies in southern Africa (Johnson & Steiner, 1997). Some of these Tabanidae and Nemestrinidae hover at flowers with very long floral tubes (Fig. 4F), and their slender mouthparts require low viscosity nectars of 25–30% (Goldblatt & Manning, 2000).

Like beetles, flies are associated with a heterogeneous group of flowers, and flies are often considered to be minor pollinators because of their small size, inconstancy, and varied food sources. However, most species of Diptera feed on nectar or other plant exudates and their sheer numbers may compensate for their low effectiveness as pollinators (Larson et al., 2001). Flies are important in the Arctic areas and at high altitude, in part because other pollinator groups are less well-represented (Hocking, 1968; Kearns, 1992; Larson et al., 2001). They tend to replace bees as pollinators in New Zealand where there are few indigenous bees (Godley, 1979). This is a widespread pattern: recently Devoto et al. (2005) examined plant–pollinator interactions along a steep rainfall gradient in southern Argentina, and found that as rainfall increased in a westerly direction there was a gradual replacement of bees by flies.

The success of flies at high latitudes and high elevations has been attributed to the moist larval habitats available, the low energy requirements of the adults, and their use of microhabitats for thermoregulation (Kearns, 1992). These microhabitats include heliotropic flowers, which offer basking sites and possibly increased nectar yield owing to the higher temperatures (Hocking, 1968). The short proboscis of most flies limits them to open flowers with exposed nectar, such as the inflorescences of Apiaceae and Asteraceae—described as flowers that "cater for the mass market" (Proctor et al., 1996). Owing to the minute volumes of nectar produced, detailed study of nectar in such flowers is a challenge. In flowers of caraway (*Carum carvi*, Apiaceae), Langenberger and Davis (2002) measured nectar volumes of 0.039 μl and 0.108 μl per floret in the male and female phases, respectively, and consistently high concentrations of 66.5% w/w or 2.5 M! This nectar is

dominated by hexose sugars, especially fructose, in spite of its high concentration.

In laboratory studies of carbohydrate feeding in Diptera, feeding behaviour varies greatly between insects fed *ad libitum* and those which are deprived of food and then offered single meals, as demonstrated by Edgecomb et al. (1994) for adult *Drosophila melanogaster* feeding on sucrose–agar diets. In general, the volumes of sugar solution ingested are positively correlated with concentration in previously starved individuals offered single meals, but insects feeding *ad libitum* show compensatory feeding and the volume imbibed is then negatively correlated with concentration. At the lower and upper extremes of diet concentration, flies ingesting very dilute fluid may use "bubbling behaviour" to evaporate excess water (Hendrichs et al., 1992), or salivary secretions may be used to dissolve solid nectar sugars. In blowflies, crop emptying rates are faster after ingestion of higher volumes or lower concentrations, and sucrose solutions are processed at half the rate of equimolar fructose solutions with half the energy content (Hainsworth et al., 1990; also see the review of Stoffolano, 1995). The utilization of different sugars by flies, especially parasitoids, is discussed by Chen and Fadamiro (2006), who found poor survival of a parasitic fly, *Pseudacteon tricuspis* (Phoridae) on the trisaccharide melezitose, which is common in aphid honeydew. Where amino acids in nectar are concerned, six essential amino acids elicit feeding responses in flies by stimulating the chemoreceptor that responds to sugars (Shiraishi & Kuwabara, 1970). Specialized fly flowers, such as those pollinated by carrion flies, have high levels of amino acids in nectar, if nectar is present (Baker & Baker, 1982), and flesh flies deprived of a protein source have been shown to select nectars containing mixtures of amino acids (Rathman et al., 1990). The responses of insects to amino acids in nectar are discussed further by Nicolson and Thornburg (2007, Chapter 5 in this volume).

3.3 Lepidoptera

Lepidoptera are equipped with an elongate proboscis and feed from narrow tubular flowers, but can also utilize many other flowers. Moths are many millions of years older than butterflies, far more speciose, and probably more effective pollinators, but nectar-related research has been mainly restricted to the conspicuous hawkmoths (Sphingidae) and butterflies of several families. Their energy requirements differ dramatically, owing to the large body size, endothermy, and hovering flight of hawkmoths. The volumes of nectar in flowers visited by hawkmoths may exceed those in hummingbird flowers belonging to the same genus, as in *Ipomoea* and *Nicotiana* (Galetto &

Bernardello, 2004; Kaczorowski et al., 2005). In contrast, slow-flying, ectothermic butterflies feed at flowers with minute volumes of nectar, often massed flowers in inflorescences, and expend little energy during feeding. The often invasive *Lantana camara* (Verbenaceae) is a classic butterfly flower, and Hainsworth et al. (1991) found that painted lady butterflies, *Vanessa cardui,* foraging at its inflorescences required 49 min to consume an average meal of 28 μl (this includes the time required to move between inflorescences and to probe empty flowers). Watt et al. (1974) investigated the use of nectar by two species of *Colias* butterflies (Pieridae) feeding on a wide range of plants, and stressed the importance of nectar as a water resource for these butterflies. Boggs (1987) reviewed the ecology of nectar feeding in Lepidoptera.

The fast hovering flight of hawkmoths and their high energy requirements make them important pollinators in warmer habitats (Raguso & Willis, 2003). They remove large volumes of nectar from pale, fragrant, long-tubed flowers opening at dusk. It has recently been suggested that high levels of CO_2 emission by flowers opening at this time may indicate the presence of abundant nectar to the moths (Guerenstein et al., 2004). The high-performance flight of hawkmoths is powered by carbohydrates—whereas early studies emphasized fat as a flight fuel in moths, conversion of carbohydrate to fat is energetically expensive, and tethered flight in the diurnal hawkmoth *Amphion floridensis* is fuelled by carbohydrate, with only unfed moths using fat (O'Brien, 1999). Many moths, and some butterflies, do not feed as adults (Boggs, 1987; Miller, 1996). Nectar feeding in moths other than Sphingidae has been little studied, except for pest species among the Noctuidae (Wei et al., 1998). While herbivory and pollination are usually studied separately, the herbivorous larvae of nectarivorous adults may specialize on the same plants, with consequences for pest outbreaks. The family Sphingidae provides some good examples of herbivorous larvae that specialize on the plants they will pollinate as adults. Adler and Bronstein (2004) recently showed that nectar supplementation in flowers of *Datura stramonium* (Solanaceae) led to increased oviposition by its pollinator, the hawkmoth *Manduca sexta*.

The suctorial proboscis of Lepidoptera evolved only once (Krenn et al., 2005). This mode of feeding requires relatively dilute nectars and for Lepidoptera the most efficient energy intake has been predicted by various authors to lie in the concentration range of 35–45% (Kingsolver & Daniel, 1995). Maximal energy intake has been measured in this range in several butterfly species, and also in the diurnal hummingbird hawkmoth *Macroglossum stellatarum* (Table 3). Addition of tylose, an inert polysaccharide,

allowed Josens and Farina (2001) to manipulate the concentration and viscosity of hawkmoth diets independently. The dependence of intake rate on concentration did not change in solutions to which tylose was added to maintain constant high viscosity, although all intake rates were reduced. If viscosity were the only physical factor affecting ingestion dynamics, solutions of equal viscosity would be ingested at the same rate. For butterflies, there is evidence that they compensate for increased food viscosity by increasing suction pressure (May, 1985; Pivnick & McNeil, 1985). A noctuid moth, *Anticarsia gemmatalis*, illustrates the ability of adult Lepidoptera to cope with a broad range of sugar concentrations under laboratory conditions; the moths regurgitate a clear, sugar-free liquid from the proboscis after feeding on sucrose solutions below 20%, or use saliva to dissolve solid sucrose (Wei et al., 1998). The Noctuidae, the largest family of moths, includes many pest species, but adult feeding has received little attention.

Sugar preferences have been tested in *Macroglossum stellatarum*, and this moth strongly prefers sucrose to fructose and fructose to glucose (Kelber, 2003). The same order of preference has been recorded in tests involving butterflies (Rusterholz & Erhardt, 1997; Romeis & Wäckers, 2000). The ecological relevance of testing pure solutions of glucose and fructose is questionable, but the use of mixed sugar solutions also shows a clear preference of the peacock butterfly *Inachis io* for high sucrose levels (Rusterholz & Erhardt, 1997). Behavioural responses to sugars are not necessarily correlated with their nutritional importance to the insects. Although glucose is not selected by butterflies in choice tests, it usually occurs in nectar at similar levels to fructose and has high nutrititional value for adult *Pieris brassicae* (Romeis & Wäckers, 2002). In three Japanese butterfly species that feed on exuded tree sap and rotting fruit, Ômura and Honda (2003) have shown that ethanol and acetic acid have synergistic effects on the butterflies' response to low hexose concentrations.

Adult feeding in Lepidoptera is not just for energy. Butterfly nectars are thought to contain relatively high levels of amino acids compared to those consumed by other pollinators (Baker & Baker, 1982). Artificial nectar that resembles that of *Lantana camara* has often been used in experimental tests (with varied results) of whether butterflies select for high levels of amino acids in nectar. Female butterflies are generally more responsive to diets enriched with amino acids than males, and female *Inachis io* were found to select low concentrations of amino acids (Erhardt & Rusterholz, 1998; Mevi-Schütz & Erhardt, 2002). Until recently, it has been assumed that female butterflies obtain nitrogen from several sources which do not include nectar: these are larval feeding, sometimes pollen feeding (*Heliconius* species), and

Table 3. Optimum nectar concentrations for various animal taxa based on empirical measurements of sucrose intake rate. (See references for experimental details.)

Taxon	Species	Conc. (% w/w)	Reference
Fruit fly	*Ceratitis capitata*	24	Warburg & Galun, 1992
Hawkmoths	*Macroglossum stellatarum*	34	Josens & Farina, 2001
Butterflies	*Thymelicus lineola*	40	Pivnick & McNeil, 1985
	Agraulis vanillae	40	May, 1985
	Phoebis sennae	30–40	May, 1985
	Vanessa cardui	31–44	Hainsworth et al., 1991
Orchid bees	*Euglossa imperialis*	35	Borrell, 2004
Bumblebees	*Bombus* species	50–65	Harder, 1986
Honeybees	*Apis mellifera*	60	Roubik & Buchmann, 1984
Stingless bees	*Melipona* species	60	Roubik & Buchmann, 1984
Ants	*Camponotus mus*	43	Josens et al., 1998
	Camponotus rufipes	40	Paul & Roces, 2003
	Pachycondyla villosa	50	Paul & Roces, 2003
Hummingbirds	*Selasphorus rufus*	40–45	Tamm & Gass, 1986
Sunbirds	*Nectarinia chalybea*	30	C.A. Beuchat & S.W. Nicolson, unpublished data
Honeyeaters	*Acanthorhynchus tenuirostris*	30–40	Mitchell & Paton, 1990
	Phylidonyris novaehollandiae	40–50	Mitchell & Paton, 1990
Bats	*Glossophaga soricina antillarum*	60	Roces et al., 1993

male spermatophores (Boggs et al., 1981). Boggs (1997) used radiotracers to examine the use of glucose and amino acids acquired during the larval and adult stages of two nymphalid butterflies. Glucose and amino acids labelled with the isotopes ^{14}C and ^{3}H were painted on leaves for the caterpillars or included in nectar solutions for the butterflies. Because the adult diet is carbohydrate-rich, incoming glucose was used in preference to stored glucose, while juvenile reserves of amino acids were used throughout adult life. Because female Lepidoptera can synthesize non-essential amino acids from nectar sugars and derive essential amino acids from the larval diet (O'Brien et al., 2002), the role of nectar amino acids in egg manufacture and adult fitness may have been underestimated. Recently, it has been shown that increased mating frequency decreases the preference of female butterflies (*Pieris napi*) for nectar-containing amino acids (Mevi-Schütz & Erhardt, 2004), and that nectar amino acids can be used to compensate for poor larval food. Increased fecundity was evident in females raised on low-quality nettle

diets and then fed nectar mimics with amino acids, but no effect was seen after rearing on high-quality larval diets (Mevi-Schütz & Erhardt, 2005). The importance of nectar amino acids for butterfly fitness thus varies with the nutritional history of females.

While female butterflies are more responsive to amino acids in nectar, mud puddling is an activity restricted almost entirely to males. This drinking at puddles or decaying organic matter is thought to be directed at the acquisition of minerals or nitrogenous compounds (Beck et al., 1999). Sodium gained by males during puddling is transferred to females at mating for use in egg production (Pivnick & McNeil, 1987). Although this behaviour suggests that sodium is scarce in nectar, it does not seem to occur in other flower-visiting orders of insects.

3.4 Hymenoptera

3.4.1 Wasps

Wasps provide their brood with animal material but many feed on nectar, honeydew and fruit juices as adults. The carbohydrate intake of social wasps (Vespidae) is supplemented with salivary secretions solicited from the larvae, and Hunt et al. (1982) suggested that this behaviour may have arisen because of nutritional similarities between the larval secretions and the primary adult food, floral nectar. Wasps have unspecialized mouthparts and are common visitors to flowers with exposed nectar and to extrafloral nectaries, where both parasitoid and predatory wasps provide anti-herbivore protection to the plants. Koptur (1992) lists many families of nectar-drinking wasps. Larger wasps from the families Pompilidae, Vespidae, and Sphecidae are more likely to drink at floral nectaries and play a role in pollination. The impact of wasp sugar feeding has been felt on a major scale in New Zealand beech forests, which have been invaded by *Vespula vulgaris* because of their abundant honeydew (Beggs, 2001).

Foraging at small flowers with accessible nectaries is common among the species-rich parasitic Hymenoptera (Jervis et al., 1993; Patt et al., 1997). Adult females of parasitoid wasps (e.g., Ichneumonidae, Braconidae, Encyrtidae, Eulophidae) feed on both host insects and sugary food such as nectar and honeydew, and this has led to the use of flowering plants and sugar sprays to increase populations of these wasps in agro-ecosystems for purposes of biological control (Idris & Grafius, 1995; Patt et al., 1997; Rogers & Potter, 2004). In this context, Wäckers (2001) carried out a detailed study of the nutritional suitability of a broad range of honeydew and nectar sugars

for adult *Cotesia glomerata* (Braconidae), and found that this parasitoid can utilize a wider range of sugars than its host, the butterfly *Pieris brassicae*.

The solitary pollen wasps (Masarinae) are a small group of sphecid wasps that resemble bees in that they provision their brood with pollen and nectar. Bees are a derived, herbivorous group of the sphecid wasps.

3.4.2 Bees

Bees, the dominant pollinators in most communities (Proctor et al., 1996), are speciose, widely distributed, and highly reliable and efficient because a females collect both nectar and pollen to feed their offspring. For most other flower visitors, pollen collection is accidental. Social bees (which are a minority among bees) need additional nectar energy to warm the nest and speed brood development (Heinrich, 1975). Endothermy allows faster foraging and makes bees important pollinators in cool temperate zones, but increases their energy requirements. The highly efficient recruitment behaviour of honeybees and stingless bees ensures that good nectar sources are exploited rapidly and surplus nectar can be accumulated (Visscher & Seeley, 1982; Nieh et al., 2003). Colonies focus on the best nectar sources in a large area by working relatively few patches at any one time and frequently adjusting the number of foragers at those patches (Visscher & Seeley, 1982). Social bees are major pollinators in European and North American systems, but less abundant in the southern hemisphere systems (Johnson & Steiner, 2000; Ollerton et al., 2006). The highly eusocial honeybees have long-lived colonies and require a succession of flowers throughout the year, so as a result are the ultimate generalists among bees (Westerkamp, 1991). Although they collect from unrelated plants (termed polylectic), flower constancy results in temporary specialization at the level of the individual insect (reviewed by Chittka et al., 1999; see also Dafni et al., 2005). When solitary bees are oligolectic, gathering pollen from a few closely related taxa, they are less specialized on nectar. The two introduced bee species that have been most adaptable in colonizing new habitats around the world are *Apis mellifera* and *Bombus terrestris*, both opportunistic generalists that may potentially compete for nectar with large numbers of solitary bee species (Goulson, 2003).

Of the 20,000 species of bees, almost all provision their young with nectar and pollen. A few mix pollen and floral oils (Buchmann, 1987). The provisioning of offspring dominates the lives of female bees. Their activity patterns, and therefore the time required to provision cells, are structured by a combination of abiotic factors (especially temperature) and the availability of floral resources (Willmer & Stone, 2004). Solitary bees usually collect pollen

and nectar simultaneously. The intensity of their foraging is illustrated by the carpenter bee *Xylocopa capitata*, which forages almost exclusively on the trees of *Virgilia divaricata* (Fabaceae) in Cape Town during spring (Louw & Nicolson, 1983). In suitable weather conditions, a female carpenter bee visits eight flowers per minute; the nectar of two of these flowers covers her flight costs, while the nectar of the remaining six is available for larval provisions. However, it is pollen rather than the concentrated nectar (53% w/w) that determines the number of foraging trips. Pollen is collected simultaneously, and a female *X. capitata* must visit about 1,700 flowers of *V. divaricata* to collect enough pollen to raise one offspring.

Bees lick nectar with hairy tongues. The importance of tongue length was emphasized by Harder (1986): it determines the maximum flower depth from which a bee can feed, the volume ingested per lick, and the licking rate. Bees are commonly divided into two broad categories based on their tongue length and resulting ability to forage at shallow and deep flowers. Short-tongued bees include the Andrenidae, Halictidae, Colletidae, and Melittidae; long-tongued bees include the Megachilidae and Apidae. The long-tongued orchid bees (Euglossini) differ from most other bees in using suction feeding (Borrell, 2004). Kingsolver and Daniel (1995) predicted that a shift from lapping to suction feeding would increase the effect of nectar viscosity on rates of ingestion, and in fact orchid bees do collect less concentrated nectars than other bees (Roubik et al., 1995; Borrell, 2004). The optimal concentration of 30–40% for these bees coincides with that predicted for suction-feeding Lepidoptera. For lapping bees the optimal concentration is higher (Table 3): the fastest energy intake occurs at 50–65% sucrose in bumblebees (Harder, 1986), and 60% in four species of *Melipona* (stingless bees) and introduced *Apis mellifera* in a tropical forest in Panama (Roubik & Buchmann, 1984). However, colonies of all four *Melipona* species were found to gather more concentrated nectar as the day progressed. Sampling the crop contents of bees can be used to provide information about nectar harvesting on a community basis (Roubik & Buchmann, 1984; Roubik et al., 1995), but this method depends on the assumption of Park (1932) that nectar carried in the crop of bees is not dehydrated. The nectar collected by honeybee foragers is regurgitated to their nestmates in a process known as trophallaxis, and the rate of transfer, like the rate of ingestion, depends on sugar concentration, being maximal at 30% w/w and decreasing at higher concentrations due to the increased viscosity (Tezze & Farina, 1999).

Although honeybees have been shown to prefer nectar concentrations of 30–50% (e.g., Waller, 1972), in practice they collect from a much wider range: for example, Seeley (1986) measured concentrations of 15–65% in

nectar loads entering a single colony over a 5-day period. Honeybees are very sensitive to differences in nectar concentration. In the same study, Seeley (1986) recorded a 30% drop in recruitment rate for a decrease of only 0.125 M (4%) in concentration (Fig. 3). The relationship between the dance rate of bees and sugar concentration is likewise non-linear, with a more pronounced change at low concentrations: bumblebees foraging at small-volume artificial flowers in the laboratory prefer 20% over 10% sucrose solutions more strongly than they prefer 50% over 40% (Waddington, 2001). The response of bees to a particular nectar concentration depends very much on the ecological context, which is an important consideration in studies involving feeder choices in a natural environment. In one of his classic papers, Lindauer (1948) showed that the threshold sucrose concentration for eliciting recruitment behaviour in honeybees declined from 2 M (~55% w/w) during the main nectar flow in spring to around 0.1 M (3.5%) in mid-summer, when bee forage became scarce and competition was intense.

The thoracic temperatures and metabolic rate of honeybees vary with both the reward rate at the food source and the motivational state of the bees. Dandelion foragers have been recorded as 10°C warmer than bees visiting sunflowers (Kovac & Schmaranzer, 1996). Graduated thermal behaviour occurs during food unloading in the hive, as well as at feeding locations. It has been shown that the temperature of dancing bees recruiting their nestmates increases with food quality and the number of brood cells, and decreases with distance of the food source from the hive and the amount of stored honey (Stabentheiner, 2001). The increased thoracic temperature of the receiver bees in turn raises their activity level, ensuring that nectar from more profitable sources is processed faster (Farina & Wainselboim, 2001). Stingless bees (*Melipona panamica*) have also been shown to regulate their thoracic temperature according to food concentration and distance from the nest, with distance having a much greater effect (Nieh & Sánchez, 2005).

As individuals, flying honeybees are in negative water balance at ambient temperatures above 31°C unless they collect either dilute nectar or water (Roberts & Harrison, 1999). When it is necessary to cool the hive by evaporation, bees will collect water or dilute nectar (Ohguchi & Aoki 83). Most desert bees are solitary and must acquire water from nectar (Willmer & Stone, 1997). In Israel, foraging choices of the mason bee *Chalicodoma sicula* are dictated by its water needs, and field measurements of its blood osmolality show rapid corrections after the ingestion of nectar of *Lotus creticus* (Willmer, 1986). Two carpenter bees (*Xylocopa*), also in Israel, depend on the water component of nectar of *Calotropis procera* (Apocynaceae), especially the smaller of the two species because it generates less metabolic water (Willmer, 1988).

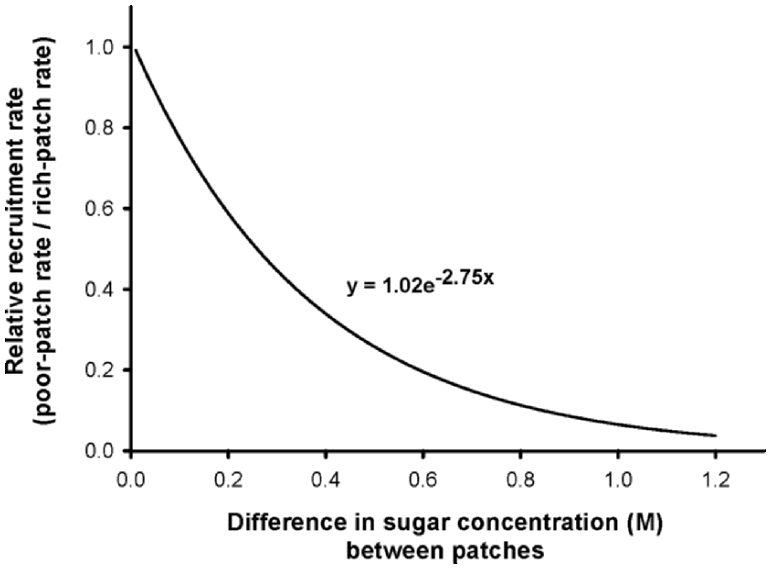

Figure 3. Recruitment rate of honeybees in relation to concentration. (From Seeley, 1986.)

Solitary *Anthophora pauperata* avoid the abundant but concentrated (70%) nectar in old flowers of *Alkanna orientalis* (Boraginaceae) in the Sinai desert of Egypt (Stone et al., 1999). In other environments with seasonal drought, honeybees also forage on dilute nectars (Percival, 1974). South African beekeepers have traditionally moved their hives north of Pretoria in winter (the dry season), in order to exploit the strong nectar and pollen flow of *Aloe greatheadii* var. *davyana* (Asphodelaceae), which has a nectar concentration of around 20% (H. Human and S.W. Nicolson, in preparation). There are several examples of introduced honeybees foraging on remarkably dilute nectars and competing with endemic birds for them: these include the nectars of *Metrosideros collina* (14–21%) in Hawaii (Carpenter, 1976), *Eucalyptus incrassata* (7%) in southern Australia (Bond & Brown, 1979), both Myrtaceae, and *Sideroxylon* (Sapotaceae, 10%) in Mauritius (Hansen et al., 2002).

Regardless of the initial concentration, honeybees must concentrate nectar to at least 82% for larval food storage, by a process of evaporation first on their tongues and later in cells (Winston, 1987). Carpenter bees and bumblebees have also been observed evaporating nectar by repeated regurgitation (Corbet & Willmer, 1980; Willmer, 1988; Heinrich, 1993). In the West Indies, carpenter bees (*Xylocopa mordax*) collect nectar of 45–50% sugar from *Passiflora* and manipulate it on their tongues to a concentration of about 62% before storing it (Corbet & Willmer, 1980). Foraging on concentrated

nectars appears to be a physiological necessity for large bees at low or moderate ambient temperatures, because their very high metabolic water production during flight far exceeds evaporative water losses (Nicolson & Louw, 1982; Bertsch, 1984).

Like moths and butterflies, honeybees have been shown to prefer sucrose to fructose and fructose to glucose (Waller, 1972; Bachman & Waller, 1977). However, the use of artificial flowers of different colours in tests of honeybee sugar preference leads to different results, because the constancy of individual bees to either blue or yellow "flowers" can override sugar preferences (Wells et al., 1992). Kearns and Inouye (1993) have discussed the relative merits of different feeder types in assessing the responses of honeybees to sugar solutions: these range from open dishes where large numbers of bees can feed simultaneously to small volume artificial flowers, which may or may not be replenished after each visit. By filling the holes of microtitre plates with different sugar solutions, Biesmeijer et al. (1999) showed that stingless bees (*Melipona* sp.) preferred sucrose to glucose and fructose, though they did not discriminate between hexose-rich and hexose-poor solutions. Bee nectars were separated by Baker and Baker (1983) into high sucrose nectars favoured by long-tongued bees and low sucrose nectars favoured by short-tongued bees, but this may be due to the slower hydrolysis of sucrose in deep flowers (Willmer & Stone, 2004). Sucrose hydrolysis is rapid in a bee's crop (Nicolson & Louw, 1982), the necessary α-glucosidase being produced in the hypopharyngeal glands rather than in the midgut as in other nectar feeders (Terra & Ferreira, 1994). The rate of crop emptying depends on sugar concentration, as in other insects, and is adjusted to the energy demands of the bee (Roces & Blatt, 1999). Passive absorption of monosaccharides from the midgut is also extremely rapid, with a favourable gradient aided by the conversion of glucose to trehalose: when hungry honeybees are fed labelled glucose, labelled trehalose can be detected in the haemolymph only 2 min later (Gmeinbauer & Crailsheim, 1993). Where the less common nectar sugars are concerned, Barker and Lehner (1974) studied the survival of honeybees on 13 different sugars, and the consumption of those sugars during cage experiments.

Like butterflies, honeybees show positive responses to amino acid mixtures mimicking the amino acid composition of nectar, but negative responses to individual amino acids when tested at high concentrations (Inouye & Waller, 1984; Alm et al., 1990). Among the amino acids that occur at relatively high concentrations in nectar, phenylalanine has a strong phagostimulatory effect on bees (Petanidou et al., 2006); honeybees prefer synthetic nectars rich in

proline (Carter et al., 2006), and glycine influences their learning behaviour (Kim & Smith, 2000).

3.4.3 Ants

Liquid carbohydrate is a major food of ants. The main sources are honeydew and extrafloral nectars, and sometimes secretions from the caterpillars of Lycaenidae, about half of which are involved in mutualistic associations with ants (Baylis & Pierce, 1993). Ants are generally considered to be poor pollinators for several reasons, including their flightlessness, frequent grooming behaviour, and production of antibiotic secretions that inhibit pollen germination; in addition they deter other pollinators and rob nectar (Peakall et al., 1991). Ants competing with honeybees for *Eucalyptus* nectar have the advantage of foraging at night when most of the nectar is secreted (Buys, 1987). One way to exclude these undesirable visitors from flowers (even if nectarless) is to attract them to extrafloral nectaries, and the distraction hypothesis suggests that extrafloral nectaries function to reduce ant visitation to flowers (Wagner & Kay, 2002). Mexican ant acacias have very active extrafloral nectaries on the leaves; they secrete a daily pulse of dilute nectar, which ensures that ants and pollinators are largely separated spatially, although they have similar temporal patterns of activity (Raine et al., 2002). Ant repellents secreted by the acacia flowers further deter ant visits, and this chemical repellency of floral tissue may be a widespread phenomenon (Ghazoul, 2001).

The more commonly accepted alternative is the protection hypothesis, the extrafloral nectar being a reward for ant (or wasp) guards for the anti-herbivore protection that they provide. Extrafloral nectaries function for much longer than the floral variety (Koptur, 1992), and herbivory can affect both the volume and composition of the extrafloral nectar. Leaf damage in *Macaranga tanarius* (Euphorbiaceae) causes increased secretion of extrafloral nectar, via a signalling pathway involving the plant stress hormone jasmonic acid, and this is later reflected in reduced herbivory (Heil et al., 2001). Similarly, the leaves of *Catalpa bignonioides* increase production of extrafloral nectar after attack by caterpillars, and ant attendance also increases (Ness, 2003). These studies show a causal link between herbivory, nectar production, and bodyguard recruitment. Smith et al. (1990) found dramatic increases in the amino acid concentration of extrafloral nectar in *Impatiens sultani* after defoliation (simulated herbivory). There was no simultaneous change in sugar concentration, which suggests a specific rather than a general physiological response. The production of extrafloral nectar is stimulated by its repeated removal as well as by real or simulated herbivory (Heil et al., 2000).

Comparisons of floral and extrafloral nectars of the same plant species are few (Baker et al., 1978; Koptur, 1994; Blüthgen et al., 2004). It has been suggested that both concentration and sugar composition may be more variable in exposed extrafloral netars (Koptur, 1992). Recent workers have analysed the sugar and amino acid composition of extrafloral nectars in some detail (Heil et al., 2000; Blüthgen et al., 2004). The extrafloral nectar of certain Mexican *Acacia* species with obligate ant associations is kept free of sucrose by postsecretory hydrolysis. This provides a biochemical mechanism for the mutualism, reinforced by the loss of gut sucrase in two *Pseudomyrmex* species and their resulting preference for hexose nectars (Heil et al., 2005). This example is an exception to the generalization that sugar types do not matter to insect consumers.

In the course of detailed research on a nectar-feeding ant community in an Australian rainforest, Blüthgen et al. (2004) compared the sugars and amino acids in a spectrum of food sources with the crop contents of ants of several species found at these sources. Ants were observed at all extrafloral but not floral nectar sources. When choice tests were conducted with artificial nectars, using the same ant community in its natural environment (Blüthgen & Fiedler, 2004b), competition between ants on the same bait was found to affect their selectivity, with preferences for higher amino acid and sugar concentrations being most distinct in the dominant ant species, *Oecophylla smaragdina* (Blüthgen & Fiedler, 2004a). Although the trisaccharide melezitose is the most common insect-synthesized sugar in honeydew and may be an attractant to ants tending aphids (Völkl et al., 1999), it was found to be less attractive than sucrose to the rainforest ants (Blüthgen & Fiedler, 2004b). This ecosystem approach to preference tests indicates broadly similar preferences across the ant community (Blüthgen & Fiedler, 2004a) and confirms earlier tests showing that amino acids in extrafloral nectar contribute to the attraction of ants (e.g., Lanza, 1991).

Nectar feeding in the ant *Camponotus mus* was investigated by weighing foragers as they crossed a small bridge between the colony and the foraging arena (Josens et al., 1998). Crop load increased with increasing sucrose concentration to a maximum at about 1.5 M (43%), then diminished because of viscosity effects. Workers carried up to 60% of their own weight in the crop, but the loads were partial for either dilute or very concentrated solutions, when the motivational state of the ants or the physical properties of the solution played a role, respectively. Duncan and Lighton (1994) used the consumption of sugar solution by honeypot ants (*Myrmecocystus*) as a convenient way to measure the cost of load carriage, but found no cost savings compared with the external load carriage, which is more common in ants. One

factor influencing the motivational state of nectar-feeding ants is colony starvation, when the ants are more inclined to accept dilute food (10% sucrose), and the food intake rate and the extent of crop filling both increase (Josens & Roces, 2000). Viscosity may be less of a problem for more primitive ponerine ants, because they lack a crop and transport nectar as a drop between the mandibles (Paul & Roces, 2003). The ponerine ant *Pachycondyla villosa* licks sugar solutions and its maximum energy intake rate occurs at a higher concentration than that of the formicine *C. rufipes*, which sucks fluid food (Table 3).

4 VERTEBRATE NECTAR CONSUMERS

Endothermic vertebrate pollinators have the advantage of being dependable over a wide range of seasons and altitudes (Wolf & Gill, 1986). Nectarivorous birds and bats, in particular, are highly mobile, and their daily and seasonal tracking of nectar resources adds to their value as pollinators (Fleming, 1992). However, rewarding them represents a significant investment by the plant, in terms of nectar production and also the substantial floral structures required to produce and contain it (Fig. 4). Extrafloral nectaries are generally small, and it is unlikely that the energetic returns from foraging on them will be worthwhile for vertebrates. Yet a remarkable exception is seen in Australian honeyeaters, which are attracted to the large red extrafloral nectaries of *Acacia terminalis*, contacting the nectarless flowers and transferring pollen while they forage (Knox et al., 1985; Stone et al., 2003).

Vertebrate nectarivores are best represented in the tropics and the southern hemisphere, where major radiations of plants have not been accompanied by corresponding bee diversity: this contrasts with the domination of the northern hemisphere pollination systems by generalist social bees (Johnson & Steiner, 2000). Both diurnal birds and nocturnal marsupials commonly visit the unspecialized brush flowers of *Eucalyptus* (Myrtaceae) and *Banksia* (Proteaceae) in Australia, and rodents in South Africa also visit Proteaceae (*Protea* species). Sussman and Raven (1978) considered pollination by non-flying mammals to be an archaic system, which persists only in the absence of bat pollinators, and this may be true of some regions (see Carthew & Goldingay, 1997). An interesting example is seen in the large-flowered Strelitziaceae, where the basal genus *Ravenala* is pollinated by lemurs in Madagascar, and the more derived *Phenakospermum* and *Strelitzia* are pollinated by bats in South America and birds in South Africa, respectively (Kress et al., 1994).

4.1 Lizards

Most reports of nectar consumption by lizards are from islands, where lizards reach higher densities than on the mainland, owing to reduced predation, and where insects may be relatively scarce. In New Zealand, arboreal geckos in the genus *Hoplodactylus* drink the copious nectar of *Phormium tenax* (Agavaceae) and *Metrosideros excelsa* (Myrtaceae), transporting pollen on their throats (Whitaker, 1987; Eifler, 1995). Diurnal geckos (*Phelsuma* sp., Fig. 4C) are generalist flower visitors and pollinators on Mauritius and other Indian Ocean islands (Nyhagen et al., 2001), and recently have been shown experimentally to prefer coloured to clear sucrose solutions, thus explaining their attraction to the coloured nectar of some Mauritian plants (Hansen et al., 2006). Tasmanian snow skinks forage on abundant nectar of *Richea scoparia* (Epacridaceae) without contacting pollen (Olsson et al., 2000), but pollination of *Euphorbia dendroides* by lacertid lizards was demonstrated by Traveset and Sáez (1997) in the Balearic Islands. It has been hypothesized that pollination by lizards may have evolved on islands as a result of the general scarcity of insects, with the consequences that island plants need pollinators and lizards need food (Olesen & Valido, 2003).

4.2 Birds

Nectarivory has evolved many times in birds, especially in tropical and subtropical areas with long flowering seasons that can sustain birds on nectar all year round. Convergent evolution is well illustrated by three major radiations of nectarivorous birds on different continents: the American hummingbirds (Trochilidae), Australasian honeyeaters (Meliphagidae), and the sunbirds and sugarbirds (Nectariniidae) of Africa and Asia. The Meliphagidae is the dominant passerine family in Australia, and the Trochilidae is the largest family of non-passerine birds—now confined to the Americas, although hummingbird fossils from the Oligocene have recently been reported from Germany (Mayr, 2004). Numerous other families, mostly passerine birds, contain species with varying dependence on nectar. These include the Hawaiian honeycreepers, flowerpiercers, tanagers, and lorikeet parrots, as well as opportunistic nectar feeders such as white-eyes, bulbuls, weavers, orioles, barbets, mousebirds, starlings, Darwin's finches, and some babblers and warblers (Nicolson & Fleming, 2003b; Lotz & Schondube, 2006). It has been estimated that around 10% of all bird species may use nectar as a resource at some time (Wolf & Gill, 1986). Even in Europe, where bird pollination is extremely rare (Ford, 1985; Ortega-Olivencia et al., 2005), opportunistic nectar consumption can be energetically important for *Sylvia* warblers returning after migratory flights (Schwilch et al., 2001). The relationship between bill length

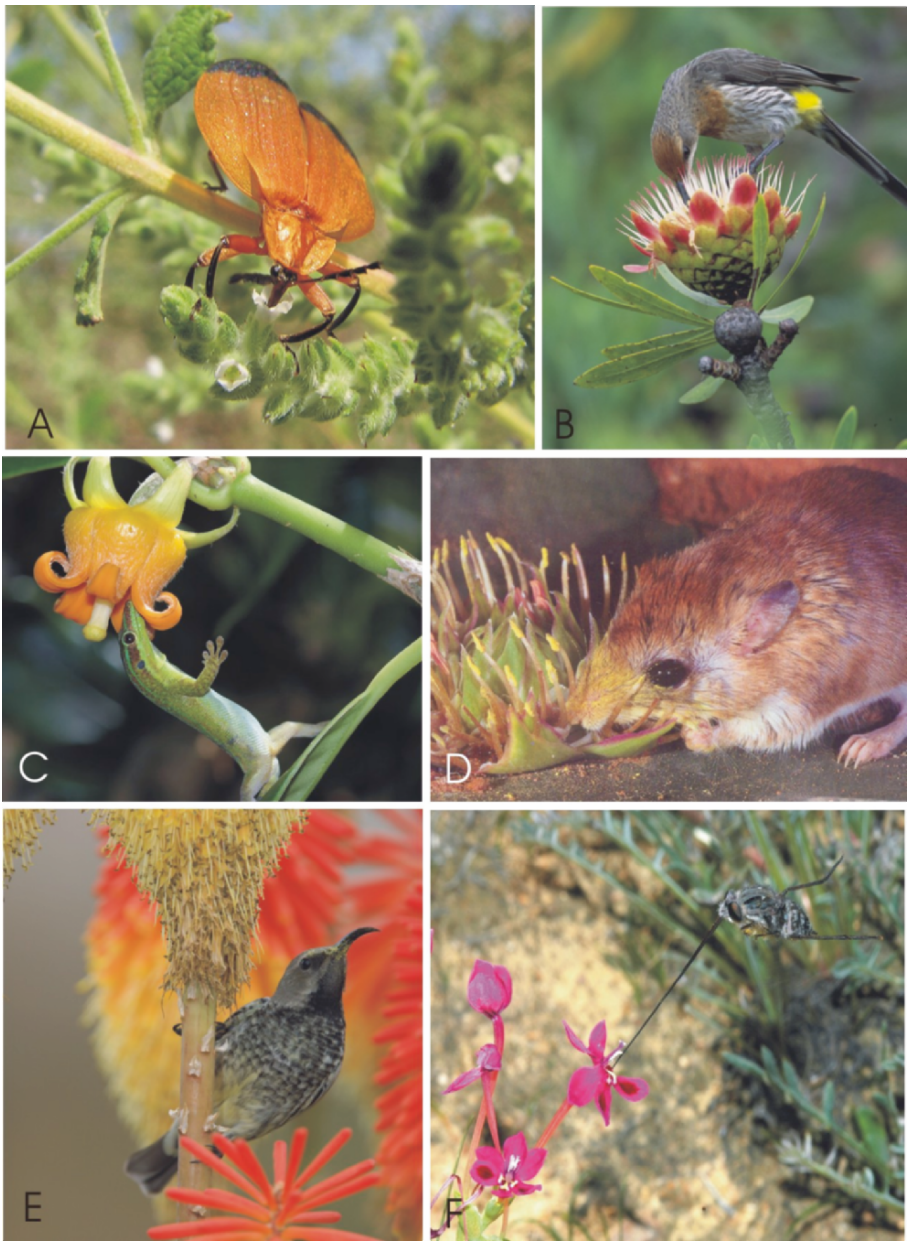

Figure 4. A. *Lycus fernandezi* (Lycidae) drinking nectar of *Aloysia wrightii* (Verbenaceae), New Mexico; B. Gurney's sugarbird *Promerops gurneyi* feeding on *Protea caffra*, South Africa; C. Diurnal gecko *Phelsuma cepediana* at flower of *Roussea simplex* (Rousseaceae), Mauritius; D. Hairy-footed gerbil *Gerbillurus paeba* foraging in flower of *Massonia depressa* (Hyacinthaceae), South Africa; E. Female black sunbird *Nectarinia amethystina* on *Kniphofia*, South Africa; F. Long-tongued fly, *Prosoeca peringueyi* (Nemestrinidae), at flower of *Lapeirousia silenoides* (Iridaceae), South Africa. (Photos: Bob Barber (A), Tim Jackson (B, E), Dennis Hansen (C), and Steve Johnson (D, F).)

and corolla length usually determines whether nectar-feeding birds are legitimate pollinators or nectar robbers, although the two categories are not distinct (Maloof & Inouye, 2000). When nectar is scarce, honeydew and sugary exudates from plants may be alternative carbohydrate sources for birds (Paton, 1980; Gaze & Clout, 1983). Stiles (1981) contributed an excellent and wide-ranging review of geographical differences in bird–flower associations. The small size of nectar-feeding birds seems to be determined by co-evolution with flowering plants and competition with the largest insect pollinators, bees and hawkmoths, which are also endotherms; and it may be the use of torpor that has enabled hummingbirds to be significantly smaller than other nectar-feeding birds (Brown et al., 1978; Cotton, 1996).

Bird flowers are usually large and robust, although sometimes massed small flowers can provide an equivalent reward (Castro & Robertson, 1997). While tubular shapes are very common, generalist brush blossoms are also important nectar sources (Stiles, 1981), especially for passerine nectarivores visiting flowers of Myrtaceae and Proteaceae in Australia and southern Africa. The copious nectar of bird flowers is characteristically dilute compared to that of insect-pollinated flowers. For example, Nicolson and Fleming (2003b) summarized data from the literature and obtained means of 25% (w/w) for the nectars of 255 hummingbird species and 21% for 158 sunbird species. These can be compared with 36% for the nectars of 156 species of bee flowers (Pyke & Waser, 1981). Various hypotheses have been proposed to account for the relatively low sugar concentration of bird nectars:

- The viscosity of nectar increases exponentially with concentration, and low viscosities enable more efficient extraction of nectar by bird tongues, especially during hovering (Baker, 1975).
- Low concentrations serve to discourage bees (Bolten & Feinsinger, 1978).
- Dilute nectars are necessary to meet the water requirements of birds, especially at high ambient temperatures (Baker, 1975; Calder, 1979).
- Nectars of "bird" flowers remain dilute because they are protected from evaporation by tubular corollas (Plowright, 1987).
- Dilute nectars may encourage birds to visit more flowers by not satiating their energy requirements immediately (Martínez del Rio et al., 2001).
- Dilute nectars are a consequence of hydrolysis of sucrose to glucose and fructose: this maintains the gradient for sucrose transport and the increased osmolality draws additional water from the nectary (Nicolson, 1998, 2002).

The question is still unresolved, and a combination of these factors is probably involved. Saying that nectar is dilute to discourage bees may be no

Figure 5. Scale-throated hermit *Phaetornis eurynome*, Brazil. (Photo: C. Purchase.)

more accurate than saying that bird flowers are red to exclude bees (Chittka & Waser, 1997). As already mentioned, bees often visit bird flowers, in spite of dilute nectar. Birds will also take concentrated nectar, such as the sunbirds that forage extensively on the viscous nectar (60% w/w) of *Lobelia telekii* in Kenya (Evans, 1996). Interestingly, hummingbirds are commonly provided with artificial nectar in both North and South America (Fig. 5), a practice that is influencing bird densities, with associated effects on the plants they pollinate (Streisfeld & Kohn, 2006).

A diet of dilute nectar, varying in concentration in time and space, has a major impact on the energy and water balance of birds, requiring close integration of the intestinal and renal systems (Beuchat et al., 1990). Nectarivorous birds exhibit compensatory feeding, increasing the volume of food consumed in response to diet dilution or when energy demands increase due to low temperatures (reviewed by Martínez del Rio et al., 2001). This is illustrated in Fig. 6 for white-bellied sunbirds, *Nectarinia talatala*, which maintain constant energy intake on sucrose solutions varying tenfold in concentration from 0.25 to 2.5 M (Nicolson & Fleming, 2003a). The ingestion of large volumes of dilute nectar results in extreme water fluxes in birds, up to five times body mass per day, and chronic diuresis. Alhough it was predicted by Beuchat et al. (1990) that hummingbirds would be able to modulate the rate of intestinal water absorption according to diet concentration, and thus

reduce the water load on their kidneys, subsequent studies have shown that this is not the case (McWhorter & Martínez del Rio, 1999; Hartman Bakken & Sabat, 2006). Water shunting directly through the intestine has, however, been demonstrated in sunbirds (McWhorter et al., 2003). Variation in fractional water reabsorption by the kidney has been shown to be important for managing water excess in all three main lineages of nectarivorous birds (Nicolson, 2006). Salts must be recovered from the large volumes of dilute urine, and measurements on the excreted fluid of both hummingbirds and sunbirds show this process to be highly efficient (Calder & Hiebert, 1983; Fleming & Nicolson, 2003). Another consequence of feeding on dilute nectar, especially at low ambient temperatures, is the energetic cost of warming large volumes of nectar to body temperature (Lotz et al., 2003).

The sugar and concentration preferences of nectar-feeding birds have attracted considerable interest, prompted initially by studies on mainly frugivorous passerine birds (reviewed by Martínez del Rio et al., 1992). For example, starlings (*Sturnus vulgaris*) strongly prefer hexose solutions when given a choice between isocaloric diets containing hexoses or sucrose, while in less frugivorous bird species the preference for hexoses is weaker; the physiological basis of these sugar preferences lies in the relative activity of the intestinal enzyme sucrase, which is completely absent in starlings (Martínez del Rio et al., 1988). Sucrose aversion, resulting from the loss of intestinal sucrase, appears to be restricted mainly to the frugivorous families of the large sturnid–muscicapid lineage of birds: the starlings, thrushes, and mockingbirds (Nicolson & Fleming, 2003b; Lotz & Schondube, 2006). Among other avian nectarivores, intestinal sucrase activity is ten times higher in hummingbirds than in a variety of passerine birds, including flowerpiercers (*Diglossa*), which are the most specialized nectar-feeding passerines in the Neotropics (Schondube & Martínez del Rio, 2004). Sucrase activity of a sunbird species, *Nectarinia osea*, has been found to be as high as that of hummingbirds (T.J. McWhorter & J.E. Schondube, unpublished data). Hexose absorption is another possible digestive constraint for specialized nectar feeders, but McWhorter et al. (2006) have recently shown that hummingbirds, like many passerine frugivores, rely substantially on paracellular uptake of hexose sugars, which suggests that sucrose hydrolysis is more likely to be limiting. As in honeybees (Gmeinbauer & Crailsheim, 1993) and the hawkmoths studied by O'Brien (1999), hummingbirds use newly ingested carbohydrate to fuel their hovering flight; another example of convergence between nectar-feeding animals (Welch et al., 2006).

It is now apparent that the sugar preferences of nectar-feeding birds are concentration-dependent (Lotz & Schondube, 2006). The sugar preferences

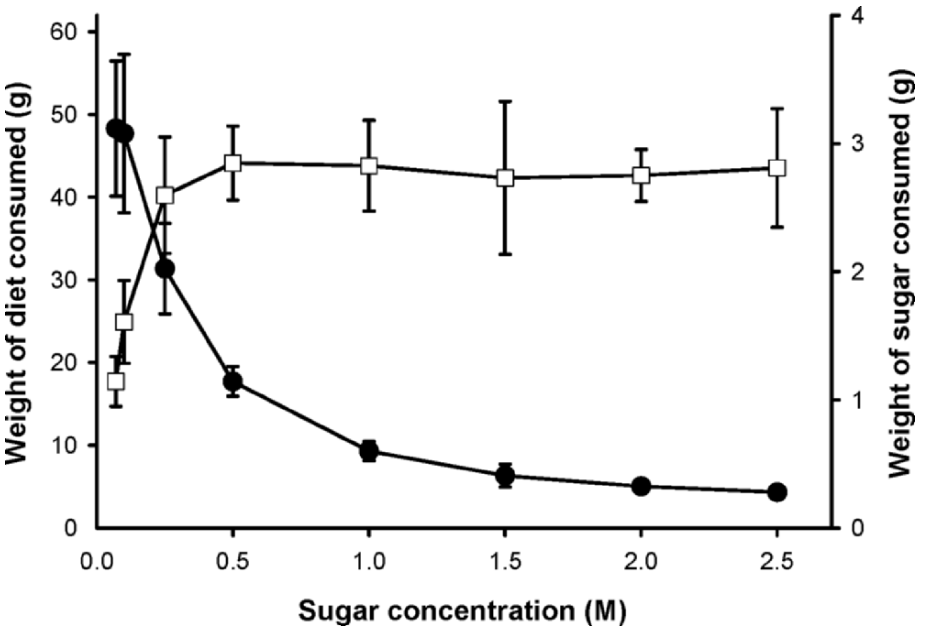

Figure 6. Compensatory feeding in the white-bellied sunbird, *Nectarinia talatala*, feeding on a broad range of sucrose concentrations. Values are means ± SE for volume consumption in ml/day (*open squares*) and sucrose consumption in g/day (*solid circles*). At sucrose concentrations below 0.25 M, the volume ingested is not sufficient to maintain energy balance. (Redrawn from Nicolson & Fleming, 2003a.)

of sunbirds (*Nectarinia talatala)* and hummingbirds (*Selasphorus platycercus*) have been compared across a range of sucrose and equicaloric hexose solutions (Fleming et al., 2004). Both species preferred hexoses when offered dilute diets (it may be difficult to hydrolyse sucrose under these conditions), but otherwise showed no significant preference. This contrasts with previous findings of sucrose preference in hummingbirds (Stiles, 1976; Martínez del Rio, 1990). Most trials with birds have used 20% w/w sugar solutions, and preferences can change when the nectars are more dilute (see also Schondube & Martínez del Rio, 2003). Moreover, when sucrose and hexose solutions of equal concentration are prepared on a % weight basis, the sucrose has a 5% higher energy content, so that apparent sucrose preference may in fact be a preference for the greater energy value of the sucrose solutions used. Hummingbirds and frugivorous tanagers are known to be remarkably sensitive to changes in sugar concentration: they can discriminate 1% differences at low concentrations (Blem et al., 2000) (Schaefer et al., 2003). Rufous hummingbirds prefer 65% sucrose over other solutions

when these are offered at realistically low volumes, resembling the volumes found in hummingbird flowers (Roberts, 1996). This is far greater than the average concentration of bird nectars (Pyke & Waser, 1981; Nicolson & Fleming, 2003b). Sunbirds fed high sucrose concentrations will drink supplementary water if it is available, diluting their food to around 30% (Nicolson & Fleming, 2003a). There is still no clear explanation for the differences between the birds' preferences in laboratory tests and the nectar properties of their food-flowers. Biophysical models of optimal nectar concentration for hummingbird feeding are briefly discussed by Nicolson and Thornburg (2007, Chapter 5 in this volume), and Table 3 shows that sucrose intake is fastest at concentrations of 30–45% for representatives of all three main nectarivore families.

So far I have considered only the water and sugar content of bird nectars. Amino acid levels in some passerine bird nectars (*Erythrina* and *Aloe*) are surprisingly high, sometimes exceeding 100 mM in total concentration (S.W. Nicolson in preparation), but white-bellied sunbirds (*Nectarinia talatala*) are generally indifferent to the inclusion of amino acids in their diets (C.D.C. Leseigneur and S.W. Nicolson, in preparation), confirming the findings of a much older study on hummingbirds (Hainsworth & Wolf, 1976). Despite low daily maintenance nitrogen requirements, nectarivorous birds are apparently unable to meet their nitrogen needs from the amino acids in nectar, and require insects or pollen as additional sources (Roxburgh & Pinshow, 2000; Van Tets & Nicolson, 2000). Pollen ingestion by birds is usually unintentional, except in the case of lorikeets, which harvest large quantities of pollen (Churchill & Christensen, 1970). Arthropod foraging by hummingbirds in Costa Rica was examined in detail by Stiles (1995). Although sunbirds feed on both nectar and arthropods, their young receive only arthropod prey. However, the rate at which female Palestine sunbirds (*Nectarinia osea*) provision their chicks increases in proportion to the sugar concentration available to the adults (Markman et al., 2002).

Another component of nectar chemistry has been shown to affect the feeding choices of *N. osea*: this is the alkaloid nicotine. It has a deterrent effect at the average levels measured in the nectar of *Nicotiana glauca* (Solanaceae) (Tadmor-Melamed et al., 2004); this is, however, an invasive plant in Israel and the responses of the plant's native hummingbird pollinators to nicotine are not known. Little is known about the effect of secondary compounds in nectar on foraging by avian nectarivores. Nectar of the South African *Aloe vryheidensis* is dark, with a bitter taste due to phenolic compounds, and attracts larger birds such as bulbuls and white-eyes, which are more effective pollinators than sunbirds (Johnson et al., 2006).

4.3 Bats

Nectarivorous bats occur mainly in the tropics and subtropics in two phylogenetically distant families; the Neotropical Phyllostomidae, derived from insectivorous Microchiroptera that radiated into flower-visiting niches, and the larger and generally less specialized fruit bats or Pteropodidae (Megachiroptera), which are restricted to Palaeotropical regions (Winter & von Helverson, 2001). They feed on similar nectars to birds, sometimes sharing flowers. The most specialized nectarivorous bats are small, long-tongued bats of the phyllostomid subfamily Glossophaginae: like hummingbirds, they hover to feed and need space for wing movement directly in front of flowers (Westerkamp, 1990). There are often no barriers between bird and bat pollination: examples are hummingbirds and phyllostomid bats pollinating *Burmeistera tenuiflora* (Campanulaceae) in Costa Rica (Muchhala, 2003), and sunbirds and fruit bats pollinating *Musa itinerans* (Musaceae) in southwestern China (Liu et al., 2002).

Depending on pollinator size, nightly nectar production by bat flowers varies from 100 μl to several millilitres; the concentration is typically around 15% w/w and the nectar, like that of many passerine bird flowers, is dominated by hexose sugars (Baker et al., 1998; Winter & von Helverson, 2001; Wolff, 2006 and references therein). It has been suggested that high nocturnal humidities probably help to keep bat nectars dilute (Búrquez & Corbet, 1998). Schondube et al. (2001) demonstrated increased intestinal sucrase activity in nectar- and fruit-feeding phyllostomid bats compared to insectivores, although this seems unnecessary for digestion of hexose nectars. The characteristic sugar composition of bat nectars is not a result of bats preferring hexoses to sucrose or digesting hexoses more efficiently (Herrera, 1999). Correlated with the water loading due to dilute nectars, the shift from insectivory to nectarivory in phyllostomid bats has also been accompanied by a decrease in renal concentrating ability (Schondube et al., 2001). Energy acquisition by phyllostomid bats has been studied under both laboratory and field conditions (von Helversen & Reyer, 1984; Winter & von Helversen, 1998, 2001). Because the nectar on which they feed is so dilute, these bats consume about 150% of their body mass in a night's foraging, visiting individual flowers repeatedly during the night and covering long distances. In captivity, this involves alternating between different feeders in indoor flight enclosures or wind tunnels, behaviour which has been very helpful for studies of their flight costs and aerodynamics (Winter & von Helversen, 1998). Hovering is apparently less expensive for both bats and hummingbirds than has been commonly assumed (Winter & von Helversen, 2001).

Bats are highly mobile pollinators and include migratory species that track nectar resources. Best known for this is the Mexican bat *Leptonycteris curasoae*, which migrates north, feeding on the nectar of columnar cacti, then returns south feeding on nectar of *Agave* species. Its seasonal movement along a nectar corridor of successively flowering plants in the families Cactaceae and Agavaceae was demonstrated by Fleming et al. (1993) using stable isotope analysis. During peak blooming, the nectar supplied by columnar cacti in the Sonoran Desert is 3–4 times greater than the energy required by nectar-feeding bats and birds, which led Fleming et al. (2001) to suggest that pollinators are limited, a situation which will favour pollinator generalization.

The Queensland blossom bat *Syconycteris australis* is a small megachiropteran (18 g) that is able to meet its energy and nitrogen requirements on a diet of nectar and pollen only (Law, 1992). Blossom bats and honeyeaters both drink the remarkably dilute (3–10%) nectar in the brush flowers of the rainforest tree *Syzygium cormiflorum* (Myrtaceae). Although they make fewer and briefer visits to these flowers, the bats are more mobile, carry more pollen, and appear to be more effective pollinators (Crome & Irvine, 1986; Law & Lean, 1999). The importance of energy (rather than nitrogen) as a limiting resource for *Syconycteris australis* was confirmed with an energy supplementation experiment that led to an increase in population size (Law, 1995). It seems more common that nitrogen is a limiting resource for nectar- or fruit-feeding vertebrates, and Thomas (1984) suggested that two frugivorous megachiropteran bats might metabolize excess carbon in their diet by increasing their flight activity.

4.4 Other mammals

Opportunistic and sometimes destructive feeding on nectar or entire flowers has been observed in many non-flying mammals with diverse diets, including rodents, marsupials, and primates (Janson et al., 1981), and even giraffes (Fleming et al., 2006). The ingestion of nectar may be incidental or deliberate. Two *Eulemur* species studied by Overdorff (1992) in Madagascar treat the same flowers differently, rufous lemurs consuming entire flowers and red-bellied lemurs licking the nectar without damaging the flowers; only the latter were considered to be potential pollinators. Nectar may sustain frugivorous primates during times of food scarcity (e.g., Gautier-Hion & Maisels, 1994) and may also be a water resource. The very dilute nectar (~10%) of *Aloe marlothii* (Asphodelaceae) can be highly desirable to baboons, *Papio hamadryas,* because it flowers during dry winters in South Africa (C.T. Symes and S.W. Nicolson, unpublished). Other plant exudates are less

seasonally variable and can also be important in mammal diets; examples are the sap and polysaccharide gums utilized by vervet monkeys (Wrangham & Waterman, 1981) and marsupial sugar gliders (Smith, 1982).

The review by Carthew and Goldingay (1997) cites 59 non-flying mammal species known to visit flowers regularly for nectar or pollen. The interaction is best known from the southern continents, especially Australia, where many marsupial species such as pygmy possums, sugar gliders, and the honey possum (Armstrong, 1979) regularly visit the generalized brush flowers of *Eucalyptus* (Myrtaceae) and *Banksia* species (Proteaceae)—also visited by birds and insects—and flower products are often an important dietary component for the animals. Rodent pollination of *Protea* species (also Proteaceae) occurs in similar ecosystems in South Africa, where the relationship between rodents and *Protea* species has been described as non–co-evolved (Wiens et al., 1983). This opinion was based on the brief flowering seasons and limited plant distributions, as well as the contrast between the morphologically specialized plants and generalist mammals. Primates may be observed easily, but small, nocturnal pollinators are difficult to study, and unfortunately there is often only circumstantial evidence for their role in pollination (Carthew & Goldingay, 1997). Based on more quantitative evidence, Fleming and Nicolson (2002) concluded that small mammals are responsible for about half of the effective seed set in *Protea humiflora*, and that its nectar and pollen are a significant nutritional resource, although limited in time and space.

The marsupial honey possum, *Tarsipes rostratus*, weighs about 9 g and is unique in being the only terrestrial mammal to feed exclusively on nectar and pollen. In southwestern Australia, isotopic turnover studies in free-living honey possums have shown that they are able to maintain energy balance on daily intakes of 7 ml of *Banksia* nectar and 1 g of pollen (Bradshaw & Bradshaw, 1999). The resulting nitrogen intake far exceeds the low nitrogen requirements of honey possums measured in the laboratory (Bradshaw & Bradshaw, 2001), so their diet does not appear to be deficient in protein.

5 WHAT HAPPENS TO NECTAR DURING POLLINATOR SHIFTS?

Adaptive radiation within plant genera is often attributed to shifts between pollination systems, and these can be linked to the energetic relationships between flowers and pollinators. For example, Raven (1979) examined pollination systems in the Onagraceae, concluding that shifts to higher reward

systems have occurred eleven times, and shifts to lower reward systems six times. The latter changes involve the loss of hummingbird pollination in a few specialized species of *Fuchsia*, and its replacement by bee and fly pollination. Pollination shifts may even involve the production of nectar in an otherwise nectarless genus, such as *Disa* (Orchidaceae), in which floral nectar is primitively absent but has evolved in species belonging to three different clades and pollinated by diverse insect taxa (Johnson et al., 1998). Of the floral traits involved in pollinator shifts, colour, and morphology have been investigated more frequently than reward and scent (see the review of Fenster et al., 2004). Earlier in this volume we looked at the question of whether nectar sugar composition is determined by pollinator type or plant phylogeny (Nicolson & Thornburg, 2007, Chapter 5 in this volume). Here I discuss some comparative studies where species in the same genus have different pollinators and where multiple nectar traits have been examined, not just sugar composition.

Southern Africa is the centre of diversity of the monocot family Iridaceae and pollination systems in the African Iridaceae tend to be specialized (Goldblatt & Manning, 2006). The large genus *Gladiolus*, with 165 species in southern Africa, has radiated widely as a direct consequence of specialization for diverse pollinators (Goldblatt et al., 2001). Long-tongued anthophorid bees are the ancestral pollinators of *Gladiolus* and the most important, but there have been multiple shifts to diverse other pollinators: long-proboscid flies (Nemestrinidae, Tabanidae), hopliine beetles (Scarabaeidae), a satyrid butterfly, moths (Noctuidae and Sphingidae), and sunbirds. Nectar properties have been measured in many *Gladiolus* species, and while volume and concentration tend to change with pollinator type (although substantial overlap remains), sugar composition is a more conservative character (Goldblatt et al., 2001). This is clearly illustrated by 20 bird-pollinated species that have originated in five out of seven sections of the genus (Goldblatt et al., 1999). *Gladiolus* is primarily insect-pollinated and its nectar is consistently high in sucrose, even in most of the bird-pollinated species: only one lineage of three species has hexose-based nectar (see also Nicolson, 2002). Most of the sunbird-pollinated species of Iridaceae retain high sucrose nectars, with the exception of three genera with elevated hexose levels (*Chasmanthe*, *Klattia,* and *Witsenia*) (Goldblatt et al., 1999).

The example of *Gladiolus* suggests that adding water may be enough to convert a bee nectar into a bird nectar (together with increased floral size). That is, the plant invests a similar amount of sugar but packages it in more or less water. For example, Kaczorowski et al. (2005) studied the variation of several floral traits with pollinators in *Nicotiana* species, all but one with high

sucrose nectars, and found that total energy is relatively stable because nectar volume and concentration vary inversely. As pointed out by Mitchell and Paton (1990), who used "equal volume" and "equal sugar" presentations to measure the nectar intake rates of honeyeaters, this is biologically more realistic than investing different amounts of sugar in the same volume of nectar. Increasing the water component of the nectar could explain the widespread shifts from insect to bird pollination in the flora of western North America (Grant, 1993), as discussed by Nicolson and Thornburg (2007, Chapter 5 in this volume).

The large genus *Penstemon* (Scrophulariaceae), with 270 species, is an excellent example because hummingbird pollination has evolved repeatedly from bee pollination (Wilson et al., 2006). Thomson et al. (2000) listed systematic rules that contrast ornithophily to melittophily in *Penstemon*, and compared pairs of closely related bird and bee species: nectar volumes were invariably higher in the bird-visited species, and nectar concentrations were usually lower. Colour and morphological characters were also clearly distinct in the two categories. Using another pair of closely related species of *Penstemon*, Castellanos et al. (2002) showed that nectar refilling rates were much faster in hummingbird-pollinated *P. barbatus* than in bee-pollinated *P. strictus*. Since the latter species produces smaller volumes of more concentrated nectar (Wilson et al., 2006), the differences in refilling rates are likely to be due to differences in water transport in the nectary. Changes in nectar volume and concentration are predicted by Wilson et al. (2006) to come first during evolutionary shifts from bee to hummingbird pollination in *Penstemon*, preceding changes in nectar sugar composition or in other floral characters such as colour or size. They suggest that birds care more about nectar, but bees are choosier about colour, given that all species of *Penstemon* produce enough nectar to interest bees.

However, for a shift to passerine or bat pollination, which is associated with the most copious and dilute floral nectars, hydrolysis of the nectar sucrose to glucose and fructose seems to be required. This maintains the gradient for sucrose transport, and the increased osmolality draws additional water from the nectary and dilutes the nectar (Nicolson, 1998, 2002). The nectar of two bat-pollinated species of Sinningieae (Gesneriaceae), which have evolved independently, is much more copious, dilute, and rich in hexoses than that of related species pollinated by moths, bees, or hummingbirds (Perret et al., 2001). Examples are more numerous for passerine pollination. The classic case of *Erythrina*, in which nectar concentration and nectar chemistry are highly labile and associated with repeated shifts from passerine to hummingbird pollination, is discussed by Nicolson and Thornburg (2007,

Chapter 5 in this volume) (but note that in this case the shift is from hexose to sucrose nectars). In the Canary Islands, sunbirds are not present but apparently ornithophilous flowers are now visited by several opportunistic nectar-feeding passerine birds such as warblers. Here Dupont et al. (2004) found that phylogenetically related plants with different pollinators differed in sugar composition, with hexose nectars being associated with bird visitation. Sugars may also have evolved readily in a few passerine-pollinated species of *Salvia* (Lamiaceae) in Africa. This genus, known for its classic "bee" flowers, has a worldwide distribution, and about a quarter of the approximately 900 species have become ornithophilous (Wester & Claßen-Bockhoff, 2006). Most of these are Neotropical and have shifted to hummingbird pollination, while retaining high sucrose nectars; Schwerdtfeger (1996) recorded 69.9% nectar sugar as sucrose in 19 bee-pollinated species of *Salvia*, and 77.7% sucrose in seven hummingbird-pollinated species. South Africa has 23 species of *Salvia*, and bird pollination has evolved in three species with large reddish flowers and dilute nectars (Wester & Claßen-Bockhoff, 2006). Nectar sugar composition has been analysed in two of them (*S. lanceolata, S. africana-lutea*) and is predominantly hexose (B-E van Wyk, unpublished data). Pollination shifts in *Salvia* from the original condition of bee pollination seem to involve adding water to the nectar for hummingbirds, but hydrolysing sucrose to hexoses for the passerine-pollinated species.

A volume increase without dilution of the nectar has occurred in ginger species (Zingiberaceae and Costaceae) flowering on the forest floor in Borneo and grouped by Sakai et al. (1999) into three pollination guilds. The nectar sugar concentration averaged 26–29%, but the daily sugar production per inflorescence was 24 and 60 times higher for species pollinated by spiderhunters (Nectariniidae) than for those pollinated by anthophorid or halictid bees, respectively. The sugar composition of nectar of these ginger flowers is unknown; it would be interesting to know whether the elevated nectar volumes required for spiderhunters are hexose-based.

In order to understand the genetic basis of pollinator shifts, it is necessary to study single floral traits in isolation, preferably using plant species for which the appropriate molecular tools are available (Galliot et al., 2006). The few studies of heritability in nectar traits concern mainly nectar production rate, which may show more plasticity than nectar chemistry (Mitchell, 2004). Changes in nectar may not always be involved in transitions between pollination systems. Schemske and Bradshaw (1999) crossed two sister species of *Mimulus* (Scrophulariaceae), hummingbird-pollinated *M. cardinalis* and bee-pollinated *M. lewisii*, and used quantitative trait locus (QTL) mapping

and field trials with hybrid plants to show that an allele that increases nectar production doubled hummingbird visitation. However, a single mutation from violet to red flower colour was later shown to be sufficient for the shift from bee pollination to hummingbird pollination, regardless of differences in nectar production (Bradshaw & Schemske, 2003); see also Beardsley et al. (2003).

More genetic tools are available for the genus *Petunia* (Solanaceae), which has distinct bee, hawkmoth, and bird pollination syndromes (Galliot et al., 2006). Using separate crosses of hawkmoth-pollinated *P. axillaris* and bee-pollinated *P. integrifolia* in a defined genetic background, Stuurman et al. (2004) analysed phenotypic and genetic differences in colour, shape, nectar reward, and scent. There are striking differences in nectar volume and concentration, showing that part of the volume difference is in relative water contributions to the nectar, and in sugar composition. Nectar volume is controlled by two QTLs, one that affects volume pleiotropically by altering flower size and another that affects nectary physiology. Their additive effects account for almost the entire difference in nectar volume between the two species. No significant QTL was detected for nectar concentration, but a single QTL decreased the proportion of sucrose, which is consistent with the activity of an invertase. In *Petunia*, volume is thus not genetically correlated with sugar composition. The gene *Nec1* is highly expressed in the nectaries of *Petunia hybrida* during active secretion of nectar (Ge et al., 2000), and future molecular analyses should provide insights into the genetic basis of nectar secretion (see also Thornburg, 2007, Chapter 6 in this volume).

6 CONCLUSION

The sugar composition of nectar may seldom be physiologically important to nectar consumers, since almost all animals are able to digest sucrose. Exceptions include certain ants feeding on extrafloral nectar of *Acacia* species (Heil et al., 2005) and frugivorous birds of the sturnid–muscicapid lineage (Martínez del Rio et al., 1992; Lotz & Schondube, 2006); both groups lack gut sucrase and thus prefer hexose nectars. In terms of nectar concentration, the concentrations leading to the highest rates of sugar intake (Table 3) are actually very similar for various suction feeders (moths, butterflies, and orchid bees), as a result of common biophysical mechanisms (Kingsolver & Daniel, 1995). In animals that lick nectar, the measured optimal concentrations are higher for bees and bats, although less so for nectar-feeding ants or birds. For all taxa, it is a paradox that these concentrations are consistently higher than those of the nectars that the animals normally consume. The

greatest discrepancy appears to be between the 60% optimum recorded for glossophagine bats and the low average nectar concentration measured for bat flowers (Roces et al., 1993). However, the same study showed that under laboratory conditions these bats drink free water, especially when rehydrating at the beginning of the night, and many pollinators may have high water requirements. Even bumblebees, which usually maintain water balance on very concentrated nectars, have been observed collecting water in warm weather (Ferry & Corbet, 1996).

The ability of nectar consumers to cope with nectars of varying concentration and composition is an advantage when nectar varies greatly in quality and quantity and when its consumers must compete for limited resources. The physiological adaptability of nectar consumers is also compatible with the concept of widespread generalization in many pollination systems (Waser & Ollerton, 2006). Differentiation is more likely to be determined by secondary compounds in nectar that attract or deter particular consumers.

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Chapter 8

ECOLOGICAL AND EVOLUTIONARY ASPECTS OF FLORAL NECTARS IN MEDITERRANEAN HABITATS

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1 NECTAR SECRETION IN MEDITERRANEAN HABITATS

Owing to its superb sweet taste, nectar has, since antiquity, been considered the drink of the gods, which underlines its importance as a major daily allurement for insects to visit flowers. This is because nectar is the major source of energy to pollinators, providing them with sugars, other nutritious substances such as amino acids, and possibly minerals. Furthermore, nectar generally constitutes the only form of water intake for such pollinators.

Unlike pollen, the other prime reward for pollinators, nectar functions solely as a reward and secretion can continue after its removal (Proctor et al., 1996). Even if the magnitude of its importance has been questioned for some areas like the Mediterranean (Herrera, 1985; Petanidou & Vokou, 1990; Petanidou & Lamborn, 2005), nectar still constitutes an irreplaceable and unique attractant for pollinators within flowering plant communities (Proctor et al., 1996), and as such is likely to be subject to selection pressures imposed by pollinators (Petanidou, 2005; Petanidou et al., 2006). Consequently, nectar may differ greatly among phylogenetically related plants pollinated by different animals (Pyke & Waser, 1981; Baker & Baker, 1982), even though genetic (Percival, 1961; Baker & Baker, 1983) and ecological constraints (Corbet, 1990; Petanidou & Smets, 1996; Petanidou et al., 1999, 2000;

S.W. Nicolson, M. Nepi, and E. Pacini (eds.), Nectaries and Nectar, 343–375.

Petanidou, 2005) may serve to limit the role of selection in shaping nectar characteristics.

The main nectar characteristics are volume, concentration, and sugar content, odour, colour, and taste, which may relate to the concentration and composition of dissolved sugar and non-sugar solids, such as amino acids, minerals, or phenolics (Thorp et al., 1975; Baker & Baker, 1983; Olesen et al., 1998; Adler, 2000a; Raguso, 2004; Petanidou, 2005; Petanidou et al., 2006). Of all nectar traits, the one that has received the most attention is quantity (volume, also in combination with sugar concentration), probably because of the ease of obtaining measurements (see Zimmerman, 1988 and Corbet, 2003 for reviews). In addition, many studies have focused on the qualitative aspect of nectars, with special attention given to the evolutionary and ecological significance of the two major components, sugars and amino acids (for reviews, see Petanidou, 2005; Petanidou et al., 2006; Nicolson & Thornburg 2007, Chapter 5 in this volume).

As nectar is an aqueous solution, its secretion depends largely on water availability. This implies that in areas with extreme water deficits, such as desert and other arid climate regions, plants may face major costs in secreting nectar. In the Mediterranean region, largely influenced by an extensive summer drought, plants are expected to face severe water stress when flowering towards the onset of, or during, the dry season. This drought regime may strongly affect secretion and other nectar attributes, and could select against nectar being produced as the sole reward (Herrera, 1985; Petanidou & Vokou, 1990). Scrutinizing previous literature, Petanidou and Lamborn (2005) discussed evidence for the importance of pollen versus nectar in Mediterranean pollination systems, which is supported by the low number of butterflies as exclusive nectar consumers (Petanidou & Ellis, 1993), the high abundance of typically low-nectar-producing species (Petanidou & Smets, 1995) and the high numbers of nectarless deceptive orchids (Dafni & Bernhardt, 1990; Dafni & O'Toole, 1994) found in this region.

In this chapter, I examine the factors that may shape nectar characteristics—such as quantity (volume) and quality (sugar and amino acid composition and concentration)—in Mediterranean habitats. These factors may be ecological (abiotic and biotic), phylogenetic, or co-evolutionary, with different pollinator guilds imposing selection. I address (i) the relative importance of the above factors in shaping nectar secretion, (ii) whether nectar is important in enhancing pollinator/bee diversity in Mediterranean communities, and (iii) whether Mediterranean communities differ from other plant communities in nectar composition. I conclude by focusing on the

importance of Mediterranean areas for bee conservation, with results drawn from this study, as well as considering the potential impact of human management on these communities, particularly the major managerial issues of invasive species, beekeeping, and bumblebee-assisted crop pollination in greenhouses.

Most of the data presented in this review are drawn from studies carried out in Mediterranean habitats, especially East Mediterranean scrub, and in particular from a 30 ha *phrygana* community at Daphni, 10 km west of the city of Athens, Greece (see Petanidou & Ellis, 1993, 1996 for site description). Most of the data used have been published elsewhere, although some new conclusions are proposed based on unpublished data sets.

2 CHARACTERISTICS OF MEDITERRANEAN NECTARS

2.1 Nectar constituents of Mediterranean nectars

In general, floral nectars are mixtures of natural products consisting primarily of carbohydrates (mono-, di-, and oligosaccharides) accompanied by a wide variety of minor components, such as amino acids, proteins, enzymes, lipids, phenolics, glycosides, salts, alkaloids, vitamins, and other organic acids, and minor compounds (Lüttge, 1977; Baker & Baker, 1982, 1983; Kearns & Inouye, 1993; Adler, 2000a; Dafni et al., 2005). Floral nectars in the Mediterranean do not differ from these norms in term of composition.

2.1.1 Sugars

Sucrose, glucose, and fructose are the "big three" sugars most commonly found in nectar (Percival, 1961; Baker & Baker, 1983; Dafni et al., 1988; see also Nicolson & Thornburg, 2007, Chapter 5 in this volume). Minor sugars, such as sorbitol, melibiose, maltose, and mannitol are usually also present (Baker & Baker, 1983). This also applies to Mediterranean nectars (Petanidou, 2005).

Based on HPLC analyses of the nectars of 73 plant species, found in *phrygana* at Daphni, sucrose was present at an average of 702.5 ± 234.2 nmoles/flower, glucose at 869.4 ± 415.9 nmoles/flower, and fructose at 905.9 ± 412.0 nmoles/flower (Petanidou, 2005). In addition to these three sugars, traces of minor sugars were also detected, such as sorbitol (9 species); melibiose (7 species); maltose and mannitol (4 species each); ribose,

mannose, and stachyose (2 species each); and arabinose, lactose, and trehalose (1 species each) (Petanidou, 2005).

Considering only the three main sugars, this phryganic community can be divided into species with "high sucrose" floral nectars (sucrose-dominant to sucrose-rich, according to the terminology of Baker and Baker (1983), which uses the sucrose/hexose ratio as a discriminating value) and "low sucrose" (hexose-dominant to hexose-rich) nectars (Petanidou, 2005). This does not imply that sugar composition of nectar is constant throughout the flower lifespan. Petanidou et al. (1996) showed that in *Capparis spinosa,* flower aging had an irreversible effect on nectar quality, which was expressed as a continuous decrease of the nectar sucrose/hexose ratio (as a result of sucrose breakdown) while the glucose/fructose ratio remained practically constant (approximately 1). The authors also concluded that the rate of sucrose breakdown was regulated (slowed down) by high sugar concentration, which implies a high invertase activity in dilute nectars versus low activity in concentrated nectars (see also Pate et al., 1985). This is very important from the nectar conservation point of view in regions with high temperatures such as the Mediterranean.

Just over half the species in Mediterranean communities have high-sucrose nectars (53.5% of the species according to Petanidou, 2005). Furthermore, species with high-sucrose nectars have the propensity to flower in spring and summer (60.8% of the species flowering then have high-sucrose nectars), whereas high-hexose nectars occur in winter flowers (63.6%) (Petanidou, 2005). The selection of high-sucrose against high-hexose nectars can be partly explained as a result of the drought constraint in the Mediterranean area, because high-hexose nectars consume more water than nectars with high concentrations of sucrose for the same amount (weight) of sugars contained, i.e., for the same sugar content (see discussion under "Water stress" on page 355; cf. Nicolson, 1998, 2002). It is unknown whether this is a specific characteristic of the Mediterranean region as no comparable data are available for other world communities.

2.1.2 Amino acids

Several amino acids have been found in floral nectars, all in much lower quantities than sugars (Baker & Baker, 1978, 1982, 1986; Gottsberger et al., 1984; Gardener & Gillman, 2001b; Chapter 5 in this volume). In the phryganic nectars, 22 amino acid compounds or groups of compounds have been detected (Petanidou et al., 1996, 2006). Cysteine and proline (including hydroxyproline) were not detected in phryganic nectars, owing to the analytical methods

Table 1. Amino acids detected in the floral nectars of *phrygana* using HPLC analysis. Values for particular amino acids, calculated from the data given in Petanidou et al. (2006), are averages over all plant species in the community (n = 73, excluding *Thymelaea hirsuta* and *Crocus cancellatus* with possible nectar contamination by pollen). Three different amino acid compounds are lumped together under "unknown". "Total amino acids" is the sum of all amino acids in the nectar. Amino acids that were not commonly detected in the nectars are flagged with * (found in less than 70% of the study species) and ** (in less than 10% of the species).

Amino acids	Mean quantity (pmoles/flower)	SE	% of total amino acids
Arginine	78	17.4	2.8
Asparagine	152	43.8	5.6
Aspartic acid	234	140.0	8.6
Glutamic acid	66	16.7	2.4
Glycine + threonine	218	35.3	8.0
Histidine + glutamine	231	61.6	8.5
Isoleucine	33	6.6	1.2
Leucine	52	10.1	1.9
Lysine	68	11.9	2.5
Methionine*	55	23.8	2.0
Ornithine	101	17.5	3.7
Phenylalanine	715	229.5	26.2
Serine	166	26.2	6.1
Tryptophan	43	11.2	1.6
Tyrosine + alanine	250	40.2	9.2
Unknown*	71	21.5	2.6
Valine	119	18.7	4.4
H-serine**	2	2.1	0.1
β-Alanine**	3	1.9	0.1
GABA (γ-aminobutyric acid)*	75	24.6	2.7
Total amino acids	*2,731*	*469.1*	*100.0*

used by the authors. (These amino acids are relatively common in some of the nectars originating from areas outside the Mediterranean: Baker & Baker, 1978; Gottsberger et al., 1984; Gardener & Gillman, 2001b). The same holds for taurine and AABA (i.e., α-aminobutyric acid), both found in the English nectars analysed by Gardener and Gillman (2001b). Of all the amino acids detected in the nectars of *phrygana*, 15 were common to nearly all nectars of the 73 species tested (Table 1, Petanidou et al., 2006). The authors compared their data set to those given by Gardener and Gillman

(2001b) and found that in general, amino acid concentration appears to be much higher in *phrygana* than in temperate systems (Petanidou et al., 2006).

Among all amino acids detected in the nectars of *phrygana*, the most prevalent was phenylalanine, both in absolute content and in concentration (Petanidou et al., 2006). Within all plant taxonomic groups, the most phenylalanine-rich nectar was that of Lamiaceae, with an average phenylalanine content of 47.2% of the total amino acids detected in all the species nectars of the family. Almost all phenylalanine-rich plants were keystone species of *phrygana* including *Stachys cretica*, *Phlomis fruticosa*, *Satureja thymbra*, *Urginea maritima*, *Asphodelus aestivus*, and *Thapsia garganica*. In a similar study carried out in an Israeli *batha* (i.e., a habitat that is equivalent to the Greek *phrygana*) Dafni et al. (1988) also found extremely high proportions of phenylalanine in the nectar of *Satureja thymbra* and *Salvia fruticosa* (71% and 52%, respectively). The high proportion of phenylalanine therefore seems to be characteristic of the phryganic plants in the Mediterranean region, as this amino acid was not detected at high levels in the nectars of other species from temperate and tropical systems (Baker & Baker, 1978, 1982, 1986; Gardener & Gillman, 2001b).

Until the early commencement of flower senescence, most if not all of the amino acids in nectar originate from phloem sap (Fahn, 1988). After the beginning of senescence, amino acids increasingly result from nectary breakdown (Petanidou et al., 1996). This type of proteolytic breakdown may be limited by sugar concentration, as found in the nectar of the Mediterranean species *Capparis spinosa* (Eisikowitch et al., 1986; Petanidou et al., 1996), which implies that sometimes nectaries may restrict amino acid flow through the nectar.

2.1.3 Minerals in floral nectars

Nectars have been found to contain K, P, Mg, Na, S, Ca, and many other minerals, with potassium prevailing in most cases (Waller et al., 1972; Kearns & Inouye, 1993; Liu et al., 2004; Dafni et al., 2005). No studies on mineral content have been performed specifically on nectars from the Mediterranean, but it is entirely probable that similar contents and concentrations are found in this region.

2.1.4 Secondary compounds

Apart from the main ingredients of sugars and amino acids, nectars often contain specific constituents or secondary compounds that may affect the

attractiveness of nectar to pollinators and could therefore play a significant role in the pollination process. Phenolic compounds, for instance, may positively contribute to the taste of nectar at very low concentrations (Baker, 1977), while at other times—especially in higher quantities—they may repel honeybees (Adler, 2000a; Hagler & Buchmann, 1993).

In the Mediterranean, several secondary compounds have been identified in the nectars of plants associated mostly with honey-making. Such compounds include grayanotoxins (in the nectar of *Rhododendron luteum*; Buys, 2000), flavonoids (e.g., kaempferol, in the nectar of rosemary *Rosmarinus officinalis*; Ferreres et al., 1998), and glycosides (e.g., amygdalin, a cyanoglycoside found in the nectar of the almond *Amygdalus communis*; London-Shafir et al., 2003; and arbutin in the nectar of the strawberry tree *Arbutus unedo*; Pryce-Jones, 1944). The presence of such substances makes nectar either toxic (e.g., in *Rhododendron luteum* and *Amygdalus communis*) or at least repellent to some visitors. The evolutionary significance of such toxic nectars remains, to a major extent, unknown.

2.1.5 Nectar viscosity

Another characteristic of Mediterranean nectars that might be related to the presence of secondary compounds is viscosity. Nectar viscosity is mainly related to sugar concentration, which is high in the region (Kearns & Inouye, 1993; Petanidou & Smets, 1995). It may also result from rapid evaporation of the exposed nectars of many species—especially those with open flowers, e.g., *Urginea maritima*, *Thapsia garganica*, *Euphorbia acanthothamnos*, and *Ruta graveolens* (Dafni & Dukas, 1986; Petanidou & Smets, 1995). Yet, the viscosity of nectar may also be due to the presence of pectic substances as a result of post-secretory hydrolytic phenomena (Saeed et al., 1975); the presence of polysaccharides may also contribute to high nectar viscosity (Josens & Farina, 2001; Dafni et al., 2005).

An interesting case of nectar viscosity has been detected in the nectar of the *phrygana* species *Phlomis fruticosa* (Petanidou, 1991; Petanidou & Smets, 1995). Repeated observations over time showed that two types of flowers appeared in a patchy distribution on the same and over several individual plants: one with viscous and another with non-viscous nectar (Petanidou, unpublished data). Interestingly, these flower types did not differ in sugar concentration measured by HPLC analysis, but flowers with viscous nectars had a significantly higher sucrose/hexose ratio and higher total amino acid content (Table 2), implying that viscosity was caused by proteolytic phenomena resulting in an amino acid excess in these nectars. A more focused

glance at the data showed that among all the amino acids and amino acid compounds detected, GABA was the only amino acid with an extremely high contribution in non-viscous versus viscous nectars (decreasing by 99% in the latter). Other amino acids (valine, phenylalanine, methionine, tryptophan, arginine, alanine + tyrosine) had much higher contributions in viscous versus non-viscous nectars, with valine showing the highest increase in viscous nectars (169%). Because GABA is an amino acid absolutely dependent on the presence of common salt (NaCl) (Keynan & Kanner, 1988; Wolfersberger, 2000), its higher content in non-viscous nectars may indicate that these nectars are additionally protected against an early breakdown by their higher NaCl content.

The evolutionary significance of viscosity as a nectar characteristic is as yet unknown, but it can be presumed that higher viscosity—if mostly due to protein hydrolysis—assists in the preservation of nectar attributes by contributing to the slowing down of disaccharide breakdown (cf. Table 2). In this way the large and long-lasting flowers of *Phlomis fruticosa* may preserve their high-sucrose nectar throughout anthesis while waiting for their relatively infrequent pollination partners, viz. long-tongued specialist bees (Petanidou, 1991; Petanidou et al., 1995). By limiting sucrose breakdown, higher viscosity favours water economy in the plant, as no excessive water is consumed to keep nectar concentrations stable in case of sucrose hydrolysis (Nicolson, 1998; Petanidou, 2005). The presence of two different types of nectar in the flowers of the same individual and within the same population of *Phlomis fruticosa*, i.e., a non-viscous type protected by the presence of GABA and NaCl, and a viscous type as a result of protein hydrolysis, highlights the importance of nectar preservation under the harsh Mediterranean conditions, an issue that undoubtedly needs further investigation.

2.2 Issues of nectar quantity and quality

In general, nectar secretion (quantity) at community level is lower in the Mediterranean compared to other regions. Cruden et al. (1983) found an average of 2.10 ± 0.67 µl nectar volume produced per flower of exclusively bee-visited species (n = 12) in the southwestern United States. In tropical systems, Opler (1983) distinguished between highly rewarding, large bee-pollinated species producing 9.75 ± 4.350 µl of nectar (n = 19), and low rewarding, small bee/wasp-pollinated species secreting only 0.63 ± 0.182 µl (n = 14) of nectar per flower. In contrast, per flower nectar yield in the Mediterranean is always low: in a Spanish *garrigue* community, Herrera (1985) could not ascertain the presence of nectar in 41% of the species studied (n = 122), concluding that in total only 35% of the species could be considered

Table 2. Different nectar attributes of viscous and non-viscous nectars of 1-day flowers of *Phlomis fruticosa* collected on 28–29 April 1992 in the phryganic habitat of Daphni, Athens. Values are averages ± SE followed by the results of statistical analyses (Mann-Whitney U tests).

Nectar attributes	Viscous nectar	Non-viscous nectar	M-W U test
Total content (nmole/flower)			
Amino acids	18.7 ± 1.75	5.22 ± 0.82	$U_{(8,16)} = 2$, $P < 0.001$
Sugars	6,880 ± 3,354.4	3,834 ± 2,504.2	$U_{(8,10)} = 20$, $P > 0.05$
Sucrose/hexose ratio			
S/(G+F) (in weight)	4.7 ± 0.72	3.3 ± 0.62	$U_{(8,10)} = 6$, $P < 0.01$
S/(G+F) (in moles)	2.5 ± 0.38	1.7 ± 0.32	$U_{(8,10)} = 6$, $P < 0.01$

nectariferous. In the Daphni *phrygana* community Petanidou (1991) found that only 12.4% of the species were nectarless (n = 133), but from the rest only 13.5% produced considerable quantities of nectar. In a more detailed study within the same *phrygana,* Petanidou and Smets (1995) and Petanidou (2005) found an average nectar secretion of 0.64 ± 0.246 μl per flower (n = 76 species). However, this substantial average was due to only a few abundantly nectar-secreting species. When three of the abundant nectar producers were removed, average secretion dropped by almost one third.

Nectar concentration (quality) in Mediterranean habitats is generally higher than in temperate communities (Beutler, 1930, 1953a, b; Cruden et al., 1983). Beutler (1930) found that the concentration of flower nectars of 18 species visited by honeybees ranged from 10–70%. von Frisch (1967) examined 65 species and found a similar range. The concentration found by Cruden et al. (1983) was 32.5 ± 2.46% (n = 12). On the other hand, Herrera (1985) found that in a Spanish *garrigue* most species had very concentrated nectars, usually higher than 60%. In the Greek *phrygana*, the community average concentration was 55.4 ± 1.69% w/w sucrose (n = 68). The species average concentration reached 76% w/w sucrose, while that of individual flowers could exceed 80% (Petanidou & Smets, 1995). In extreme cases (e.g., *Urginea maritima, Anthyllis hermanniae*), nectar may even crystallize in all flowers within a population and it cannot be sampled using capillaries (Petanidou, personal observations). Regardless of the small volumes detected in *phrygana*, the total amount of per flower nectar sugars is comparable to other temperate systems (Cruden, et al., 1983; Petanidou & Smets, 1995). The low nectar volume in *phrygana* coupled with a high energetic content is almost certainly related to water limitations in the Mediterranean area.

2.3 Plant species with no nectar

An important feature of Mediterranean plants is the absence of nectar from plants that one expects to be nectar-rewarding, based on their floral display. Such non-nectariferous species occurring in the Mediterranean region can be grouped into three major categories:

1. Species with nectarless deceit flowers, very commonly found in Orchidaceae in the region (Dafni & Bernhardt, 1990; Dafni & O'Toole, 1994). In deceptive pollination, pollinators are offered no floral reward, i.e., no nectar or pollen, for their visits to orchids (Dafni, 1984). Considering the high number of deceptive orchids in the Mediterranean area, it may be concluded that such a rewardless investment service may be of highly selective significance here. For instance, orchids comprise ~4% of the total angiosperm flora of the island of Lesvos, Greece, which may increase to 5% if all orchid subspecies are considered (Bazos, 2005, personal communication).

2. Species with differential investment in advertisement versus little or no reward. This group includes genera with showy flowers that have little or no nectar, and that use "discovery advertisement" sensu Dafni (1996), such as geophytes with autumnal flowering and hysteranthous foliage (e.g., *Colchicum, Cyclamen, Crocus*). It also includes plants with big and showy flowers blooming in spring, like *Acanthus spinosus* and *Bellardia trixago*, both with large white flowers (Petanidou, 1991). Although the rationale for the existence of such nectarless species would fit with that of deceptive pollination in the Mediterranean, the selection process towards flower emptiness is totally unknown for either species.

3. Species with differential investment in pollen versus nectar, both as advertisement and reward, which includes anemones (*Anemone*), poppies (*Papaver*, *Glaucium flavum*), and nightshades (*Solanum*). Such "pollen flowers" are common in the Eastern Mediterranean and very important to bees for pollen alone (Proctor et al., 1996). Other genera bearing less conspicuous and consequently less competitive pollen flowers (e.g., *Hypericum*), may be favoured by flowering during a less competitive period, i.e., towards summer, and then receiving pollinator services through necessity (Petanidou, 1999).

3 FACTORS SHAPING NECTAR SECRETION AND OTHER CHARACTERISTICS

The volume and concentration of nectar secreted by a flower depend on the following three factors: (i) ambient humidity and temperature (Corbet et al., 1979), (ii) selective reabsorption of solutes or water (Búrquez & Corbet, 1991; Nicolson, 1995), and possibly (iii) changes in the concentration at which nectar is secreted (Corbet, 2003). This means that nectar volume and concentration depend both on external conditions (climate, weather) and plant characteristics (e.g., related to structural and physiological attributes of plants and flowers). I discuss these factors in more detail below.

In a series of studies, Petanidou and Vokou (1990, 1993) and Petanidou and Smets (1995) argued that the severe water deficit and very high temperatures characterizing the Mediterranean summer and spring may have detrimental effects on nectar secretion rates and volumes (see also Herrera, 1985). As nectar secretion evidently continues even under extremely harsh conditions, one question is: to what extent are Mediterranean plants adapted to secrete nectar under unfavourable conditions, i.e., in high temperatures or low humidity?

3.1 Temperature

The nectar secretion rate increases with temperature, with an optimum dependent on the species in question (Fahn, 1949; Shuel, 1952; Beutler, 1953b; Huber, 1956; Corbet, 1990; Jakobsen & Kristjánsson, 1994). Petanidou and Smets (1996) hypothesized that because Mediterranean plants are adapted to high temperatures their optimal nectar secretion takes place at higher temperatures than that of temperate plants. In other words, relatively high temperatures could induce nectar secretion in Mediterranean plants. They tested their hypothesis on thyme *Thymus capitatus*, a typical *phrygana* plant, flowering under controlled temperature and humidity. Interestingly, nectar secretion in thyme flowers increased with temperature up to 38ºC as long as plants were not water-stressed or light-limited. The optimal temperature for nectar secretion was found to be 32.5ºC, much higher than optimal temperatures known for temperate species, e.g., *Oenothera biennis* (optimal temperature 24ºC), *Borago officinalis* (23.5ºC), and *Trifolium repens* (10ºC and 18ºC) (Shuel, 1952; Huber, 1956; Jakobsen & Kristjánsson, 1994). Petanidou and Smets (1996) also observed that in the open and under temperate summer conditions (i.e., low temperatures and solar irradiance) nectar secretion in thyme depended more on changes in light levels than on temperature. The authors concluded that temperature stress may stimulate nectar secretion

in plants adapted to Mediterranean conditions. Such an adaptation may be most pronounced in summer-flowering species, which are visited by a large number of nectar-consuming insect species, as is the case with *Thymus capitatus* (Petanidou, 1991).

3.2 Humidity

A considerable part of the Mediterranean region is made up of coastal areas, where the sea has a dominant effect on terrestrial habitats. Pérez-Bañón (2000) found that in such habitats it is not primarily the temperature, but the differential humidity that positively affects nectar secretion, both in volume and sugar content. Working on *Medicago citrina*, a leguminous shrub in the archipelago of Columbretes, in Spain, the author discovered that the low relative humidity had a dramatic effect on nectar secretion. Nectar secretion was measured (i.e., volume, concentration, and sugar content per flower) on several mid-March mornings and ambient temperature and relative humidity were also recorded throughout the day. Amongst all parameters tested, the ones found to affect nectar secretion were (i) the mean of the maximal temperatures recorded over the 24 h preceding sampling, and (ii) the mean relative humidity recorded 2 h before sunrise (6:00–8:00). Further analysis of the data showed that mean relative humidity had a positive effect on both nectar volume and sugar content, which was more significant than that of temperature at all flower ages tested. The conclusion is that, in island communities, atmospheric humidity may play a very crucial role in nectar secretion that is otherwise limited by extreme water drought, evidently more important than temperature itself (Búrquez & Corbet, 1998).

3.3 Light intensity

Mediterranean plants are generally adapted to high light intensities and their nectar secretion is not expected to be limited by solar irradiance under normal Mediterranean conditions. Under unfavourable light conditions nectar secretion may decrease dramatically. Experimenting on *Thymus capitatus*, I found that, with a few exceptions, flowers in the sun secreted more nectar of a higher concentration than flowers growing mostly in the shade, implying limitation by solar irradiance (Petanidou, unpublished data). When *T. capitatus* plants were grown under typical temperate conditions (i.e., under low temperature and light intensity), solar irradiance was the most significant limiting factor for nectar secretion, not low temperature (Petanidou & Smets, 1996). The experiment was repeated with *Ballota acetabulosa*, another labiate species sympatric and co-flowering with *T. capitatus*, which differs in its microhabitat preference by usually growing in more shaded areas. *Ballota*

appeared to perform optimally under temperate conditions where neither temperature nor solar irradiance appeared to limit nectar secretion (Petanidou & Smets, 1996).

3.4 Water stress

Because water is suspected to be a permanent limiting factor for nectar secretion in the Mediterranean, it is probably logical to assume that the highest nectar yields occur in the years of highest precipitation. Although there are no hard data, there is some support for this hypothesis from observations made in the wild on *Capparis spinosa*, *Thymus capitatus*, *Prasium majus*, *Satureja thymbra*, *Asphodelus aestivus*, and *Ornithogalum exscapum* (Petanidou & Smets, 1996; Petanidou et al., 1996; Petanidou, 1999).

To address the question of nectar secretion under water stress experimentally, Petanidou et al. (1999) studied the effect of irrigation on nectar secretion in three Lamiaceae species typical of *phrygana* (*Satureja thymbra, Stachys cretica,* and *Thymus capitatus*). Experimenting on potted plants taken from the wild, along with control measurements carried out on naturally growing non-irrigated potted plants, they found that after treatment only *T. capitatus* produced higher nectar volumes and total sugars per flower. Nectar yield in *S. thymbra* did not change with irrigation, whereas *S. cretica* showed dissimilar trends depending on the irrigation time within the flowering period. The authors concluded that irrigation may promote nectar secretion only in flowering periods that are unfavourable for growing, e.g., in summer. During such periods available water resources are probably allocated solely to nectar secretion—which may add up to considerable quantities—rather than to vegetative growth and excessive flower production, as may occur during spring.

Under typical Mediterranean conditions high-sucrose nectars predominate over high-hexose nectars, which implies that the former have been selected for (Petanidou, 2005). An explanation may be given by the overriding effect of drought, the most ecophysiologically effective constraint in the region. High-hexose nectars consume more water than high-sucrose nectars for the same amount (weight) of sugars (Nicolson, 1998, 2002). Therefore, by having high-sucrose nectars, Mediterranean plants avoid excessive water loss from hundreds of ephemeral flowers. In addition, by having high-sucrose nectars hidden within deep flowers, plants avoid nectar loss through evaporation during the hot and dry period of the year (Petanidou, 2005).

3.5 Nutrient stress

Lack of nutrients combined with water shortage may constitute another stress limitation to nectar secretion in Mediterranean plants. A first attempt to investigate the effect of nutrient application on nectar secretion was made by Shuel (1955) on non-Mediterranean plants. He concluded that nectar secretion is higher under low nitrogen supply. This conclusion was experimentally confirmed by Petanidou et al. (1999) in an extensive study carried out on Mediterranean plants. The authors investigated the effect of artificial nutrient supply on nectar secretion in three Lamiaceae species, both potted and naturally growing. Interestingly, they found similar results to those for irrigation (increased nectar secretion in the case of *Thymus capitatus*, no change in *Satureja thymbra*, and mixed trends in *Stachys cretica*). They concluded that irrigation is more important than nutrient supply in increasing per-flower nectar secretion, implying that the most influential external factor in shaping the physiology of nectar secretion in the Mediterranean is primarily drought, not nutrient scarcity. Owing to the production of surplus flowers on artificially fertilized plants (as a result of extended vegetative growth), a much higher number of nectarless flowers were found than on untreated plants (Petanidou et al., 1999). The presence of empty flowers has been considered to be of evolutionary significance, as it may enhance insect movements between flowers and plants, increasing visitation rates, reducing geitonogamy and thus increasing plant fitness (Brink & de Wet, 1980; Bell, 1986; Gilbert et al., 1991; Sakai, 1993). The results of Petanidou et al. (1999) indicate that this may apply particularly to regions experiencing long periods of drought, such as the Mediterranean.

Another effect of nutrient supply is the alteration of the chemical composition of nectar. Petanidou et al. (1999) found that nutrient application results in nectars having higher sucrose/hexose ratios than controls (although in *Thymus capitatus* the results were not significant). In the same series of experiments, amino acid concentration of nectars remained statistically unchanged after treatment with fertilizer. Treated flowers and controls, however, differed markedly in the relative abundance of certain amino acids, which were different among the three study species. In a similar study carried out in the UK, Gardener and Gillman (2001a) found that the concentration of total amino acids together with those of glutamine and proline increased significantly with increasing fertilizer treatment in *Agrostemma githago*, whereas the concentration of GABA decreased. Fertilizing also resulted in a significant decrease of the relative abundance of about half of the amino acids in the nectar of *Agrostemma*, with the exception of glutamine, which increased. The results of both studies show that the nectar complement can

be influenced by soil conditions (cf. also Shuel, 1952, 1955; Shuel & Shivas, 1953), which may alter nectar attractiveness, and therefore have important implications at the plant–pollinator interface.

3.6 Ecological succession

Time is an important parameter in the framework of pollination ecology and several nectar secretion attributes appear to depend on flowering time, at least within a genetically related group of plants. Among all attributes Petanidou et al. (2000) studied in the Lamiaceae, only nectar concentration seemed to increase with flowering time, whereas the majority of attributes were affected negatively—flower depth and corolla width, the size of the nectary and its stomata, as well as the volume and sugar content of the nectar.

There is evidence that nectar secretion changes with time, not only in the course of the flowering season, but also within ecological time. A very interesting case is the change of nectar yield that some plants show in the course of post-fire succession in Mediterranean habitats (Petanidou & Ellis, 1996; Petanidou, 1999; Potts et al., 2003).

Firstly, this change may be a consequence of changes in the community structure, with annuals being gradually replaced by perennials as the system ages, combined with the fact that perennials bear more alluring flowers (i.e., more nectar-rewarding) than annuals (Petanidou & Smets, 1995; Petanidou, 1999). Potts et al. (2003) quantified some key parameters of both pollen and nectar forage at the community level in different ages of post-fire communities and showed that changes in floral reward structure reflected the general shift from annuals (generally low-reward open-access flowers) to perennials (mostly high-reward and restricted access flowers) as post-fire regeneration ensues. In particular, the authors found that nectar volume, water content, concentration, and the diversity of nectar-foraging niches are all greatest in the first post-fire stage of succession, i.e., immediately after fire, with a steady decrease as regeneration proceeds (Table 3). This is slightly different to what Petanidou and Ellis (1996) suggested—relatively low per-flower nectar quantity in the first post fire years. A similar decline with ecosystem age after fire was found in energy availability in nectar and pollen, and the relative importance of pollen to nectar energy (Potts et al., 2003).

Secondly, within the core of the main flowering season, perennials are much more competitive than annuals, the latter offering about half the nectar yield of the former (as per day sugar equivalent) (Petanidou & Smets, 1995; Petanidou & Ellis, 1996).

Thirdly, and most surprising, is the fact that some perennial plants may increase their nectar secretion during the mature phryganic stage whereas annuals/biennials may experience reduced secretion in the course of succession in Mediterranean communities (e.g., *Capparis spinosa*, *Phlomis fruticosa*, and *Stachys cretica* versus *Lamium amplexicaule* and *Salvia verbenaca*; Petanidou, 1999). Comparing the nectar standing crop of *Satureja thymbra* in burnt and unburnt areas in Israel, Potts et al. (2001) found similar results, with nectar standing crops two times higher in unburnt than in burnt habitats. As a result, in the course of ecosystem succession, perennials may become more attractive to bees and to other pollinating insects than annuals within the community, thus promoting their fitness through differential seed set. The conclusion drawn from all the above studies is that floral communities and associated rewards not only shape pollinator community structure, but also have significant implications for the process of succession.

4 MATCHING NECTARS AND FLOWER TYPES

High-volume nectars have generally been associated with deep and tubular flowers because of their smaller surface:volume ratio, which diminishes water loss through evaporation (Corbet et al., 1979; Plowright, 1987; Dafni, 1991). Freely exposed nectar in open flowers tends to equilibrate with ambient humidity (Corbet et al., 1979; Nicolson, 1998, 2002). Similarly, nectar concentration is more constant in deep flowers compared to open ones, which contain smaller volumes of nectar where concentration can fluctuate rapidly (Corbet, 2003).

These principles apply equally to the Mediterranean *phrygana*, where nectar volume is found to be positively correlated with flower depth ($R = 0.312$, $P < 0.01$), whereas nectar concentration shows a negative association ($R = –0.485$, $P < 0.000$) (Petanidou & Smets, 1995; Petanidou, 2005). Both floral depth and nectar volume are highly related to sucrose/hexose ratios in nectar at the community level ($R = 0.441$, $R = 0.426$, respectively; $P < 0.001$; Petanidou, 2005). One can conlude that the *phrygana* community is made up of two major sets of flower types and nectars (although intermediate values do exist): deep flowers with high, albeit dilute, volumes of sucrose-dominant to sucrose-rich nectars (such flowers may act as “nectar reservoirs” by slowing the rate of sucrose breakdown); and shallow or open flowers with less volume but more concentrated, hexose-rich nectars (Petanidou, 2005).

Among all the *phrygana* plant families, the Lamiaceae have the highest average nectar yield per flower and Asteraceae the lowest (Petanidou & Smets,

Table 3. Summary of the nectar attributes related to ecosystem post-fire succession. (Data are from Petanidou and Ellis (1996), Potts et al. (2003, 2004), Petanidou and Lamborn (2005)).

Attributes	**Freshly burnt site (1–2 year *phrygana*)**	**Intermediate age (15–25 year scrub)**	**Mature pine forest (>50 years)**
Nectar component			
*Volume**	low–medium–high	medium–low	medium
Per flower volume	relatively low	higher	higher
*Concentration**	medium–high	medium	medium–low
*Water content**	low–medium–high	medium–low	medium
Nectar niche diversity	high	lower	low
Energy reward*			
Pollen	high	medium	low
Nectar	medium–high	medium–high	medium
Pollen: nectar	high	medium	low
Plant component			
Plant diversity	high	medium	medium
Floral abundance	high	medium	medium
Plant groups	many annuals	fewer annuals	more perennials
Bee component			
Diversity	high	medium	low
Abundance	high	medium	low
Guilds	Mainly short-tongued bees; many Andrenidae and Apidae	Mixed guilds including long-tongued bees (Megachilidae); fewer Andrenidae and Apidae	Mixed guilds; many Andrenidae and Apidae

* per unit habitat area

1995). Dissimilar flower shapes, therefore, differ in their nectar volume, with gullet types secreting the highest and bowl- and head-shaped secreting the lowest volumes. Bowl-shaped flowers possess more concentrated nectars compared to gullet-shaped flowers (Petanidou & Smets, 1995).

Structural traits of flowers appear to play a major role in shaping nectar characteristics, at least within a phylogenetically related group of plants. This is true for flower size (i.e., corolla length and width) that is related to nectary size (cf. also Chapters 3 and 4 in this volume) and nectar yield (Dafni et al., 1988; Dafni, 1991; Petanidou et al., 2000; Galetto & Bernardello, 2004). The size of the nectaries and nectarostomata (i.e., the openings on the nectary

through which the nectar is secreted) is positively correlated with nectar volume (Dafni et al., 1988; Petanidou et al., 2000). On the other hand, the number of nectarostomata does not appear to play a significant role in controlling nectar volume (Petanidou et al., 2000), which is in agreement with other continental (Teuber et al., 1980; Davis & Gunning, 1991), but not tropical species (Galetto, 1995; but see Galetto & Bernardello, 2004). Among all nectary attributes only the stomatal size affects nectar concentration and this is a negative relationship (Petanidou et al., 2000). Species with small nectarostomatal openings secrete more concentrated nectars, at least within the Lamiaceae (Petanidou et al., 2000). It is unknown whether this is a peculiarity of Mediterranean nectaries, or a general characteristic of all Lamiaceae, or even broader groups.

5 NECTAR AND THE POLLINATOR INTERFACE

5.1 Relating consumers to deep-flower nectars

Tubular, deep, and closed flowers can protect nectar from nectar thieves and unwanted insects, such as short-tongued visitors who will have limited access (Baker & Baker, 1983; Dafni, 1991; McCall & Primack, 1992; Menzel & Shmida, 1993; Potts et al., 2001). The presence of numerous hairs and stamens in the flowers of several Mediterranean species, such as those within the genera *Cistus* and *Capparis*, may have a role similar to long corollas in restricting air movement and excluding insects (Petanidou & Ellis, 1996; Petanidou, 2005). In this respect, the presence of honey leaves or honey pockets (i.e., petal scales where nectar is accumulated) in bowl-shaped flowers in some Mediterranean genera is probably related to a similar nectar-protective function (e.g., *Fritillaria, Nigella, Ranunculus*).

5.2 Nectar sugars and pollinators

High-sucrose nectars prevail in the Mediterranean, not only at the community level and during the major flowering season (spring), but also during the harshest season, i.e., summer (Petanidou, 2005). In addition to the reasons mentioned on page 346, the propensity of sucrose-rich nectar species to flower in spring–summer, versus hexose-rich species to flower in winter can be explained on the basis of:

- Co-evolution of plants with insects. By containing easy-to-digest monosaccharides (Nicolson, 1998), high-hexose nectars are more adapted to consumption by an extensive array of mainly non-specialized pollinators

(e.g., short-tongued bees, wasps, beetles, butterflies, flies; cf. Petanidou, 1991). On the other hand, high-sucrose nectars are better adapted to more specialized pollinators such as long-tongued bees which are apt to perform sucrose digestion (hydrolysis). The dominance of hexose-rich nectars in winter coincides with the prevalence of non-specialized pollinator guilds (e.g., syrphid, anthomyiid, and other flies). Similarly, sucrose-rich nectars prevail in spring and summer together with their selective agents, the long-tongued bees. The presence of any high-hexose nectars during spring and summer is probably related to mixed guilds of insects that are active during that period (Petanidou, 2005).

- Trade-off between plant water economy and co-evolution with insect diet. For the same carbohydrate reward offered to pollinators, high-sucrose nectars utilize less water than high-hexose ones. Considering that calorific value is more important to bees (at least to honeybees; cf. Wells et al., 1992) than the type of sugars contained in the nectar (i.e., mono-, disaccharides), it could be concluded that in bee-dominated communities, such as those of the Mediterranean, selection favours high-sucrose over high-hexose.

5.3 Nectar amino acids and pollinators

Phenylalanine (present in 9.5% of the study species) and GABA (present in 63% of the species) were the only amino acids in the phryganic community that were consistently correlated with pollinator guilds and families (Petanidou et al., 2006). The effect was expressed as the relationship between the phenylalanine content of plant nectars (= % of total amino acid content) versus the number of species in pollinator guilds or families visiting them. Phenylalanine appeared to be positively related to long-tongued bees and megachilids. GABA could be correlated with to a broader array of insects—long-tongued bees, anthophorid and andrenid bees, as well as anthomyiid and syrphid flies.

On the other hand, several amino acids appeared to be sporadically repellent to a few insect groups. Asparagine appeared to repel many insect groups: beetles, bugs, anthomyiid flies, wasps, short-tongued bees and colletids, but only megachilids among the long-tongued bees). These characteristics seemed to be a result of co-evolution with bees—long-tongued bees, especially Megachilidae, seem to have played the major selective role for phenylalanine-rich nectars (Petanidou et al., 2006). This could be related to the fact that phenylalanine is an essential amino acid in bee diets (de Groot, 1953), an explanation that fits well with the classic ideas of Baker and Baker (1973a, b, 1978, 1986). Petanidou et al. (2006), however, go further by

arguing that phenylalanine's most important effect for bees is its strong phagostimulatory quality, which is unique among many amino acids tested in other studies (Inouye & Waller, 1984). This quality certainly adds to the taste of nectar (Gardener & Gillman, 2002), hence influencing bee preferences and the plant–pollinator food web structure at the community level. Having such a potential, and owing to the high number of bee species in the Mediterranean, it is not surprising that phenylalanine dominates the nectars of plant species that are characterized by prevailing melittophily in this region, especially in the Lamiaceae (Dafni et al., 1988; Petanidou & Ellis, 1993, 1996; Petanidou & Vokou, 1993; Michener, 2000).

There are a few interesting species exceptions within the phenylalanine-rich family of Lamiaceae. The first is *Thymus capitatus* with a detected phenylalanine content at community average levels both in Israel and Greece (Dafni et al., 1988; Petanidou et al., 2006). A possible explanation is that *T. capitatus* constitutes a "pollinator sink" within both communities, visited by mixed insect guilds (123 insect species in the Greek *phrygana* of which bees comprise only 24%; Petanidou, 1991; Petanidou & Potts, 2006). It might be that plants flowering outside the main blooming season under conditions of little or no competition for pollinators, i.e., during the Mediterranean summer or early spring (Petanidou, 1991, 2004), are less challenged to produce extra phagostimulants, therefore have low levels of phenylalanine in their nectars (e.g., *Lamium amplexicaule* in Athens and *Rosmarinus officinalis* in Israel, as well as *T. capitatus* in both countries) (Dafni et al., 1988; Petanidou et al., 2006). The exception of *L. amplexicaule* could also be explained by the partly cleistogamous character of its flowers (Lord, 1982).

The case of GABA that is related positively with some insect guilds visiting the phryganic plants may be similar to that of phenylalanine. Petanidou et al. (2006) argue that phagostimulation may be related to the probable co-presence of NaCl, a salt on which GABA strongly depends (Keynan & Kanner, 1988; Wolfersberger, 2000). There is some evidence that NaCl has a positive effect in attracting honeybees probably by improving nectar taste (Taber, 1991; Fulton, 1997; Gardener & Gillman, 2002). Perhaps in an area like the Mediterranean where sweetness can be of limited discriminatory value (all nectars are concentrated, see also next paragraph), it is the combination of GABA–NaCl that constitutes the most important nectar phagostimulant for several pollinating guilds (flies, bees, and beetles) that might have acted as selective agents for GABA-rich nectars.

In addition to the effect that particular amino acids may have in attracting pollinators to Mediterranean flowers, Petanidou et al. (2006) found that total

amino acid content of nectar constitutes a very significant trait to which some pollinator guilds (anthophorids, megachilids, and apids) respond. This supports the general ideas of Baker and Baker (1982, 1986) that amino acids may have co-evolutionary significance in floral nectars. The novel finding by Petanidou et al. (2006), however, is that this positive effect is also applicable to solitary bees, as well as social honeybees and tropical stingless bees discussed by earlier studies (Roubik et al., 1995; Gardener & Gillman, 2002). The most important finding, however, is that total amino acid content of nectars versus sugar content is the most significant factor in shaping plant–pollinator interactions in the Mediterranean habitats. The explanation for this is probably related to the Mediterranean climate, as in such hot and dry habitats, characterized by very high sugar concentrations (Petanidou & Smets, 1995), the sweet taste of nectar may probably be too "strong" to function as species-specific discriminator and allurement. In these habitats, the high contribution of amino acid content, together with the presence of individual phagostimulants (e.g., phenylalanine, and possibly NaCl–combined GABA) may have been selected in addition and constitute the nectar traits that are specifically important in attracting particular insect guilds (Petanidou et al., 2006). It will be interesting to see if these trends are also found in other mediterranean regions and habitats, such as Chile, South Africa, and the Californian coastal scrub.

5.4 Nectar minerals and pollinators

Among all minerals present in floral nectars special attention was given to potassium, which was found to discourage honeybees from visiting onion flowers (Waller et al., 1972; Liu et al., 2004). Moreover, there is some evidence today that sodium (in the form of NaCl) has a positive effect on attracting honeybees (Taber, 1991; Fulton, 1997); NaCl may improve nectar taste significantly (Gardener & Gillman, 2002).

Plants contain a high K/Na ratio, which is reflected in the haemolymph of herbivorous insects, including the highly evolved bees, as a result of co-evolution with higher plants (Boné, 1944; Duchateau et al., 1953). This may also influence nectar–pollinator relationships (Hiebert & Calder, 1983). Given the deterrent character of potassium opposed to the attractive character of sodium (Waller et al., 1972; Liu et al., 2004), I hypothesize that, in general, highly attractive nectars are selected on the basis of their high Na/K ratio, especially those visited by highly evolved pollinators. Within *phrygana*, I expect that this will mostly apply to plant species visited by long-tongued bees, primarily Megachilidae and Anthophoridae. No doubt, future research on nectar attractiveness will explore these questions and hypotheses.

5.5 Nectar secondary compounds and pollinators

The presence of secondary compounds in floral nectars may enhance plant fitness both inside and outside the Mediterranean (see "Secondary compounds" on page 348). This may be achieved through different methods:

- Increasing pollinator visitation to plants defended against herbivore attack (Adler, 2000a, 2000b)
- Attracting more specialized pollinators (Masters, 1991)
- Influencing the preferences of foragers (e.g., phenol and alkaloid compounds; cf. Waller et al., 1972; Hagler & Buchmann, 1993)
- Increasing floral constancy of legitimate pollinators and inhibiting nectar thieves (Stephenson, 1981, 1982)
- Increasing interflower and interplant movements to avoid ingesting excessive levels of secondary compounds (London-Shafir et al., 2003).

5.6 Floral nectar, floral diversity, and bee diversity

Several studies have attempted to explain bee diversity using single quantitative nectar parameters at a community level, and it has been shown that changes in nectar levels influence bee visitation to flowers (see Proctor et al., 1996; Potts et al., 2003, 2004 for reviews). The most commonly used attributes have been spatial and temporal patterns of nectar volume and concentration. Although such parameters of nectar reward structure may define the suite of flower visiting, they fail to give a full picture of how the community is organized on the basis of these single aspects of nectar reward. In order to describe the nectar reward structure of Mediterranean communities, Potts et al. (2004) used a complex approach to quantify "nectar resource diversity" which they defined as the variety of nectar volume and concentration combinations available in a community. They found that the variation in bee species richness within a habitat is much better explained by such a parameter than by other nectar variables such as volume, concentration, energy value, and water content, which have little predictive value per se. In fact, the authors demonstrated that nectar resource diversity may be a fundamental factor organizing nectarivorous communities. Using a series of Mediterranean habitats differing in successional stage and structure, they found that nectar resource diversity is highly correlated with floral species richness and particularly with the species richness of annuals. In addition, nectar resource diversity is highly correlated with bee diversity, which illustrates the importance of this parameter in determining the flower–visitor web structure in Mediterranean communities. This is a key finding in view of the management of these communities, demonstrating the importance of mosaic structure

combined in several successional stages in order to attain the maximal floral and bee diversity in a habitat (see also Petanidou & Ellis, 1996).

5.7 What types of nectars do pollinators prefer?

In the Mediterranean, as in many other regions and habitats, nectar profile (i.e., secretion and characteristics) is sometimes strongly related to the plant's main pollinator guilds (Baker & Baker, 1983; Petanidou, 2005; Petanidou et al., 2006). This may be reflected in differences in the nectar profile of genetically closely related plant species that are pollinated by different insect guilds. The pollination of several species and subspecies of *Capparis* in Israel is a case in point. The nectar of *C. ovata*, a hawkmoth-pollinated species, is higher in volume and concentration than that of *C. spinosa*, a bee-pollinated species, which occurs in the same localities and has a similar flower morphology (Dafni et al., 1987). In addition, the two subspecies of *C. spinosa* in Israel were found to have different nectar profiles: a hawkmoth-pollinated subspecies with high nectar yield and a bee-pollinated one with lower nectar volume and concentration (Eisikowitch et al., 1986).

Within the Mediterranean, however, cases like *Capparis* are not common, as this genus represents an exception for many reasons. Aided by a very efficient water economy, *Capparis* is a thriving genus in the Mediterranean region where it manifests diverse pollination systems and exceptional traits such as summer flowering and showy nocturnal flowers with unusually high nectar rewards (Rhizopoulou, 1990; Petanidou et al., 1996; Rhizopoulou et al., 1997). In fact, *Capparis* provides the most abundant nectar reward within Mediterranean scrub, with a recorded nectar volume of 15.21 μl per flower—about 24 times as much as the community average (0.64 μl) (Petanidou & Smets, 1995). On the other hand, a phryganic community encompasses an outstanding diversity of ordinary plant species that, unlike *Capparis*, follow the "system rules," by flowering mainly within the major blooming period (i.e., spring, from March to May) and having small, diurnal, and low-nectar-yielding flowers (Petanidou et al., 1995; Petanidou & Smets, 1995). Such habitats also contain an exceptional diversity of flower-visiting insects of particular taxonomic and ecological guilds (Petanidou & Ellis, 1993, 1996). This raises the question of whether, at the community level, there is a possibility of matching insect guilds and plant species on the basis of their nectar attributes. Petanidou et al. (2006) attempted to address this question in a community study and their results are summarized below:

1. Relationships with insects are more significant in distinguishing plant assemblages characterized by particular nectar traits than other plant

attributes, both taxonomic and ecological (flowering season, life form). This means that plant–pollinator co-evolution is probably more important in shaping nectar traits than ecological constraints or phylogenetic affinities.
2. In general, the discriminating nectar trait for the response of most insect guilds is amino acid composition, not sugar composition or nectar volume.
3. Bee response is mostly shaped by amino acid composition, whereas the response of other anthophilous insect guilds is mainly shaped by sugar composition.
4. Among all amino acids, asparagine and H-serine always have a negative effect on insect guilds, whereas phenylalanine has a positive effect (on long-tongued bees), as does GABA (on long-tongued bees and other anthophilous insect guilds).
5. Among major sugars, only fructose has a general positive influence on different insect guilds, especially on short-tongued bees and insects other than bees (hoverflies, anthomyiid flies, beetles, and wasps), while sucrose has a positive influence on long-tongued bees and glucose a negative influence on wasps.

In conclusion, even in a generalized system like *phrygana* (Petanidou & Potts, 2006), it appears that the nectar traits of plant species play an important role in organizing the community and its plant–pollinator resources. Perhaps we are at the beginning of unravelling the thread of the nectar secrets encompassing both gastronomy and the satisfaction of insects' physiological needs (Gardener & Gillman, 2002).

6 NECTAR AND MANAGEMENT OF MEDITERRANEAN HABITATS

6.1 Introduced and invasive plants: effects on wild flowers and bees

Invasive plants represent a major threat to world biodiversity and especially to the Mediterranean, one of the world hot spots for biodiversity (di Castri et al., 1990). Such plants often bear "more attractive" flowers, i.e., larger or more rewarding, which may bring about competition for pollination with the native flora, and may result in reduced seed set in native species (Memmott & Waser, 2002). The reduced seed set and biological fitness of the native species will have detrimental repercussions at the levels of both economics

(e.g., reduced fruit or seed yield in agricultural systems) and nature conservation (e.g., local extinction of species).

Copiously offered nectar is often the most effective fee for an introduced alien species to establish and become invasive. Especially in the Mediterranean, where nectar is not abundant, efficient invasive plants are expected to be those offering high nectar or pollen yields. Such high nectar yields may also be available at the population level in situations of extremely high number of flowers that some plants or populations may have. This is the case for *Bunias orientalis*, an extremely successful invasive plant species in central Europe, which is a food source for bumblebees and honeybees, but has negative impacts on the fitness of native plant species (Schurkens & Chittka, 2001). Examples like this must raise the attention of managers and decision makers before any site management is implemented. In this respect, the deliberate introduction of the American species *Phacelia tanacetifolia* as a nectar source plant in central Europe and the Mediterranean is astounding, especially when considering the cost of irrigation in an area suffering from extreme drought (Petanidou, 2003).

6.2 Invasive bees: beekeeping, bumblebee management, and wild bee conservation

The diet of all bee species consists exclusively of pollen and nectar collected from flowers, although it may occasionally be supplemented by other substances, such as honeydew, plant sap, waxes, resins, and water (Michener, 1974). As a consequence, pollen and nectar are the most sought-after foods within a community, and the source of competition among bees and other flower-visiting insects, at least in periods when these resources are limited. Among all bees, honeybees and bumblebees are notorious for exploiting floral rewards, and a bulky literature has accumulated on their competitive efficiency against solitary bees (see Goulson, 2003, for a review).

Both honeybees and bumblebees possess undoubted foraging abilities. Apart from having relatively long tongues, these large and hairy animals thermoregulate in flight and retain heat within their large nests, therefore being able to exploit all sources of nectar in the community by foraging earlier in the morning than many native, solitary bee species or under unfavourable weather conditions, thus reducing the food base of other bees (Corbet et al., 1993; Dafni & Shmida, 1996; Willmer & Stone, 2004). In addition, they are generalists with large and long-lived colonies and so are able to adapt to a succession of different flower sources as they become available. Having such assets, it is no wonder that honeybees and bumblebees have

proved to be highly competitive in various communities and most adaptable in colonizing new habitats far from the places of their origin. Considering that the colonized areas may be limited in nectar resources, these bees can constitute a threat to the local pollinator fauna, especially to small solitary bees in the cases where their foraging host breadths overlap. This applies particularly to the almost omnipresent *Apis mellifera*, which has been observed frequenting the majority of plant species within any one geographic region, visiting nearly 40,000 different plant species (Crane, 1990). The situation is also alarming within the Mediterranean, where honeybees are extensively managed for honey production not only in agricultural lands, but also in marginal lands, woodland and scrubland, as well as in protected areas. As an example, within the 30-ha *phrygana* community in Athens, *A. mellifera* was recorded visiting 103 out of the 133 available plant species (Petanidou & Potts, 2006). In such cases, honeybees could also be displacing native bees by just reducing their resource base (Petanidou & Ellis, 1996; Forup & Memmott, 2005).

Bumblebees (*Bombus* spp.), whose natural range is largely confined to the temperate northern hemisphere, have recently been introduced to various countries to enhance crop pollination. In the Mediterranean region, especially in typical Mediterranean habitats where bumblebees are relatively uncommon (Petanidou & Ellis, 1993), this fashion started in the 1980s and continues to date on an enormous scale, mainly in order to assist pollination in greenhouses. Following escapes from commercial colonies, such introductions lead to unwanted invasions, which may spread over large areas (Dafni & Shmida, 1996; Dafni, 1998).

It has been argued that depletion of nectar on a daily basis before native bees begin to forage, may result in a significant asymmetry in competition in favour of these introduced species (Goulson, 2003). On Mt Carmel in Israel, Dafni and Shmida (1996) reported declines in abundance of medium- and large-sized native bees (and also of honeybees) following the arrival of *Bombus terrestris* in 1978. Hingston and McQuillan (1999) recorded displacement of two species of *Chalicodoma* (Megachilidae) in Tasmania by introduced *B. terrestris*, which the authors consider a threat to Australian ecosystems (Hingston & McQuillan, 1998). Based on measurements of the high competitiveness of *B. terrestris* to native bees, it has been suggested that unregulated movements of non-native populations of the species within Europe should be banned without a full risk assessment (Ings et al., 2005). The impacts of *A. mellifera* introductions are similar: Goulson et al. (2002) found higher abundances of native bees in honeybee-free sites in Tasmania; Forup and Memmott (2005) observed some changes in floral host breadth of

long-tongued bees as a result of colonization by honeybees, although they found no effect on short-tongued bees. In New Caledonia, unique systems of pollination mutualism have been endangered by the introduction of honeybees (Kato & Kawakita, 2004).

Mediterranean habitats are known for their high solitary bee diversity (O'Toole & Raw, 1991; Petanidou & Ellis, 1993; Michener, 2000; Petanidou & Lamborn, 2005), which in turn is associated with high nectar niche diversity, especially in low scrub systems (Potts et al., 2004). Because very few Mediterranean species secrete copious nectar, with the majority producing relatively little, invasions by bumblebees will affect the diversity of medium- to long-tongued solitary bees negatively, as has happened in Israel (Dafni & Shmida, 1996). Similarly, under the pressure of intense beekeeping it is expected that the diversity of solitary bees as a whole will decline. Introduced bees are widespread, and because of this, deleterious effects are expected to occur on a large scale, and in some areas may be irretrievably severe. In this respect, areas managed almost solely by uncontrolled grazing (or rather, overgrazing) and intense beekeeping, especially in the East Mediterranean, are a priority risk (Rackham & Moody, 1992; Petanidou et al., 2001). They encompass not only marginal and wild habitats, but also abandoned agricultural lands, frequently terraced slopes, and hills that are nowadays unprofitable for primary production. That these areas are frequently isolated, and often on islands, may be an even worse omen (Roubik & Wolda, 2001).

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Index to scientific names

This index includes all the scientific names for bacteria, fungi, protozoa, animals, and plants mentioned in this book. Genus and species names are in *italics*, family names are in CAPITALS. Other groups, such as orders and subfamilies, as well as plant families that have been included in other families, are in Roman type. Plant families follow the Angiosperm Phylogeny Group (APG II, 2003) classification. **Bold** page numbers indicate figures and tables.

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Subject index

This index lists common names, place names, and subjects mentioned in the text. **Bold** page numbers indicate figures and tables.