

## The diversity, ecology and evolution of extrafloral nectaries: current perspectives and future challenges

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• **Background** Plants in over one hundred families in habitats worldwide bear extrafloral nectaries (EFNs). EFNs display a remarkable diversity of evolutionary origins, as well as diverse morphology and location on the plant. They secrete extrafloral nectar, a carbohydrate-rich food that attracts ants and other arthropods, many of which protect the plant in return. By fostering ecologically important protective mutualisms, EFNs play a significant role in structuring both plant and animal communities. And yet researchers are only now beginning to appreciate their importance and the range of ecological, evolutionary and morphological diversity that EFNs exhibit.

• **Scope** This Highlight features a series of papers that illustrate some of the newest directions in the study of EFNs. Here, we introduce this set of papers by providing an overview of current understanding and new insights on EFN diversity, ecology and evolution. We highlight major gaps in our current knowledge, and outline future research directions.

• **Conclusions** Our understanding of the roles EFNs play in plant biology is being revolutionized with the use of new tools from developmental biology and genomics, new modes of analysis allowing hypothesis-testing in large-scale phylogenetic frameworks, and new levels of inquiry extending to community-scale interaction networks. But many central questions remain unanswered; indeed, many have not yet been asked. Thus, the EFN puzzle remains an intriguing challenge for the future.

**Key words:** Angiosperms, ants, ant–plant interactions, extranuptial, extrafloral nectaries, extrasoral, ferns, herbivory, interaction networks, mutualisms, nectar, plant defence, protection.

### INTRODUCTION

Within the vast repertoire of plant defence mechanisms, extrafloral nectaries (EFNs) attract aggressive arthropods that protect developing leaves, shoots and flowers from herbivores. Plants in over one hundred families bear EFNs; EFN-bearing species occur in a wide range of habitats and climates and latitudes worldwide, from tropical forests to deserts. EFNs display a remarkable diversity in morphology, density and location on plants, and have diverse ecological functions. They commonly attract ants, as the nectar secreted by EFNs is a valuable, carbohydrate-rich food resource. Other arthropods – some, but not all of which protect the plant from herbivores – also feed on extrafloral nectar. By fostering ecologically important protection mutualisms, EFNs potentially boost the success of certain plant species, thus shaping plant community composition. They are equally important in structuring communities of arthropods, including herbivores, predators and parasitoids.

In spite of their ubiquity and ecological importance, the evolutionary history of EFNs is complex and poorly understood. The last account of the taxonomic distribution of EFNs was published by Koptur (1992), though for many years a website has been maintained and updated regularly by K. H. Keeler (*World list of plants with extrafloral nectaries*; <http://biosci-labs.unl.edu/Emeriti/keeler/extrafloral/Cover.htm>). Reviews of EFNs over the past two decades have focused on their ecological

role (Heil and McKey, 2003; Rico-Gray and Oliveira, 2007) with relatively less consideration paid to their structure and morphology (but see, for example, Vogel, 1997, 1998a, b). Recently, however, significant methodological advances have permitted novel explorations of the diversity, ecology and evolution of EFNs. For example, advances in molecular phylogenetics have dramatically increased our understanding of phylogenetic relationships at many taxonomic levels, resulting in new plant classification systems (e.g. Angiosperm Phylogeny Group, 1998, 2009) and frameworks to test evolutionary hypotheses (e.g. Huelsenbeck and Rannala, 1997; Huelsenbeck *et al.*, 2001; Emerson, 2002; Mathews, 2009). Here, we provide an overview of new insights, highlight major gaps in our current knowledge, and outline future research directions on the diversity, ecology and evolution of extrafloral nectaries.

### EFN DIVERSITY

EFNs are highly diverse, whether one considers their phylogenetic distribution, biogeography, structure (e.g. location on a plant and morphology), phenology, ecology or genetics. In the face of such diversity, it is striking that extrafloral nectar itself is more-or-less similar in composition across disparate plant taxa (Rico-Gray and Oliveira, 2007). This is in strong contrast to floral nectar, which differs widely in association with different types of flower visitors (e.g. Hansen *et al.*, 2007; Nicolson,

2007; Nicolson and Thornburg, 2007; Pacini and Nepi, 2007). Very recent advances in proteomic research are allowing scientists to unravel the complex synthesis pathways underlying extrafloral nectar secretion (Orona-Tamayo *et al.*, 2013). Although extrafloral nectar *per se* is beyond the scope of this article, several recent publications offer outstanding reviews and overviews of research done in nectar biology in general (e.g. Nicolson *et al.*, 2007; Heil, 2011; Escalante-Pérez and Heil, 2012).

#### Phylogenetic distribution

Some 90 angiosperm families and almost a dozen fern genera were recorded by Koptur (1992) to include at least one species that bears EFNs. Since then, numbers of known EFN-bearing taxa have dramatically increased. In this Highlight, Weber and Keeler (2013) carefully analyse the systematic distribution of EFN-bearing species, based on 135 years of published records in combination with a mega-phylogeny of plants and the current classification of angiosperms (Angiosperm Phylogeny Group, 2009). They report EFNs in a total of 3941 species distributed across 745 genera and 108 families, four of which are fern families, but none in bryophytes, gymnosperms, early angiosperms or magnoliids. Interestingly, almost half of all EFN-bearing species belong to only three angiosperm families. The legume family (Fabaceae) stands out, with 30 % of the EFN-bearing species; it has long been known for the richness of its interactions with ants (McKey, 1989). The second- and third-ranked families are Passifloraceae and Malvaceae, respectively, together comprising 20 % of the remaining EFN species.

Fern nectaries have long intrigued scientists, including Darwin and his son (Darwin, 1876; Darwin, 1877). Because ferns lack flowers, their nectaries do not really qualify as ‘extrafloral’; they are sometimes termed ‘extrasoral’. In this Highlight, Koptur *et al.* (2013) review the literature on the diversity, ecology and evolution of nectaries in ferns, and provide new evidence for a protective mutualism in a tropical fern species bearing extrasoral nectaries.

#### Biogeographic distribution

EFN-bearing plants occur in tropical and subtropical regions as well as in many temperate regions, including a wide range of habitats, from wet tropical rainforests to deserts (see Rico-Gray and Oliveira, 2007, and citations therein). EFN-bearing plants can make up a considerable proportion of the total vegetation, especially in tropical rainforests, with almost 30 EFN-bearing species in one hectare of Australian rainforest (Blüthgen and Reifenthat, 2003), but also in Brazilian cerrados (e.g. Machado *et al.*, 2008) and Mexico’s seasonal forests (e.g. Diaz-Castelazo *et al.*, 2005). Compared to rainforests and savannah-like habitats, EFNs found within the vegetation of deserts and other arid lands have been poorly documented. Although one might expect them to be rare in dry climates due to the costs associated with producing liquid rewards, vegetation surveys in the Sonoran Desert (south-western USA) show that many members of the dominant plant families, notably the legumes and cacti, in fact bear functional EFNs (Marazzi *et al.*, 2011). Only a handful of studies have examined the

distribution of EFN plants along altitudinal (Keeler, 1979) or latitudinal gradients (Stott and Pemberton, 1998).

#### Location on a plant and morphology

EFNs are also diverse with respect to their morphology and distribution on an individual plant. This diversity is reflected in the multitude of classifications that have been proposed to describe them (e.g. Caspary, 1848; Delpino, 1868–1875; Zimmermann, 1932; Fahn, 1979; Schmid, 1988; Vogel, 1997). Almost any above-ground plant part can bear EFNs, from vegetative parts such as leaves and stipules, to parts of the inflorescences such as pedicels, and even the outer floral organs not directly involved in pollination. Morphologically, EFNs can range from simple glandular trichomes and cryptic non-structural to structural secretory tissue embedded within EFN-bearing plant parts, to conspicuous, complex vascularized or non-vascularized glands on the surface of the EFN-bearing organ. In anatomically specialized EFNs, at least three different kinds of tissues can be recognized: the epidermis, the nectary parenchyma, and the subnectary parenchyma (including the vascular bundles branching off from the leaf vascular system). In some EFN-bearing legumes with a well-developed nectary parenchyma, an additional fourth structure of one or two layers of cells can be observed between the nectary and the subnectary parenchyma (e.g. Melo *et al.*, 2010; Marazzi *et al.*, 2013).

In this Highlight, Marazzi *et al.* (2013) analyse EFN diversity in location and morphology in the legume genus *Senna* (in which over 80 % of its 350 species bear EFNs). The authors suggest a novel way to view the diversity of EFN morphology, based on the level of morphological differentiation from the organ on which they are borne, which they term ‘individualization’. *Senna* includes EFNs representing two extremes: non-individualized EFNs, in the form of cryptic EFNs embedded within the tissue of the bearing organ (newly described in *Senna*); and highly individualized EFNs, in the form of conspicuous, gland-like EFNs on the surface of the bearing organ. Interestingly, the two morphologies do not appear to co-occur in a single species. Furthermore, they characterize two unrelated *Senna* clades.

#### Phenology of EFN production

EFNs may vary in their abundance and distribution on a plant over the course of its development. This temporal dimension is one of the least documented aspects of EFN diversity; very few studies have followed individual plants over their lifetimes, or have examined seedlings at all (e.g. Kelly, 1986; Rogers *et al.*, 2003; but see McKey, 1984; Kwok and Laird, 2012). Marazzi *et al.* (2013, this issue) show that *Senna* EFNs are present from early seedling stages to the adult plant. In one species they report an unexpected shift in EFN location during early plant ontogeny, which they attribute to possible changes in the program of leaf development during seedling stage.

#### Genetic mechanisms

Phenotypic diversity usually results from diversity in the genetic organization, regulation and/or expression of the underlying developmental programs. In the case of nectaries, such underlying programs are poorly understood. The gene *CRABS*

*CLAW* (*CRC*) from the small *YABBY* family of transcription factors (Bowman and Smyth, 1999; Bartholmes *et al.*, 2012) appears to be an early-functioning regulator of the development of both floral and extrafloral nectaries in core eudicots (Lee *et al.*, 2005a, b). While the location of floral nectaries may be determined by *CRC* along with several upstream *MADS* box floral homeotic genes and other unknown regulatory genes (Lee *et al.*, 2005a), the development of EFNs may involve the recruitment of different transcriptional control networks than those needed in floral nectaries (Lee *et al.*, 2005b). Comparative analyses of EFN anatomy and development in *Passiflora* (Passifloraceae) suggest that a shared developmental program is acting during leaf development to create diverse EFNs, and that their final location and morphology mainly depend on the maturity of the leaf tissue where the shared program is active (Krosnick *et al.*, 2011). This means that the program responsible for EFN development is closely associated with the developmental program of the EFN-bearing organ. Both need to be investigated in order to understand the intrinsic nature of EFN diversity.

### EFN ECOLOGY

EFNs secrete nectar that attracts ants and other small arthropods; ants and a few other visitors then protect the plant by disturbing, attacking, removing or killing insect herbivores and seed predators. A recent comprehensive review of the ecology of ant–plant defensive mutualisms is provided by Rico-Gray and Oliveira (2007; and see their appendix 6.1). Here, we briefly summarize current understanding, with a focus on the ecological role of EFNs.

#### *The benefits of EFNs*

EFN-mediated ant–plant associations were among the first interactions to gain widespread attention from early evolutionary biologists. Although they secrete nectar, EFNs were recognized early on to be unrelated to pollination. They had long been observed to attract ants, but whether or not this had any adaptive significance was widely debated. ‘Protectionists’ argued that EFNs functioned to attract herbivore-attacking ants, whereas ‘exploitationists’ held that they merely secreted waste products and their visitors were merely benign (Beattie, 1985). It was not until ant-exclusion experiments were conducted in the 1960s and 1970s that evidence for ants’ defensive function began to accumulate (reviewed in Bentley 1977; Koptur, 1992; Bronstein, 1998; Rico-Gray and Oliveira, 2007). Many outstanding experiments of this type have now been conducted, not only in flowering plants but also in ferns. In this Highlight, Koptur *et al.* (2013) demonstrate that modern ferns bearing functional leaf nectaries are able to attract protective ants and engage in mutualistic relationships. Recent meta-analyses have confirmed that most EFN-bearing plants do in fact benefit from ant protection (Chamberlain and Holland, 2009; Trager *et al.*, 2010), although with such analyses, one should keep in mind that negative results are often not published.

The protective role of EFNs is not always certain, and the mere presence of EFNs should not be taken to imply that a defensive mutualism necessarily exists. Ant visitors vary greatly in the quality of defence they provide (Ness *et al.*, 2006; Miller,

2007; Palmer *et al.*, 2010); some avoid contact with herbivores, and in fact may run from danger (e.g. Letourneau *et al.*, 1983). Some of the other arthropods that feed at EFNs confer protection from herbivores (Koptur, 2005), including certain parasitoid wasps (Wäckers, 2001; Wäckers and Bonifay, 2004), predatory wasps (Cautle and Rico-Gray, 2003), and spiders (Ruhren and Handel, 1999; Whitney, 2004), but many others are freeloaders on this resource (e.g. Pemberton, 1993; Nicolson, 2007). In some cases, EFN visitors as a whole have been shown not to confer protection from herbivory (e.g. O’Dowd and Catchpole, 1983; Heads and Lawton, 1985; Rashbrook *et al.*, 1993; Nogueira *et al.*, 2012a). This observation has suggested that EFNs may serve alternative or additional adaptive functions for plants. For example, Becerra and Venable (1991) proposed that extrafloral nectar might function to lure ants from floral nectar and thus prevent them from interfering with pollination (see also Wagner and Kay, 2002; Rosenzweig, 2002). Wagner and Nicklen (2010) provided evidence that resource provision by EFNs prompts ants to build their nests near the plant, thereby enhancing plant nutrition.

Some studies have shown that extrafloral nectar secretion is modulated in response to herbivory, adding weight to the interpretation of EFNs as part of plants’ defensive repertoires. In particular, in certain facultative ant–plant associations, extrafloral nectar production can be induced in response to artificial damage to leaves or to herbivory (e.g. Koptur, 1989; Mondor *et al.*, 2006; Escalante-Pérez *et al.*, 2012), or can increase in response to light and ant presence (Radhika *et al.*, 2010; Bixenmann *et al.*, 2011). Production of EFNs themselves may also increase after herbivory (Pulice and Packer, 2008). Nectar induced in response to herbivory has in some cases been shown to be enriched in either amino acids (Smith *et al.*, 1990) or sucrose (Ness, 2003). Disentangling the role of the phytohormone jasmonic acid in activating and modulating extrafloral nectar production (see Heil, 2011; Escalante-Pérez and Heil, 2012) will provide new insights in the very nature of EFN-mediated protection mutualisms.

#### *The importance of extrafloral nectar to arthropods*

Regardless of whether or not plants benefit from their actions, it is clear that extrafloral nectar is a critical food resource for small arthropods, including ants (Davidson, 1997; Blüthgen *et al.*, 2000, 2004; Blüthgen and Fiedler, 2004a, b; Schmid *et al.*, 2010), contributing to colony growth (Lach *et al.*, 2009; Byk and Del-Claro, 2010) and comprising up to 90 % of the total food collected by some species (but usually around 10 %; Tillberg and Breed, 2004). Indeed, extrafloral nectar has been hypothesized to contribute to the ecological dominance of ants worldwide (Davidson, 1997).

Extrafloral nectar is a valuable resource for ants for at least two reasons. First, EFNs are conspicuous, easily located, and – at least at some temporal scale – persistent food sources. In these respects, they differ substantially from other food items, notably animal prey, which in contrast to EFNs are ephemeral and may be difficult to locate. Second, the mono- and disaccharides (and small quantities of amino acids) found in extrafloral nectar are effective fuels for ant activity. The chemistry of extrafloral nectar, its nutritional value for consumers, and the taxonomic range of

those consumers lie beyond the scope of this article (but see, for example, [Nicolson, 2007](#)).

Research on EFN-mediated mutualisms has always been strongly phytocentric. However, it is also possible to take an ‘ants-eye view’ of these interactions. [Lanan and Bronstein \(2013\)](#) have examined how ant-colony-level foraging activity is distributed across a large population of a Sonoran Desert cactus (*Ferocactus wislizeni*) that bears EFNs. They demonstrated that individual colonies of the ant *Crematogaster opuntiae* remain associated with individual cacti for months or years at a time, with the ants aggressively fending off intrusions from other colonies and other species. Location of a cactus relative to the nearest *C. opuntiae* nest opening, as well as to the nearest conspecific cactus, determines the likelihood that it is well-attended by defenders when under attack. The availability of extrafloral nectar as an easily accessible, plentiful source of carbohydrates can also change the behaviour of these cactus-visiting ants, making them more aggressive towards insect herbivores ([Ness et al., 2009](#)). The relative competitive abilities among *F. wislizeni*-visiting ant species can explain their relative quality as plant defenders, as well as the likelihood that they will be able to displace other ants from individual cacti ([Morris et al., 2005](#); [Ness et al., 2006](#)). Thus, ant-centred research can open up new perspectives on the benefits that plants receive from EFNs.

#### *Specialization and generalization in ant–EFN mutualisms*

The papers in this Highlight focus on EFN structure and function in plants that have rather generalized mutualisms with plant defenders. In these interactions, ants do not live on the plant itself, and extrafloral nectar is the only plant-produced nutritional reward (apart from occasionally stolen floral nectar). However, it is important to note the existence of another kind of ant–plant defensive mutualism. In so-called myrmecophytic mutualisms (reviewed by [Heil and McKey, 2003](#); [Rico-Gray and Oliveira, 2007](#)), plants maintain intimate associations with individual ant colonies, commonly housing them in specialized chambers such as hollow stems or thorns. Myrmecophytic plants provide all of the resident ants’ food needs, some but not all of it in the form of extrafloral nectar. In particular, many provide highly nutritious ‘food bodies’. For example, some neotropical *Acacia* species produce so-called Beltian bodies, which are vascularized and contain high concentrations of protein lipids, in addition to carbohydrates ([Heil et al., 2004](#)). Recent research indicates that the chemistry of myrmecophytes’ extrafloral nectar may be quite different from close relatives with more generalized ant–plant defensive mutualisms. Their composition makes myrmecophyte EFN considerably less attractive to generalists that would probably be less effective partners ([Heil et al., 2005, 2009, 2010](#)). Further discussion of these highly specialized interactions and the role of EFNs within them can be found in [Heil et al. \(2010\)](#).

#### *Community ecology of ant–EFN mutualisms*

At the community scale, large numbers of ants and plants may be linked into EFN-mediated interaction networks. These complex interconnections have fascinated ecologists interested in ecological networks ([Bascompte and Jordano,](#)

[2007](#)). In this Highlight, [Díaz-Castellazo et al. \(2013\)](#) summarize the major results of their ongoing, uniquely long-term (20-year) study of one such network in Veracruz, Mexico. At present, 76 EFN-bearing plant species in 61 genera in 29 families interact with 54 ant species from 20 genera. Long-term analyses reveal shifts not only in plant and ant species identified within the network, but an increase in overall specialization of the ant community as a consequence of reduced abundance of two dominant, competitively superior generalists. Network organization is key to the maintenance of species diversity at the community scale ([Bascompte et al., 2003](#)); its persistent, relatively stable structure provides a hopeful indication that this particular ant–plant community network is fairly resilient to disturbance.

There is growing evidence that introduced EFN-bearing plant species can in some cases be readily incorporated into native ant–plant networks, suggesting that EFNs may be a trait that facilitates colonization of new habitats ([Lach et al., 2010](#)). Sometimes there may be detrimental consequences for the native plant community (which may be outcompeted by the invaders, losing the attention of its mutualist coterie). Similarly, invasive ants can be facilitated by the presence of native EFN-bearing flora, at the expense of native ants ([Savage et al., 2009](#); [Savage and Rudgers, 2013](#), this issue). Finally, invasive ants and plants can form highly successful mutualisms ([Koptur, 1979](#); [Eichhorn et al., 2011](#)), a phenomenon that some are concerned may result in ‘invasional meltdown’ ([Simberloff and Von Holle, 1999](#); [Green et al., 2011](#)) at the community level, as invasives facilitate each other’s population growth.

Interaction networks formed by EFN-bearing plants and their ants of course do not occur in isolation, but rather are embedded within much broader communities linked by trophic relationships. In this Highlight, [Savage and Rudgers \(2013\)](#) consider the case in which ants feed at both EFNs and honeydew-producing, phloem-feeding insects on the same plant. The former trophic interaction benefits the plant, whereas the latter harms it. The authors point out that the net effects on plants could not have been predicted from studying one form of interaction in isolation.

#### *EFN-mediated mutualisms in agriculture*

The ecological function of ants and EFNs has also been explored beyond natural communities. In agricultural systems, ants have been suggested to be promising biological control agents (e.g. [de la Fuente and Marquis, 1999](#); see also [Rico-Gray and Oliveira, 2007](#)), and crop plants with EFNs (including cashew nuts and peaches) have been shown to benefit from EFN-mediated ant protection (e.g. [Rickson and Rickson, 1998](#); [Mathews et al., 2009](#)). For instance, in some peach (*Prunus persica*) cultivars in which EFN expression has been suppressed, the plants experience significantly higher damage from herbivory, leading to reduced fruit production ([Mathews et al., 2009](#)). EFNs represent a valuable food resource for other beneficial insects, including ladybird beetles (e.g. [Pemberton and Vandenberg, 1993](#); [Lundgren, 2009](#)), making EFN-bearing plants suitable for pest control not only in agricultural systems, but for landscape gardening as well (e.g. [Mizell, 2001](#); [Wäckers and van Rijn, 2005](#)).

### Linking ecology with morphological diversity

Given the adaptive nature of EFNs, it is plausible to think that their exceptional morphological diversity could at least in part be related to differences in their ecological interactions with mutualists. Ants are known to exhibit preferences for certain types of EFNs, visiting certain morphologies more often than others (Díaz-Castelazo *et al.*, 2005). It is reasonable to suppose that plants with EFN morphologies preferred by ants benefit more from the protective service of ants than plants with EFNs that are least preferred and less visited (as speculated by Marazzi *et al.*, 2013, this issue). Comparative experimental studies are necessary to test whether plants with more attractive EFN locations and morphologies experience higher fitness benefits as a consequence.

## EFN EVOLUTION

The remarkable diversity and ecology of EFNs raise many questions regarding how such structures evolved. To address these questions, we must reconstruct their evolutionary history and assess levels of homology. We can then test hypotheses about their role in the evolution of the plants bearing them, of the ants feeding on them, and of the interactions they facilitate. Molecular phylogenetics offers powerful new tools to explore all these aspects in a historical framework (Butler and King, 2004; Weber and Agrawal, 2012). There are not only conventionally sized phylogenies, with up to a few hundred taxa, to use for this purpose, but also ‘mega-phylogenies’, with several hundred to several thousands of taxa (e.g. Bininda-Emonds, 2004; de Queiroz and Gatesy, 2007; Smith *et al.*, 2009). Phylogenetic studies of EFN evolution are still in their infancy (see Weber and Keeler, 2013, in this issue). Here, we summarize progress made in our understanding of EFN evolution and evolutionary role in a phylogenetic framework.

### Morphological evolution and homology

Trends and patterns in the evolution of EFNs at higher taxonomic levels have barely been explored in a phylogenetic context. To explain the vast phylogenetic and phenotypic diversity of EFNs, scientists have been invoking many independent origins and convergent or parallel evolution towards a similar ecological role (Beattie, 1985; Rico-Gray and Oliveira, 2007). Indeed, in this Highlight Weber and Keeler (2013) estimate that EFNs evolved independently at least 457 times in plants. As suggested in recent analyses of the huge diversity of EFNs in legumes using a mega-phylogeny of the family and the ‘precursor model’ of trait evolution (Marazzi *et al.*, 2012), it is possible that many EFNs are best explained as having arisen from shared evolutionary (genetic or developmental) precursors, rather than by multiple *de novo* origins and recent losses. This would be consistent with the idea that recurring origins of very similar traits in close relatives and striking convergences in more distant relatives may be associated with more cryptic genetic and developmental precursors (e.g. Sanderson and Hufford, 1996; Wake *et al.*, 2011).

Most of the existing work on EFNs in a phylogenetic framework actually focuses at or below the genus level (e.g. Marazzi and Sanderson, 2010; Weber *et al.*, 2012;

Marazzi *et al.*, 2013, this issue). Analyses at this relatively narrow but usually more thoroughly sampled taxonomic scale allow us to gain qualitative insight into the evolutionary history of EFNs in a specific system, unravelling ancestral EFN conditions as well as revealing levels of homology. Furthermore, combining such reconstructions with ontogenetic studies may provide additional insights about shared underlying developmental programs and the levels of homology among EFNs (Krosnick *et al.*, 2011; Marazzi *et al.*, 2013). In any case, it is likely that there were several kinds of ancestral EFN morphologies. For instance, there is still no support for the hypothesis that colleters, which secrete a viscous fluid to protect developing meristems, represent ancestral structures from which certain specialized EFNs are derived (Thomas, 1991).

### Factors influencing the evolutionary history of EFNs

Few studies to date have tested the adaptive nature of EFNs in a phylogenetic framework (Nogueira *et al.*, 2012b; Weber *et al.*, 2012). Using a densely sampled phylogeny of the tribe Bignonieae (Bignoniaceae), Nogueira *et al.* (2012b) showed that the inferred pattern of EFN evolution is better described by an adaptive model of evolution (incorporating both selection and drift) than by a neutral model of evolution (i.e. pure drift; see Butler and King, 2004). The authors suggested that two main factors underlying the evolution of EFNs and associated protective mutualisms are changes associated with the geographic distribution of plants and the emergence of new morphological structures in the same organ (or related ones) bearing the nectaries. In Bignonieae, the shift in habitat from forests to savannahs and especially the emergence of stipitate glandular trichomes (another defence trait) clearly influenced the evolution of EFNs, leading to a reduction in EFN abundance on the plants. Nogueira *et al.* (2012b) proposed that EFNs in drier environments would thus confer lower benefits to plants bearing them. In support of their hypothesis, experimental studies on two Bignonieae species from savannah habitats showed that although EFNs attracted ants, these provided no protective function, suggesting that they were retained due to phylogenetic inertia (Nogueira *et al.*, 2012a). Phylogenetic inertia has also been invoked to explain the occurrence of functional EFNs in plants in habitats lacking ants (Keeler, 1985).

While EFNs in Bignonieae are phylogenetically labile, in *Viburnum* (Adoxaceae; Weber *et al.*, 2012), as well as *Senna* (Marazzi and Sanderson, 2010), EFNs are phylogenetically conserved: in both genera they evolved once, then have been retained despite shifts among geographic regions and environments. Furthermore, in their study, Weber *et al.* (2012) showed that *Viburnum* species with both EFNs and leaf domatia, a trait mediating protective mutualistic interactions with mites, actually receive more benefits than any of the species with either one of the (indirect) defence traits. Therefore, while additional defence traits may reduce benefits from EFN-mediated mutualisms in some cases (e.g. Nogueira *et al.*, 2012b), they may actually enhance them in others (e.g. Weber *et al.*, 2012). In either case, the evolutionary history of EFNs is best understood when simultaneously considering all factors, biotic and abiotic, that can potentially influence their evolution.

## EFNs and plant diversification

Given the benefits that plants receive from mutualisms, it is plausible to expect that the evolution of traits that mediate these interactions influence at least to some degree the evolution of the plants that bear them. For example, many plants worldwide offer an elaiosome, a lipid-rich tissue attached to the seeds, to the ants that carry the seeds to their nest. Elaiosomes have recently been shown to be associated with increased diversification of plant lineages that possess them (Lengyel *et al.*, 2009, 2010). In the case of EFNs, evidence for the potentially key evolutionary role of EFNs has been provided in *Senna* (Marazzi and Sanderson, 2010). The large *Senna* EFN clade – characterized by conspicuous gland-like EFNs and comprising approx. 80 % of all species in the genus – evolved about 40 million years ago and diversified faster than its sister clade and the other older clades of the genus lacking such EFNs. To explain the distinctive geographic concentration of the EFN clade in South America, the authors speculated that EFNs may have promoted the colonization of new habitats appearing with the early uplift of the Andes. It is still unclear, however, whether EFNs represent key innovations in plant defences, and whether ant–plant protection mutualisms do indeed drive plant diversification.

## CONCLUSIONS

The exceptional diversity of extant EFNs, the complexity of their ecological interactions, and the multitude of their evolutionary histories make EFNs a particularly significant puzzle in plant biology. A wide range of studies – illuminating both narrow and broad taxonomic scales, unravelling both details and large-scale patterns, testing one-to-multiple hypotheses, and making fruitful use of phylogenies – have allowed us in recent years to dramatically increase our understanding. Yet, we acknowledge that major pieces of the puzzle are still missing. It seems very likely that there are more EFNs and EFN-bearing plant species than are currently known. In particular, small, cryptic EFNs are probably much more widespread than currently thought, and probably more important ecologically than currently recognized. Here, we have focused upon new research directions in the study of EFN diversity, ecology and evolution; the papers that follow in this Highlight section address these issues in depth. However, many questions remain to be addressed. Among the most poorly understood phenomena are the following. What are the evolutionary origins of EFNs? What genetic machinery underlies EFN development? How do ants locate EFNs? How expensive are EFNs, and extrafloral nectar, to produce, and are these costs partially responsible for their patchy distribution in nature? As we fill in the gaps in our knowledge of EFN biology, we will inevitably discover other missing pieces. The complex natural history of EFNs makes them endlessly fascinating, and their study offers exciting research opportunities for scientists from disparate fields of research.

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## LITERATURE CITED

- Angiosperm Phylogeny Group.** 1998. An ordinal classification for the families of flowering plants. *Annals of the Missouri Botanical Garden* **85**: 531–553.
- Angiosperm Phylogeny Group.** 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APGIII. *Botanical Journal of the Linnean Society* **161**: 105–121.
- Bartholmes C, Hidalgo O, Gleissberg S.** 2012. Evolution of the *YABBY* gene family with emphasis on the basal eudicot *Eschscholzia californica* (Papaveraceae). *Plant Biology* **14**: 11–23.
- Bascompte J, Jordano P.** 2007. Plant–animal mutualistic networks: the architecture of biodiversity. *Annual Review of Ecology, Evolution and Systematics* **38**: 567–593.
- Bascompte J, Jordano P, Melián CJ, Olesen JM.** 2003. The nested assembly of plant–animal mutualistic networks. *Proceedings of the National Academy of Sciences USA* **100**: 9383–9387.
- Beattie AJ.** 1985. *The evolutionary ecology of ant–plant mutualisms*. Cambridge: Cambridge University Press.
- Becerra JX, Venable DL.** 1991. The role of ant–Homoptera mutualisms in the evolution of extrafloral nectaries. *Oikos* **60**: 105–106.
- Bentley BL.** 1977. Extrafloral nectaries and protection by pugnacious bodyguards. *Annual Review of Ecology and Systematics* **8**: 407–427.
- Bininda-Emonds OR.** 2004. The evolution of supertrees. *Trends in Ecology & Evolution* **19**: 315–322.
- Bixenmann RJ, Coley PD, Kursar TA.** 2011. Is extrafloral nectar production induced by herbivores or ants in a tropical facultative ant–plant mutualism? *Oecologia* **165**: 417–425.
- Blüthgen N, Fiedler K.** 2004a. Preferences for sugars and amino acids and their conditionality in a diverse nectar-feeding ant community. *Journal of Animal Ecology* **73**: 155–166.
- Blüthgen N, Fiedler K.** 2004b. Competition for composition: lessons from nectar-feeding ant communities. *Ecology* **85**: 1479–1485.
- Blüthgen N, Reifenrath K.** 2003. Extrafloral nectaries in an Australian rainforest: structure and distribution. *Australian Journal of Botany* **51**: 515–527.
- Blüthgen N, Verhaagh M, Goitia W, Jaffe K, Morawetz W, Barthlott W.** 2000. How plants shape the ant community in the Amazonian rainforest canopy: the key role of extrafloral nectaries and homopteran honeydew. *Oecologia* **125**: 229–240.
- Blüthgen N, Gottsberger G, Fiedler K.** 2004. Sugar and amino acid composition of ant-attended nectar and honeydew sources from an Australian rainforest. *Austral Ecology* **29**: 418–429.
- Bowman JL, Smyth DR.** 1999. *CRABS CLAW*, a gene that regulates carpel and nectary development in *Arabidopsis*, encodes a novel protein with zinc finger and helix-loop-helix domains. *Development* **126**: 2387–2396.
- Bronstein JL.** 1998. The contribution of ant-plant protection studies to our understanding of mutualism. *Biotropica* **30**: 150–161.
- Butler MA, King AA.** 2004. Phylogenetic comparative analysis: a modeling approach for adaptive evolution. *American Naturalist* **164**: 683–695.
- Byk J, Del-Claro K.** 2011. Ant–plant interaction in the Neotropical savanna: direct beneficial effects of extrafloral nectar on ant colony fitness. *Population Ecology* **53**: 327–332.
- Casparry R.** 1848. De nectariis. Adolphum Marcum. *Botanische Zeitung* **6**: 628–630.
- Chamberlain SA, Holland JN.** 2009. Quantitative synthesis of context dependency in ant–plant protection mutualisms. *Ecology* **90**: 2384–2392.
- Cuautle M, Rico-Gray V.** 2003. The effect of wasps and ants on the reproductive success of the extrafloral nectaried plant *Turnera ulmifolia* (Turneraceae). *Functional Ecology* **17**: 417–423.
- Darwin C.** 1876. *The effects of cross and self-fertilisation in the vegetable kingdom*. London: John Murray.
- Darwin F.** 1877. On the nectar glands of the common brakefern. *Botanical Journal of the Linnean Society* **15**: 407–409.
- Davidson DW.** 1997. The role of resource imbalances in the evolutionary ecology of tropical arboreal ants. *Biological Journal of the Linnean Society* **61**: 153–181.
- Delpino F.** 1868–1875. Ulteriori osservazioni sulla dicogamia nel regno vegetale. I & II. *Atti della Società Italiana di Scienze Naturali*, XI, XII.
- Díaz-Castelazo C, Rico-Gray V, Ortega F, Angeles G.** 2005. Morphological and secretory characterization of extrafloral nectaries in plants of coastal Veracruz, Mexico. *Annals of Botany* **96**: 1175–1189.
- Díaz-Castelazo C, Sánchez-Galván IR, Guimarães PR Jr, Raimundo RLG, Rico-Gray V.** 2013. Long-term temporal variation in the organization of an ant–plant network. *Annals of Botany* **111**: 1285–1293.

- Eichhorn MP, Ratliffe LC, Pollard KM. 2011. Attraction of ants by an invasive *Acacia*. *Insect Conservation and Diversity* 4: 235–238.
- Emerson BC. 2002. Evolution on oceanic islands: molecular phylogenetic approaches to understanding pattern and process. *Molecular Ecology* 11: 951–966.
- Escalante-Pérez M, Heil M. 2012. Nectar secretion: its ecological context and physiological regulation. *Secretions and Exudates in Biological Systems* 12: 187–219.
- Escalante-Pérez M, Jaborsky M, Lautner S, et al. 2012. Poplar extrafloral nectaries: two types, two strategies of indirect defenses against herbivores. *Plant Physiology* 159: 1176–1191.
- Fahn A. 1979. *Secretory tissues in plants*. London: Academic Press.
- de la Fuente MAS, Marquis RJ. 1999. The role of ant-tended extrafloral nectaries in the protection and benefit of a neotropical rainforest tree. *Oecologia* 118: 192–202.
- Green PT, O'Dowd DJ, Abbott KL, Jeffery M, Retallick K, Nally RM. 2011. Invasional meltdown: invader–invader mutualism facilitates a secondary invasion. *Ecology* 92: 1758–1768.
- Hansen DM, Olesen JM, Mione T, Johnson SD, Müller CB. 2007. Coloured nectar: distribution, ecology, and evolution of an enigmatic floral trait. *Biological Reviews* 82: 83–111.
- Heads PA, Lawton JH. 1985. Bracken, ants and extrafloral nectaries. III. How insect herbivores avoid ant predation. *Ecological Entomology* 10: 29–42.
- Heil M. 2011. Nectar: generation, regulation and ecological functions. *Trends in Plant Science* 16: 191–200.
- Heil M, McKey D. 2003. Protective ant–plant interactions as a model system in ecological and evolutionary research. *Annual Review in Ecology, Evolution and Systematics* 34: 425–453.
- Heil M, Baumann B, Kruger R, Linsenmair K. 2004. Main nutrient compounds in food bodies of Mexican *Acacia* ant-plants. *Chemoecology* 14: 45–52.
- Heil M, Rattke J, Boland W. 2005. Post-secretory hydrolysis of nectar sucrose and specialization in ant/plant mutualism. *Science* 308: 560–563.
- Heil M, González-Teuber M, Clement LW, Kautz S, Verhaagh M, Bueno JCS. 2009. Divergent investment strategies of *Acacia* myrmecophytes and the coexistence of mutualists and exploiters. *Proceedings of the National Academy of Sciences USA* 106: 18091–18096.
- Heil M, Orona-Tamayo D, Eilmus S, Kautz S, González-Teuber M. 2010. Chemical communication and coevolution in an ant-plant mutualism. *Chemoecology* 20: 63–74.
- Huelsenbeck JP, Rannala B. 1997. Phylogenetic methods come of age: testing hypotheses in an evolutionary context. *Science* 276: 227–232.
- Huelsenbeck JP, Ronquist F, Nielsen R, Bollback JP. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* 294: 2310–2314.
- Keeler KH. 1979. Distribution of plants with extrafloral nectaries and ants at two elevations in Jamaica. *Biotropica* 11: 152–154.
- Keeler KH. 1985. Extrafloral nectaries on plants in communities without ants: Hawaii. *Oikos* 44: 407–414.
- Kelly CA. 1986. Extrafloral nectaries: ants, herbivores and fecundity in *Cassia fasciculata*. *Oecologia* 69: 600–605.
- Koptur S. 1979. Facultative mutualism between weedy vetches bearing extrafloral nectaries and weedy ants in California. *American Journal of Botany* 66: 1016–1020.
- Koptur S. 1989. Is extrafloral nectar production an inducible defense? In: Bock J, Linhart J. eds. *Evolutionary ecology of plants*. Boulder, CO: Westview Press, 323–339.
- Koptur S. 1992. Extrafloral nectary-mediated interactions between insects and plants. In: Bernays E. ed. *Insect–plant interactions, volume IV*. London: CRC Press, 81–129.
- Koptur S. 2005. Nectar as fuel for plant protectors. In: Wackers F, van Rijn PCJ, Bruin J. eds. *Plant-provided food for carnivorous insects*. Cambridge: Cambridge University Press, 75–108.
- Koptur S, Palacios-Rios M, Díaz-Castelazo C, Mackay WP, Rico-Gray V. 2013. Nectar secretion on fern fronds associated with lower levels of herbivore damage: field experiments with a widespread epiphyte of Mexican cloud forest remnants. *Annals of Botany* 111: 1277–1283.
- Krosnick S, Gasser C, Potter D. 2011. *Passiflora* as a model system for studying nectary diversification: insights and implications. *Abstract book of the XVI International Botanical Conference (IBC2011, Melbourne, Australia)*: 63–64. Available at [http://www.ibt2011.com/downloads/IBC2011\\_Abstract\\_Book.pdf](http://www.ibt2011.com/downloads/IBC2011_Abstract_Book.pdf).
- Kwok KE, Laird RA. 2012. Plant age and the inducibility of extrafloral nectaries in *Vicia faba*. *Plant Ecology* 213: 1823–1832.
- Lach L, Hobbs ER, Majer EJD. 2009. Herbivory-induced extrafloral nectar increases native and invasive ant worker survival. *Population Ecology* 51: 237–243.
- Lach L, Tillberg CV, Suarez AV. 2010. Contrasting effects of an invasive ant on a native and an invasive plant. *Biological Invasions* 12: 3123–3133.
- Lanan MC, Bronstein JL. 2013. An ant's-eye view of an ant–plant protection mutualism. *Oecologia*, in press. doi:10.1007/s00442-012-2528-0.
- Lee JY, Baum SF, Alvarez J, Patel A, Chitwood DH, Bowman JL. 2005a. Activation of CRABS CLAW in the nectaries and carpels of *Arabidopsis*. *Plant Cell* 17: 25–36.
- Lee JY, Baum SF, Oh SH, Jiang CZ, Chen CJ, Bowman JL. 2005b. Recruitment of CRABS CLAW to promote nectary development within the eudicot clade. *Development* 132: 5021–5032.
- Lengyel S, Gove AD, Latimer AM, Majer JD, Dunn RR. 2009. Ants sow the seeds of global diversification in flowering plants. *PLoS ONE* 4: pe5480.
- Lengyel S, Gove AD, Latimer AM, Majer JD, Dunn RR. 2010. Convergent evolution of seed dispersal by ants, and phylogeny and biogeography in flowering plants: a global survey. *Perspectives in Plant Ecology, Evolution and Systematics* 12: 43–55.
- Letourneau DK. 1983. Passive aggression: an alternative hypothesis for the *Piper–Pheidole* association. *Oecologia* 60: 122–126.
- Lundgren JG. 2009. Nutritional aspects of non-prey foods in the life histories of predaceous Coccinellidae. *Biological Control* 51: 294–305.
- Machado SR, Morelato LPC, Sajo MG, Oliveira PS. 2008. Morphological patterns of extrafloral nectaries in woody plant species of the Brazilian cerrado. *Plant Biology* 10: 660–673.
- Marazzi B, Sanderson MJ. 2010. Large-scale patterns of diversification in the widespread legume genus *Senna* and the evolutionary role of extrafloral nectaries. *Evolution* 64: 3570–3592.
- Marazzi B, McMahon MM, Lanan M, Bronstein JL. 2011. Diversity and evolution of extrafloral nectaries in Sonoran desert plants. *Abstract book of the XVI International Botanical Conference (IBC2011, Melbourne, Australia)*: 616 (e-poster P0882). Available at [http://www.ibt2011.com/downloads/IBC2011\\_Abstract\\_Book.pdf](http://www.ibt2011.com/downloads/IBC2011_Abstract_Book.pdf).
- Marazzi B, Ané C, Simon M, Delgado Salinas A, Luckow M, Sanderson MJ. 2012. Locating evolutionary precursors on a phylogenetic tree. *Evolution* 66: 3918–3930.
- Marazzi B, Conti E, Sanderson MJ, McMahon MM, Bronstein JL. 2013. Diversity and evolution of a trait mediating ant–plant interactions: insights from extrafloral nectaries in *Senna* (Leguminosae). *Annals of Botany* 111: 1263–1275.
- Mathews CR, Bottrell DG, Brown MW. 2009. Extrafloral nectaries alter arthropod community structure and mediate peach (*Prunus persica*) plant defense. *Ecological Applications* 19: 722–730.
- Mathews S. 2009. Phylogenetic relationships among seed plants: persistent questions and the limits of molecular data. *American Journal of Botany* 96: 228–236.
- McKey D. 1984. Interaction of the ant-plant *Leonardoxa africana* (Caesalpinaceae) with its obligate inhabitants in a rainforest in Cameroon. *Biotropica* 16: 81–99.
- McKey D. 1989. Interactions between ants and leguminous plants. In: Stirton CH, Zarucchi JL. eds. *Advances in legume biology. Monographs in Systematic Botany from the Missouri Botanical Garden* 29: 673–718.
- Melo Y, Machado SR, Alves M. 2010. Anatomy of extrafloral nectaries in Fabaceae from dry-seasonal forest in Brazil. *Botanical Journal of the Linnean Society* 163: 87–98.
- Miller TEX. 2007. Does having multiple partners weaken the benefits of facultative mutualism? A test with cacti and cactus-tending ants. *Oikos* 116: 500–512.
- Mizell RF. 2001. *Many plants have extrafloral nectaries helpful to beneficials*. University of Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, EDIS. <http://edis.ifas.ufl.edu/in175>.
- Mondor EB, Tremblay MN, Messing RH. 2006. Extrafloral nectary phenotypic plasticity is damage- and resource-dependent in *Vicia faba*. *Biology Letters* 2: 583–585.
- Morris WF, Wilson WG, Bronstein JL, Ness JH. 2005. Environmental forcing and the competitive dynamics of a guild of cactus-tending ant mutualists. *Ecology* 86: 3190–3199.
- Ness JH. 2003. *Catalpa bignonioides* alters extrafloral nectar production after herbivory and attracts ant bodyguards. *Oecologia* 134: 210–218.

- Ness JH, Morris WF, Bronstein JL. 2006. Integrating quality and quantity of mutualistic service to contrast ant species visiting *Ferocactus wislizeni*, a plant with extrafloral nectaries. *Ecology* **87**: 912–921.
- Ness JH, Morris WF, Bronstein JL. 2009. For ant-protected plants, the best defense is a hungry offense. *Ecology* **90**: 2823–2831.
- Nicolson SW. 2007. Nectar consumers. In Nicolson SW, Nepi M, Pacini E. eds. *Nectaries and Nectar*. Dordrecht: Springer, 289–342.
- Nicolson SW, Thornburg RW. 2007. Nectar chemistry. In: Nicolson SW, Nepi M, Pacini E. eds. *Nectaries and nectar*. Dordrecht: Springer, 215–264.
- Nicolson SW, Nepi M, Pacini E. 2007. *Nectaries and nectar*. Dordrecht: Springer.
- Nogueira A, Guimaraes E, Machado SR, Lohmann LG. 2012a. Do extrafloral nectaries present a defensive role against herbivores in two species of the family Bignoniaceae in a Neotropical savanna? *Plant Ecology* **213**: 289–301.
- Nogueira A, Rey PJ, Lohmann LG. 2012b. Evolution of extrafloral nectaries: adaptive process and selective regime changes from forest to savanna. *Journal of Evolutionary Biology* **25**: 2325–2340.
- O'Dowd DJ, Catchpole EA. 1983. Ants and extrafloral nectaries: no evidence for plant protection in *Helichrysum* spp.—ant interactions. *Oecologia* **59**: 191–200.
- Orona-Tamayo D, Wielsch N, Escalante-Pérez M, et al. 2013. Short-term proteomic dynamics reveal metabolic factory for active extrafloral nectar secretion by *Acacia cornigera* ant-plants. *The Plant Journal* **73**: 546–554.
- Pacini E, Nepi M. 2007. Nectar production and presentation. In: Nicolson SW, Nepi M, Pacini E. eds. *Nectaries and nectar*. Dordrecht: Springer, 167–214.
- Palmer TM, Doak DF, Stanton ML, et al. 2010. Synergy of multiple partners, including freeloaders, increases host fitness in a multispecies mutualism. *Proceedings of the National Academy of Sciences USA* **107**: 17234–17239.
- Pemberton RW. 1993. Observations of extrafloral nectar feeding by predaceous and fungivorous mites. *Proceedings of the Entomological Society of Washington* **95**: 642–643.
- Pemberton RW, Vandenberg NJ. 1993. Extrafloral nectar feeding by ladybird beetles (Coleoptera: Coccinellidae). *Proceedings of the Entomological Society of Washington* **95**: 139–151.
- Pulice CE, Packer AA. 2008. Simulated herbivory induces extrafloral nectary production in *Prunus avium*. *Functional Ecology* **22**: 801–807.
- de Queiroz A, Gatesy J. 2007. The supermatrix approach to systematics. *Trends in Ecology & Evolution* **22**: 34–41.
- Radhika V, Kost C, Mithöfer A, Boland W. 2010. Regulation of extrafloral nectar secretion by jasmonates in lima bean is light dependent. *Proceedings of the National Academy of Sciences* **107**: 17228–17233.
- Rashbrook VK, Compton SG, Lawton JH. 1993. Ant–herbivore interactions: reasons for the absence of benefit to a fern with foliar nectaries. *Ecology* **73**: 2167–2174.
- Rickson FR, Rickson MM. 1998. The cashew nut, *Anacardium occidentale* (Anacardiaceae), and its perennial association with ants: extrafloral nectary location and the potential for ant defense. *American Journal of Botany* **85**: 835–849.
- Rico-Gray V, Oliveira PS. 2007. *The ecology and evolution of ant–plant interactions*. Chicago: University Chicago Press.
- Rogers WE, Siemann E, Lankau RA. 2003. Damage induced production of extrafloral nectaries in native and invasive seedlings of chinese tallow tree (*Sapium sebiferum*). *The American Midland Naturalist* **149**: 413–417.
- Rosenzweig ML. 2002. The distraction hypothesis depends on relatively cheap extrafloral nectaries. *Evolutionary Ecology Research* **4**: 307–311.
- Ruhren S, Handel SN. 1999. Jumping spiders (Salticidae) enhance the seed production of a plant with extrafloral nectaries. *Oecologia* **119**: 227–230.
- Sanderson MJ, Hufford L. 1996. *Homoplasy: the recurrence of similarity in evolution*. New York: Academic Press.
- Savage AM, Rudgers JA. 2013. Non-additive benefit or cost? Disentangling the indirect effects that occur when plants bearing extrafloral nectaries and honeydew-producing insects share exotic ant mutualists. *Annals of Botany* **111**: 1295–1307.
- Savage AM, Rudgers JA, Whitney KD. 2009. Elevated dominance of extrafloral nectary-bearing plants is associated with increased abundances of an invasive ant and reduced native ant richness. *Diversity and Distributions* **15**: 751–761.
- Schmid R. 1988. Reproductive versus extra-reproductive nectaries – historical perspective and terminological recommendations. *Botanical Review* **54**: 179–232.
- Schmid VS, Schmid S, Steiner J, Zillikens A. 2010. High diversity of ants foraging on extrafloral nectar of bromeliads in the Atlantic rainforest of southern Brazil. *Studies on Neotropical Fauna and Environment* **45**: 39–53.
- Simberloff D, Von Holle B. 1999. Positive interactions of nonindigenous species: invasional meltdown? *Biological Invasions* **1**: 21–32.
- Smith LL, Lanza J, Smith GC. 1990. Amino acid concentrations in the extrafloral nectar of *Impatiens sultani* increase after simulated herbivory. *Ecology* **71**: 107–115.
- Smith S, Beaulieu J, Donoghue M. 2009. Mega-phylogeny approach for comparative biology: an alternative to supertree and supermatrix approaches. *BMC Evolutionary Biology* **9**: 37.
- Stott P, Pemberton RW. 1998. The occurrence and abundance of plants with extrafloral nectaries, the basis for antiherbivore defensive mutualisms, along a latitudinal gradient in East Asia. *Journal of Biogeography* **25**: 661–668.
- Thomas V. 1991. Structural, functional and phylogenetic aspects of the colleter. *Annals of Botany* **68**: 287–305.
- Tillberg CV, Breed MD. 2004. Placing an omnivore in a complex food web: dietary contributions to adult biomass of an ant. *Biotropica* **36**: 266–271.
- Trager MD, Bhotika S, Hostetler JA, et al. 2010. Benefits for plants in ant–plant protective mutualisms: a meta-analysis. *PLoS ONE* **5**: pe14308.
- Vogel S. 1997. Remarkable nectaries: structure, ecology, organophyletic perspectives. I. Substitutive nectaries. *Flora* **192**: 305–333.
- Vogel S. 1998a. Remarkable nectaries: structure, ecology, organophyletic perspectives. II. Nectarioles. *Flora* **193**: 1–29.
- Vogel S. 1998b. Remarkable nectaries: structure, ecology, organophyletic perspectives. IV. Miscellaneous cases. *Flora* **193**: 225–248.
- Wäckers FL. 2001. A comparison of nectar-and honeydew sugars with respect to their utilization by the hymenopteran parasitoid *Cotesia glomerata*. *Journal of Insect Physiology* **47**: 1077–1084.
- Wäckers FL, Bonifay C. 2004. How to be sweet? Extrafloral nectar allocation by *Gossypium hirsutum* fits optimal defense theory predictions. *Ecology* **85**: 1512–1518.
- Wäckers FL, van Rijn PC. 2005. *Food for protection: an introduction*. In: Wäckers FL, van Rijn PCJ, Bruin J. eds. *Plant-provided food for carnivorous insects: a protective mutualism and its applications*. New York: Cambridge University Press, 1–14.
- Wagner D, Fleur Nicklen E. 2010. Ant nest location, soil nutrients and nutrient uptake by ant-associated plants: does extrafloral nectar attract ant nests and thereby enhance plant nutrition? *Journal of Ecology* **98**: 614–624.
- Wagner D, Kay A. 2002. Do extrafloral nectaries distract ants from visiting flowers? An experimental test of an overlooked hypothesis. *Evolutionary Ecology Research* **4**: 293–305.
- Wake DB, Wake MH, Specht CD. 2011. Homoplasy: from detecting pattern to determining process and mechanism of evolution. *Science* **331**: 1032–1035.
- Weber MG, Agrawal AA. 2012. Phylogeny, ecology, and the coupling of comparative and experimental approaches. *Trends in Ecology & Evolution* **27**: 394–403.
- Weber MG, Keeler KH. 2013. The phylogenetic distribution of extrafloral nectaries in plants. *Annals of Botany* **111**: 1251–1261.
- Weber MG, Clement WL, Donoghue MJ, Agrawal AA. 2012. Phylogenetic and experimental tests of interactions among mutualistic plant defense traits in *Viburnum* (Adoxaceae). *The American Naturalist* **180**: 450–463.
- Whitney KD. 2004. Experimental evidence that both parties benefit in a facultative plant–spider mutualism. *Ecology* **85**: 1642–1650.
- Zimmerman JG. 1932. Über die extrafloralen Nektarien der Angiospermen. *Beihfte zum Botanischen Zentralblatt* **49**: 99–196.