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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 Reifennrath, 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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