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HIGHLIGHT ON ECOLOGY AND EVOLUTION OF EXTRAFLORAL NECTARIES

The phylogenetic distribution of extrafloral nectaries in plants

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• *Background and Aims* Understanding the evolutionary patterns of ecologically relevant traits is a central goal in plant biology. However, for most important traits, we lack the comprehensive understanding of their taxonomic distribution needed to evaluate their evolutionary mode and tempo across the tree of life. Here we evaluate the broad phylogenetic patterns of a common plant-defence trait found across vascular plants: extrafloral nectaries (EFNs), plant glands that secrete nectar and are located outside the flower. EFNs typically defend plants indirectly by attracting invertebrate predators who reduce herbivory.
• *Methods* Records of EFNs published over the last 135 years were compiled. After accounting for changes in taxonomy, phylogenetic comparative methods were used to evaluate patterns of EFN evolution, using a phylogeny of over 55 000 species of vascular plants. Using comparisons of parametric and non-parametric models, the true number of species with EFNs likely to exist beyond the current list was estimated.
• *Key Results* To date, EFNs have been reported in 3941 species representing 745 genera in 108 families, about 1–2 % of vascular plant species and approx. 21 % of families. They are found in 33 of 65 angiosperm orders. Foliar nectaries are known in four of 36 fern families. Extrafloral nectaries are unknown in early angiosperms, magnoliids and gymnosperms. They occur throughout monocotyledons, yet most EFNs are found within eudicots, with the bulk of species with EFNs being rosids. Phylogenetic analyses strongly support the repeated gain and loss of EFNs across plant clades, especially in more derived dicot families, and suggest that EFNs are found in a minimum of 457 independent lineages. However, model selection methods estimate that the number of unreported cases of EFNs may be as high as the number of species already reported.
• *Conclusions* EFNs are widespread and evolutionarily labile traits that have repeatedly evolved a remarkable number of times in vascular plants. Our current understanding of the phylogenetic patterns of EFNs makes them powerful candidates for future work exploring the drivers of their evolutionary origins, shifts, and losses.

Key words: Extrafloral, extranuptial, foliar, nectary, extrafloral nectary, phylogeny, taxonomy, distribution, mutualism, angiosperms, rosids, asteriids.

INTRODUCTION

Extrafloral nectaries (EFNs) are plant glands that secrete sugar, water and amino-acids (first called ‘extranuptual nectaries’; Delpino, 1886). Unlike floral nectaries, which function primarily in pollination, EFNs are commonly implicated in indirect plant defence, as they attract invertebrate predators whose presence and activity can reduce herbivory (Bentley, 1977). A large body of research has focused on understanding the ecology and physiology of EFNs, and they are frequently featured in studies of facultative mutualisms and indirect plant defence (Heil and McKey, 2003; Bronstein *et al.*, 2006). However, our ability to formulate and evaluate hypotheses about the evolutionary origins and ecological drivers of EFNs is limited by the lack of detailed knowledge concerning their phylogenetic distribution across plant clades. Here, we synthesize reports of EFNs in the literature and analyse the phylogenetic patterns of EFNs across vascular plants using modern comparative methods. Our goal is to provide a comprehensive evaluation of how many plants have EFNs and how they are distributed and evolving across plants, to identify regions of the plant tree-of-life that are in particular need of finer-scale studies of EFNs, and to facilitate the formulation of general hypotheses about the drivers of EFN evolution.

EFNs are highly diverse morphologically and include glandular structures that differ considerably in their location, size and form (Fig. 1). They have been described on almost every above-ground plant part, including leaves, petioles, bud bracts, stipules, stems, cotyledons, fruits and the outside of sepals (Elias, 1983). They include structures that range from single-cell nectar-secreting hairs, ‘formless’ glandular tissue, complex raised cups and shallow bowl-like depressions, and they range from highly vascularized to completely lacking vascularization (Elias, 1983). The high diversity of plant structures that fall under the name EFN is due in part to their definition, which is generally based on ecological function (nectaries not involved in pollination; Delpino, 1886), rather than their location, structure or developmental origin *per se* (nectaries on plant parts not related to the flower; Caspary, 1848). The present, weakly defined categories make the formulation of general hypotheses about the drivers of EFN evolution challenging, as different types of EFNs may not be homologous or may be influenced by different ecological and developmental factors. By incorporating a phylogenetic

FIG. 1. EFNs are diverse within and between taxa, and are found throughout vascular plants. (A) Ants feeding on the foliar nectaries of a fern frond, *Dyrnaria quercifolia*. (B, C) Monocots: sepal nectaries on *Dendrobium gatton* (B) and an unidentified orchid (C). (D) Malvales: sepal nectaries on *Hibiscus* sp. (E, F) Solanales: ant feeding on the foliar/petiolar nectaries on *Ipomoea carnea* (E) and sepal nectaries on *Ipomoea alba* (F). (G–I) Dipsacales: foliar nectaries on *Viburnum coriaceum* (G), marginal nectaries on *V. cinnamomifolium* (H) and petiolar nectaries on *V. opulus* (I). Photographs: (A) by Eric Belita; (B) by Suzanne Koptur; (C–F) by K.H.K., (G, H) by Patrick Sweeney, copyright 2011 Peabody Museum of Natural History; (I) by Gary Fawless, Cofrin Center for Biodiversity.

perspective in studies of EFN diversity, however, we can begin to disentangle the evolutionary history of origin, loss and morphological transition in EFNs, leading to informed hypotheses about the homology of various forms of this functionally defined trait. Furthermore, establishing the phylogenetic distribution of a trait will facilitate future testing of specific hypotheses concerning the ecological drivers of EFN evolution. For example, are there evolutionary correlations between EFN types and ecological factors previously predicted to influence EFN evolution, such as resource availability (McKey, 1989; Schupp and Feener, 1991; Heil and McKey, 2003), and mutualist abundance or aggressiveness (see Schupp and Feener, 1991)? Or, does the origin or loss of EFN correlate with other plant traits predicted to influence their ecology, such as a vine-like growth habit (Koptur, 1992), or the presence of additional mutualist rewards such as domatia (Weber *et al.*, 2012)?

Reports of EFNs have been published since at least the 1870s (Belt, 1874; Darwin, 1877; Poulsen, 1877), but potentially as early as 1762 (Hall cited in Bentley, 1977). Many reports are from taxonomic species descriptions, where EFNs are frequently noted as characters relevant to identification (e.g. Killip, 1938; Fryxell, 1978). EFNs have also been the specific subject of numerous morphological and anatomical studies (e.g. Bonnier, 1879; Lüttge, 1971). Several studies have extensively documented EFN prevalence by surveying specific habitats and locations, including Costa Rican forest and riparian habitats (Bentley, 1976) and the cerrado of southeast Brazil (Oliveira and Leitao-Filho, 1987; Machado *et al.*, 2008), east Asia (Pemberton, 1998) and various temperate

habitats (Keeler, 1980). In some cases, detailed surveys of EFN presence and morphology have been conducted within specific plant clades, e.g. *Macaranga* (Euphorbiaceae) (Fiala and Maschwitz, 1991), *Viburnum* (Adoxaceae) (Weber *et al.*, 2012) and *Senna* (Leguminosae, Cassiinae) (Marazzi *et al.*, 2006; Marazzi and Sanderson, 2010). Together, these sources contribute to the growing list of plant taxa known to have EFNs.

Over the past century, there have been several reviews of the taxonomic distribution of plants reported to have EFNs at the time of publication. Delpino (1886) calculated that 2900 species had 'extranuptial nectaries' and discussed their taxonomic and geographic distribution. Later, Zimmermann (1932) published an assessment of the distribution of plants with EFNs together with a categorization of nectary structure. Almost 50 years later, Bentley (1977) provided an abridged version of this report and included additional tropical families newly found to harbour EFNs. Elias (1983) added still more families to the growing list in his evaluation of terminology used to describe different morphological types of EFNs. In 1992, Koptur provided the most recent published synthesis of families and genera with EFNs (Koptur, 1992). However, all authors of these past reviews stressed that the number of species reported to have EFNs was likely to grow as new taxa were examined, and that our understanding of how EFNs are distributed across plants families is likely to change as additional species are evaluated. Indeed, the continued accumulation of additional reports of EFNs published over the last two decades, as well as substantial updates in plant systematics over the last century, merit a re-evaluation of how many plants have EFNs, and how they are dispersed across the plant phylogeny.

Previous reviews have noted that EFNs appear to be a phylogenetically widespread plant trait and hypothesized that they have been gained and lost many times independently across the plant tree of life (Bentley, 1977; Bronstein *et al.*, 2006). Such a pattern would suggest that EFNs exhibit high evolutionary convergence, consistent with their distribution being influenced by forces such as adaptation to ecological factors. Recent technological advances in building and analysing large phylogenies allow us to evaluate these hypotheses in a broad phylogenetic context. Thus, by consolidating published reports of EFNs over the last 135 years, we provide a summary of the taxonomic distribution of plants reported as having EFNs and assess their evolutionary patterns using widely sampled phylogenies of vascular plants. In particular, we analyse the taxonomic and phylogenetic patterns of plants reported to have EFNs, discuss what insights these patterns provide concerning the drivers of EFN evolution, and provide statistical estimates of how these patterns may change with the discovery of new EFN taxa in the future.

MATERIALS AND METHODS

To document the taxonomic distribution of plants described as having EFNs, K.H.K. began compiling published reports and personal observations of plants with EFNs in the late 1970s (Keeler, 2008). This 'World List of Plants with Extrafloral Nectaries' (hereafter, World List), includes information on the genus, species and family of all plants reported to have EFNs over the last 135 years, from 1877 (Poulsen, 1877) to January 2012. This list incorporates previously published lists of EFN-bearing taxa (Zimmermann, 1932; Schnell *et al.*, 1963; Elias, 1983; Koptur, 1992). When available, the position of the EFN on the plant, the species' common name, and the plant's growth habit were also included. All information in the World List was deposited in an open-source, online database (Keeler, 2008) that is publicly available as a resource for those studying EFNs.

Because the taxonomic nomenclature of plants in the World List has changed since many of the original reports were published, we updated all names of plants documented as having EFNs to their current Angiosperm Phylogeny Group (Angiosperm Phylogeny Group, 2009) taxonomic classifications. To do this, we cross-referenced the World List with the International Plant Names Index (http://www.ipni.org/index.html), the World Checklist of Selected Plant Families (http://apps.kew.org/wcsp/) and Tropicos (http://www.tropicos.org) using Plantminer (Gustavo *et al.*, 2010). All synonyms were replaced with current accepted names for analyses. We further amended the list by omitting EFN reports in which we had low confidence. We omitted (*a*) families with only a single, unpublished report of EFNs: Aristolochiaceae and Clusiaceae; (*b*) families with a single, published report of EFNs that could not be confirmed: Alismataceae (Schnell *et al.*, 1963), Ancistrocladaceae (Metcalfe 1951 in Koptur, 1992), Annonaceae (Koptur, 1992), Bruneliliaceae (Watson and Dallwitz, 1992 onwards), Caryophyllaceae (Bentley, 1977), Goodeniaceae (Bentley, 1977), Hydrangeaceae (Zimmermann, 1932), Icacinaceae (Koptur, 1992), Musaceae (Koptur, 1992), Olacaeae (Metcalf and Chalk, 1971) and Stryracaceae (Vesque, 1886); and (*c*) genera without any species identification, if they could not be confirmed by other means (26 genera). Additionally, Nepi *et al.* (2009) call the nectaries of *Gnetum cuspidaturm* extrafloral, but because Kato *et al.* (1995) demonstrated their function in pollinator reward, so we omit them here. While these omissions risk potentially discarding taxa with EFNs, they conservatively deal with potential false-positive EFN reports.

Estimating the total number of species with EFNs

Because our understanding of the taxonomic and phylogenetic distribution of EFNs is likely to change as more species with EFNs are discovered, we used a model comparison framework to estimate the number of unreported cases of EFNs likely to exist beyond the current publication list. In particular, we utilized a general methodology designed for the estimation of total number of classes in a population from observed frequency count data (Bunge, 2011). For each species in the World List (other than the omitted cases above), we obtained a 'frequency count' based on the number of times EFNs have been reported for that species in the literature. We obtained the publication count for each species in the World List using the following search terms in Google Scholar: the genus name, the species name and either (*a*) 'extrafloral nectar*', (*b*) 'extranuptial nectar*' (*c*) 'foliar nectar*' or (*d*) 'bract* nectar*'. This count included any works in the Google Scholar database as of 1 March 2012 that fitted our search criteria, including academic books, journal, conference abstracts, dissertations, theses and peer-eviewed articles. Our search did

not discriminate between multiple publications from the same or different authors (e.g. two publications from the same laboratory were treated the same as two publications from two different laboratories). Using this publication 'count data', we estimated the total number of species with EFNs, represented as the sum of the number of reported and the estimated number of as-yet-unreported species with EFNs, using CatchAll (Bunge, 2011), a program for analysing frequency count data from incidence-based samples. CatchAll uses maximum likelihood estimates and a combined heuristic/statistical model-selection algorithm to compare diversity estimate models across multiple levels of outlier deletion. We compared five non-parametric models (Good-Turing, Chao1, ACE, ACE1 and Chao–Bunge gamma-Poisson), and five parametric models (Poisson, single exponential mixed Poisson, and mixtures of two, three and four exponentials mixed Poisson) to find the best fitting estimate of total EFN richness (Bunge *et al.*, 2012).

Phylogenetic methods

To visualize the large-scale phylogenetic distribution of plant families reported to contain at least one species with EFNs, we mapped the presence/absence of species with EFNs on the family-level mega-tree from the Angiosperm Phylogeny Group (APGIII). The APGIII tree (Angiosperm Phylogeny Group, 2009) is a compilation of previously published plant phylogenies, and is intended to give the current best estimate of relationships among all plant families. We incorporated branch lengths using the program Phylocom (BLADJ; Webb *et al.*, 2008) according to age estimates from Wikström *et al.* (2001) based on fossil records and non-parametric rate-smoothing estimates. Patterns of EFN distribution across plant families were graphically displayed using iTOL (Letunic and Bork, 2007).

While the APGIII tree allows for a broad view of EFN distribution across seed plants, a phylogeny with finer-scale resolution is required to evaluate metrics such as phylogenetic signal. Thus, we calculated phylogenetic signal using a trimmed version of a consensus tree of maximum likelihood phylogenies of 55 437 seed plant species constructed using the gene regions *atpB*, *matK*, *trnK*, *trnL*, *rbcl* and *ITS* (Smith *et al.*, 2009). Taxa were selected for inclusion in this phylogeny in an unrelated study (Smith *et al.*, 2009) and thus should be neutral with respect to EFN presence (for a similar approach, see Sage *et al.*, 2011). For analyses, the 55 337 species tree was trimmed so that each genus was represented by only one tip (9745 genera) using the drop.tip function in the R package APE (Paradis *et al.*, 2004) which preserves topology and branch lengths. Using the trimmed phylogeny, we evaluated the phylogenetic signal of EFNs via the estimation of Fritz & Pervis' D for binary traits, which is a measure of sister-clade differences in a discrete character state for a given phylogeny (Fritz and Purvis, 2010). An estimated D of 1 represents a distribution of binary traits that is random with respect to the phylogeny, where as a D of 0 represents a distribution expected under Brownian motion (Fritz and Purvis, 2010). Similarly, a D of greater than 1 is more over-dispersed than expected at random, while a negative D is more phylogenetically clumped than expected under Brownian motion (Fritz and Purvis, 2010). Using the R package caper (R Development Core Team, 2010; Orme *et al.*, 2011), we calculated D for the presence of EFNs and, in order to assess significance, compared our estimate with simulated distributions of D under (*a*) randomly reshuffled trait values across the tips of the tree, and (*b*) trait evolution under Brownian motion. Each simulation included 1000 permutations. This approach preserves the phylogenetic relationships of our taxa as well as the number of species assigned to each character state, while varying the distribution of character states across the tree.

We inferred the number of gains and losses needed to explain the distribution of EFNs on the trimmed phylogeny from Smith *et al.* (2009) using stochastic character mapping (Huelsenbeck *et al.*, 2003) in SIMMAP (Bollback, 2006) and maximum parsimony criteria in GLOOME (Cohen *et al.*, 2010). Maximum parsimony calculates the fewest number of evolutionary gains and losses needed to explain the distribution of EFNs on the phylogeny, where the relative cost of gains and losses are equal. Stochastic character mapping infers the probability and expected number of gains and losses in EFNs based on the phylogeny and an underlying probabilistic model of character evolution. Unlike parsimony methods, stochastic character mapping can incorporate branch-length information and allows for multiple state changes to occur on a single branch. We utilized a beta distribution (starting alpha = 1, $k = 31$) on the bias prior for the two-state frequencies prior, and a gamma distribution (starting alpha = 1·25, beta = 0·25, $k = 90$) on the overall rate prior. The number of evolutionary events (gains $0 \rightarrow 1$, and losses $1 \rightarrow 0$) was estimated via the simulation of 1000 stochastic mutational maps, with each map having 20 draws from the prior distribution. We estimated the rate of gain and loss of EFNs across the phylogeny using maximum likelihood optimization and compared one- and two-rate Markov models (Pagel, 1994) in the diversitree package in R (R Development Core Team, 2010).

RESULTS

We found reports of EFNs in 3941 species of vascular plants representing 745 genera in 108 families (Table 1). Foliar nectaries have been reported in four fern families (39 species from seven genera), but are not known from bryophytes, gymnosperms, early angiosperms and magnoliids. EFNs occur in a variety of monocotyledons (260 species from 82 genera representing 15 families), including some true grasses (22 species from five genera), various dioscorea (71 species) and many orchids (77 species in 45 genera). EFNs are most common in eudicots (3642 species in 654 genera representing 89 families), with over half of all species with reported EFNs (2342 species) belonging to the rosid I clade (Table 1). EFNs have not been reported in the Apiales. The families with the most EFNs are Fabaceae (1069 out of approx. 19 500 species in Fabales), Passifloraceae (438 out of approx. 935 species in Malpighiales) and Malvaceae (301 out of approx. 4225 species in Malvales), while the genera with the most EFNs are *Passiflora* (322 species, Passifloraceae), *Inga* (294 species, Fabaceae) and *Acacia* (*sensu lato* 204 species, Fabaceae).

TABLE 1. *Taxonomic distribution of EFN reports in major clades of vascular plants*

Major clade(s)/order	Families with EFN/total families (%)	Genera with EFN/total genera (%)	Species with EFN/total species (%)	EFN location*
Early tracheophytes	**0** (0)	**0** (0)	**0** (0)	–
Ferns				
Cyatheales	**1**/8 (12·5)	**2**/15 (13)	**2**/663 (0·3)	L
Polypodiales	**3**/15 (20)	**7**/252 (2)	**37**/6962 (0·1)	L
Gymnosperms	**0**/15 (0)	**0**/79 (0)	**0**/850 (0)	–
Early angiosperms	**0**/7 (0)	**0**/12 (0)	**0**/175 (0)	–
Magnoliids	**0**/5 (0)	**0**/154 (0)	**0**/2929 (0)	–
Monocotyledons				
Alismatales	**2**/14 (14)	**4**/166 (2·4)	**9**/4560 (0·20)	L, Pt, Sm, Pd, Br
Asparagales	**4**/14 (28)	**51**/1122 (4·5)	**106**/26070 (0·40)	L, Se, Pd, F
Commelinales	**1**/5 (20)	**1**/68 (1·5)	**1**/812 (0·12)	Se
Dioscoreales	**1**/5 (20)	**2**/21 (9·5)	**71**/1037 (6·8)	L, Pt
Liliales	**2**/11 (18)	**3**/67 (4·5)	**7**/1558 (0·45)	L, Sm, Se
Poales	**3**/17 (18)	**11**/997 (1·1)	**35**/18325 (0·22)	L, Pt, Se, Pd, Br
Zingiberales	**2**/8 (25)	**10**/92 (11)	**31**/2111 (1·5)	L, Pt, Pd, Se, Br
Early eudicots				
Proteales	**1**/4 (25)	**6**/85 (7·1)	**6**/1710 (0·41)	L
Ranunculales	**2**/7 (29)	**3**/199 (1·5)	**4**/4445 (0·09)	L
Rosid I: Fabidae				
Cucurbitales	**1**/7 (14)	**23**/129 (18)	**41**/2295 (1·8)	L, Pt, Sm, Pd, Se
Fabales	**3**/4 (75)	**113**/754 (15)	**1020**/20055 (5·2)	L, Pt, Sp, Sm, Pd, Se
Fagales	**1**/8 (12·5)	**1**/33 (3·0)	**2**/1055 (0·19)	Br
Malpighiales	**13**/39 (33)	**136**/716 (19)	**1028**/15935 (6·5)	L, Pt, Sp, Sm, Pd, Se
Oxalidales	**2**/7 (29)	**2**/60 (3·3)	**2**/1815 (0·17)	L
Rosales	**3**/9 (33)	**18**/261 (6·9)	**249**/7725 (3·2)	L, Pt, Sp, Sm, Se
Rosid II: Malvidae				
Brassicales	**2**/17 (12)	**3**/398 (0·75)	**6**/4765 (0·13)	L, F
Crossosomatales	**1**/7 (14)	**1**/12 (8·3)	**1**/66 (1·5)	L
Malvales	**4**/10 (40)	**59**/338 (17)	**305**/6005 (5·3)	L, Pt, Sp, Pd, Se
Myrtales	**5**/9 (56)	**18**/380 (4·5)	**69**/11027 (0·63)	L, Pt, Sm, Pd
Picramniales	**1**/1 (100)	**1**/2 (50)	**1**/46 (2·2)	L
Sapindales	**5**/9 (56)	**32**/471 (6·6)	**57**/6070 (0·92)	L, Pt, Sm, Sp, Se, F
Other core eudicots				
Caryophyllales	**9**/34 (26)	**32**/811 (3·9)	**116**/11510 (0·95)	L, Pt, Sm, Se Ae, F
Ericales	**8**/25 (32)	**22**/346 (6·9)	**170**/11515 (1·5)	L, Pt, Se, Pd
Santalales	**1**/13 (7·7)	**1**/151 (0·66)	**2**/1992 (0·10)	Pd
Saxifragales	**2**/15 (13)	**2**/112 (1·8)	**4**/2470 (0·16)	Se
Vitales	**1**/1 (100)	**3**/14 (21)	**8**/850 (0·94)	St, Sm
Asterid I: Solanidae				
Gentianales	**3**/5 (60)	**37**/1118 (3·3)	**55**/16637 (0·34)	L, Pt, Sm, Pd, Se
Lamiales	**13**/23 (52)	**96**/1059 (9·6)	**292**/23810 (1·2)	L, Pt, Sm, Pd, Se, F
Solanales	**2**/5 (40)	**19**/165 (12)	**107**/4080 (2·7)	L, Pt, Sm, Pd, Se
Asterid II: Asteridae				
Aquifoliales	**1**/5 (20)	**1**/21 (4·8)	**1**/536 (0·19)	L
Asterales	**3**/11 (27)	**22**/1743 (1·5)	**46**/26870 (0·19)	L, Se
Dipsacales	**1**/7 (14)	**2**/45 (4·4)	**48**/1090 (4·4)	L, Pt, St

Numbers of families, genera and species in each order are from (Stevens, 2001 onwards) APGIII. If Stevens (2001 onwards) reports a range of counts for a clade, we used the highest number given. Early angiosperms include the Amborellales, Nymphaeales and Austrobaileales. The only family with EFNs that is not included here is Boraginaceae, an unplaced Asteroid family that includes two EFN species from the same genus (*Cordia dentata* and *C. spinescens*).

* Where available, the EFN location is provided: L, leaf; Pt, Petiole; Sp, stipules; Sm, stem; Pd, pedicels; peduncles or stems of inflorescence; Sl, sepals/calyx/perianth/tepals/floral bracts/cataphylls; Br, Leaf bracts/leaf buds; F, fruit.

Estimating the total number of species with EFNs

Comparisons of frequency count estimation models based on searches of publication records suggest that the total number of plants with EFNs are best explained by a model with a finite mixture of three geometric distributions (Table 2). The goodness-of-fit statistics GOF5 (the corrected χ^2 *P*-values) for the four best models were well over 0·01, indicating they displayed good fit to the data. The non-parametric and other parametric models (Poisson, inverse Gaussian, negative binomial and log-normal mixed Poisson) provided inferior fits. The best-fitting model estimated the total number of species with EFNs to be 8184 species (s.e. $\pm$ 392) species. The next three best-fitting models provided slightly higher, but quantitatively similar, estimates of total number of species with EFNs (8314 $\pm$ 460, 8318 $\pm$ 492 and 8482 $\pm$ 423, respectively).

TABLE 2. *Predicted total number of species with EFNs from model estimates*

Model*		Tau	Estimated total species	s.e.	Lower CB	Upper CB
Best model	3 exponentials mixed Poisson	102	8184	392	7473·4	9013·9
Model 2	4 exponentials mixed Poisson	240	8318	492·8	7440·3	9379
Model 3	2 exponentials mixed Poisson	17	8483	423·4	7716·6	9380·6
Non-P 1	Chao1	2	5348	146	5081	5654·3
Non-P 2	ACE1	10	7336	281·4	6821·1	7926·1

Tau, The upper frequency cut-off; s.e., the standard error of the estimate; Lower and Upper CB, the lower and upper 95 % confidence bound.
* The model with the best overall fit is listed first, followed by five alternative models: Best model, Model 2 and Model 3 = top three selected parametric models; Non-P 1 and Non-P 2 = top non-parametric models. 2–4 exponentials mixed Poisson = models with stochastic abundance distribution as a mixture of multiple exponentials and a two-geometrics mixed Poisson distribution.

Phylogenetic patterns

Plant species reported to possess EFNs are widely scattered across vascular plant lineages (Fig. 2). The distribution of EFNs across vascular plants exhibited a moderate level of phylogenetic signal ($D = 0{\cdot}56$). Simulation tests indicated that, while the phylogenetic pattern differed significantly from the Brownian expectation (probability of estimating D under Brownian evolution $<0{\cdot}001$), it also differed significantly from 1 (probability of estimating D with random phylogenetic structure $<0{\cdot}001$).

Phylogenetic analyses strongly support a high number of repeated gain and loss of EFNs across plant clades. Parsimony methods revealed that a minimum of 457 independent gains and 41 losses are required to explain the distribution of EFNs across a broadly sample seed plant phylogeny. Using stochastic character-mapping methods, however, the expected number of gains and losses was estimated to be much higher: 701 gains and 316 losses, respectively.

DISCUSSION

The phylogenetic and taxonomic distribution of EFN reports in plants

To date, EFNs have been reported in approx. 1·0–1·8 % of flowering plant species, depending on total plant species estimates (Govaerts, 2001; Scotland and Wortley, 2003). However, we estimate that the number of unreported cases of EFNs is as high as the number already reported, suggesting that, in total, EFNs may be present in approx. 2·0–3·6 % of flowering plants. Almost all the currently reported species with EFNs are angiosperms (99·7 %, the remaining 0·3 % being ferns) and 93 % of those are eudicots. The majority of EFNs are found in two orders of rosids: the Malpighiales (26·0 % of all EFN reports) and the Fabales (25·8 % of all reports). Although EFNs are widespread, almost half of the angiosperm orders (33 out of 65) lack them, and EFNs appear to be entirely absent in gymnosperms, early angiosperms and early ferns. Reports of species with EFNs represent at least 457 independent lineages that are widely distributed across vascular plants. Models of their evolution strongly support a high number of independent evolutionary gains and losses of EFNs across plant clades, especially in more-derived eudicot families. Taken together, our study confirms that EFNs are a relatively common and phylogenetically widespread plant trait. Their broad distribution, repeated evolution and moderate phylogenetic signal across vascular plants are consistent with ecological research suggesting that selection and trait conservatism have a role in shaping their distribution.

Phylogenetic analyses using Smith's broadly sampled vascular plant phylogeny (Smith, 2009) revealed that the distribution of EFNs displays a moderate phylogenetic effect. We found that EFN evolution deviates significantly from the pattern expected under strict Brownian motion, a result that reflects the many single reports of EFN-bearing species in otherwise EFN-free genera, families or even orders. On the other hand, the distribution of EFNs across plants also deviates significantly from a pattern that is random with regard to phylogeny, and obvious instances of phylogenetic clustering do exist. Most notably, the majority of all species with EFNs (59 %) are found in one major clade of rosids (Table 1). EFNs are also frequently phylogenetically clustered within smaller clades. For instance, they are present on all species of at least two small (non-monotypic) families (the Ebenaceae and the Thomandersiaceae), and in all, or virtually all, species of some genera (*Passiflora*, Passifloraceae; *Inga*, Fabaceae; *Populus*, Salicaceae; *Gossypium*, Malvaceae). In at least two genera with a mix of EFN and non-EFN species, clade-specific phylogenetic studies have demonstrated that the EFN-bearing species form monophyletic groups [*Senna* (Marazzi and Sanderson, 2010); *Viburnum* (Weber *et al.*, 2012)]. Additionally, in some families and genera, e.g. Fabaceae, Polygonaceae and *Senna*, there is enough clustering of plants with EFNs to utilize the trait as an informative taxonomic character (Fryxell, 1978; Marazzi *et al.*, 2006; Sanchez *et al.*, 2009).

Our phylogenetic assessment strongly agrees with previous work suggesting that EFNs are an evolutionarily 'labile' trait that has evolved convergently many times in plants (Bentley, 1977; Elias, 1983; Koptur, 1992). Furthermore, EFNs repeatedly arise on the same plant parts in distantly related clades, in particular the stipules, bracts, lower leaf surface, leaf margins and leaf petioles (see Table 1). This pattern is striking, and future investigations into its causes (i.e. adaptation, constraint) are warranted. While the results described here offer a first estimation of the total number of evolutionary events needed to explain EFN distributions in plants, they should be considered as rough estimates of the actual number of EFN evolutionary gains and losses that have occurred during the

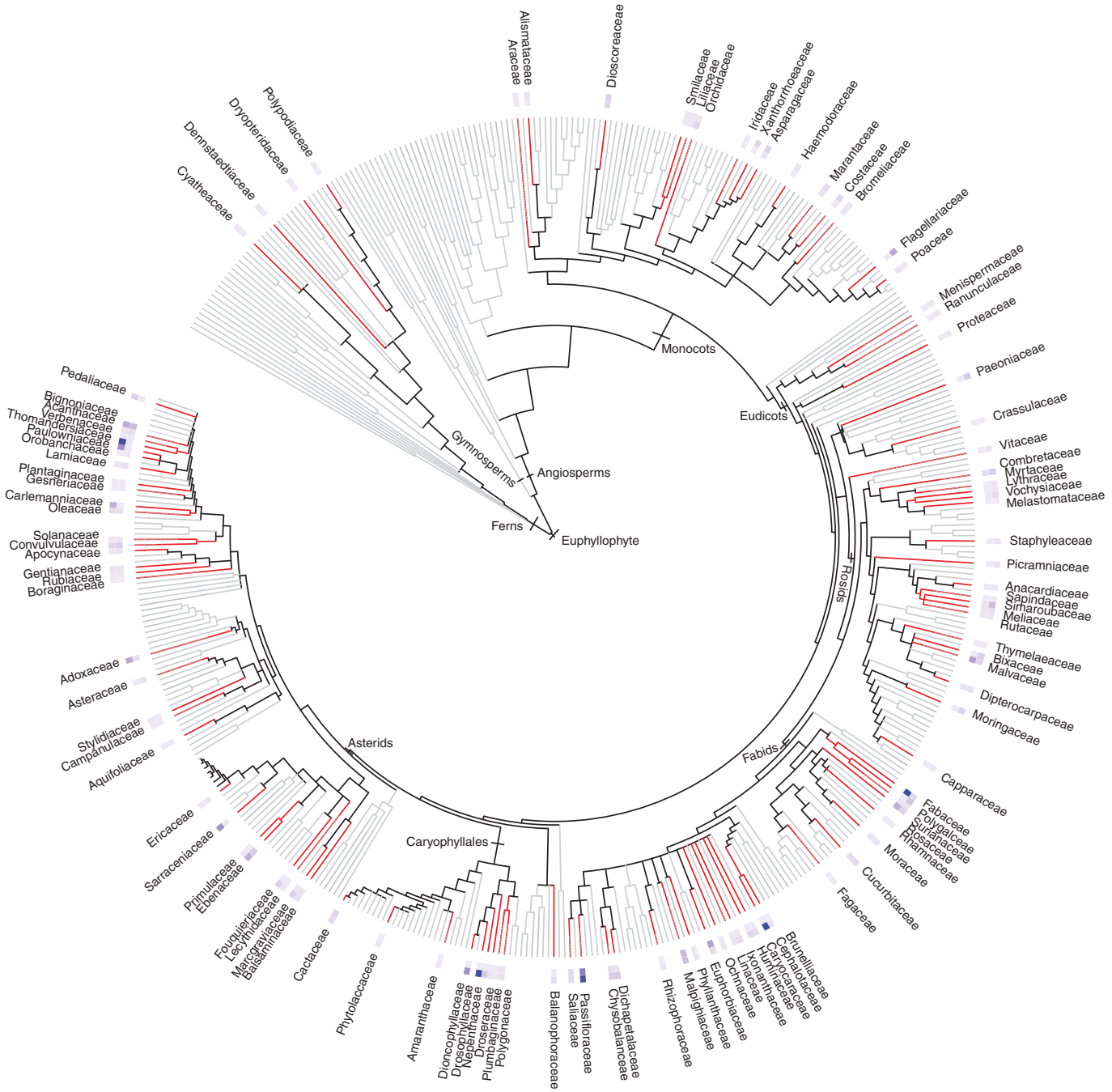

FIG. 2. The phylogenetic distribution of plant families containing species reported to have EFNs (Angiosperm Phylogeny Working Group, 2009). Branch lengths are according to fossil information from Wilkström *et al.* (2001). Family names are only given for those families with EFNs. Ancestral branches leading only to families containing reports of EFNs are coloured in red. Branches whose daughters include EFNs and non-EFN families are indicated in black. Blue boxes surrounding the phylogeny are shaded according to the number of species with EFNs in that family (inner ring), and the percentage of species with EFNs in that family (outer ring), with light shading representing low numbers and dark shading representing high numbers. The number of species with EFNs in a family (inner ring) range from 1069 species (Fabaceae) to 0 species. The percentage of species with EFNs (outer ring) ranges from 100 % (Cephalotaceae, Thomadersiaceae, Drosophyllaceae) to 0 %.

evolutionary history of living plant lineages. Ideally, the accumulation of targeted studies conducted at narrower taxonomic scales, with finer phylogenetic resolution, will further refine our understanding of the precise number and type of evolutionary transitions that have occurred in EFN evolution across the vascular plant phylogeny.

Future directions in studying EFN evolution

The list of plant taxa with EFNs has been steadily growing since the first reports before 1900. Here, new published reports of EFN species along with changes in taxonomy bring the list up to 108 families of flowering plants containing 745 genera. The accumulation of this type of character and natural-history

TABLE 3. *Areas in need research in the study of EFN evolution and distribution in plants*

General area	Examples of hypotheses	Suggested approach
Elucidating patterns of EFN evolution	(1) EFNs have a higher rate of evolution in certain clades (e.g, legumes, McKey, 1989)	Evaluate EFNs presence or confirm absence on herbarium and live specimens, place distribution into an explicit phylogenetic context (e.g. Marazzi *et al.*, 2006; Marazzi and Sanderson, 2010), fit multi-rate and single-rate models of character evolution.
Ecological and environmental drivers of EFN evolution	(1) EFNs are tropical adaptations (Koptur, 1992) (2) EFNs evolve in response to resource availability (McKey, 1989; Schupp and Feener, 1991; Heil and McKey, 2003) (3) Mutualist abundance and/or aggression is a driver of EFN gain/loss (see Keeler, 1985; Schupp and Feener, 1991) (4) EFNs evolved as defences against ant-homoptera mutualisms (Becerra and Venable, 1989)	Comparative phylogenetic studies that test for correlations between EFNs and putative adaptive factor (e.g. Weber *et al.*, 2012). Are origins and losses in EFNs correlated with: Moves into and out of the tropics? Carbon-rich habitats with continuously flushing leaves? Habitats with high mutualist abundance/ aggression? Susceptibility to ant-tended homoptera? A coupling of identifying phylogenetic patterns and experimental manipulations may be particularly useful in untangling ecological drivers of EFN evolution (Weber and Agrawal, 2012).
Evolutionary interactions between EFNs and other plant traits (trade-offs, synergisms & constraints)	(1) EFNs are more likely to evolve in vines than other growth habits (Bentley, 1977; Koptur, 1992) (2) EFNs evolutionarily trade-off with other defence traits (Rehr *et al.*, 1973; Levin, 1976; Stewart and Keeler, 1988) (3) Some EFNs, especially small laminar EFNs have evolved with mite domatia, providing 'Bed and breakfast' for predaceous and fungivorous mites (Weber *et al.*, 2012).	Comparative phylogenetic studies that test for correlations between EFNs and traits while accounting for shared history (e.g., Rudgers *et al.*, 2004; Weber *et al.*, 2012). Negative correlations are consistent with fitness trade-offs or constraints, while positive correlations suggest synergisms or adaptive trait pairings.
Assessing EFN origin and homology among nectary types	(1) EFNs within eudicots are homologous and share common genetic controls (Lee *et al.*, 2005*a*) (2) EFNs and floral nectaries are homologous (3) Hydathodes are evolutionary precursors to EFNs (Elias and Gelband, 1977)	Phylogenetically informed developmental genetics (e.g. Lee *et al.*, 2005*a*)

Examples of specific hypotheses for each area are provided, along with a suggested approach and citations of example studies, where available.

information is crucial for synthetic investigations of their distribution and evolutionary history. As new species with EFNs continue to be discovered, our understanding of their broad distributional patterns will undoubtedly continue to shift. Indeed, model selection methods estimate that that the number of unreported cases of EFN may be higher than the number of species already reported. Thus, we suggest that studies documenting EFN presence and absence in additional plant groups will be particularly valuable in the future of EFN evolutionary biology (Table 3). These studies will be especially important in large families already rich in plants reported to have EFNs, such as the Bignoniaceae (140 out of 800 species reported with EFN), Euphorbiaceae (286 out of 5735 species) and Malvaceae (293 out of 4425 species). We hypothesize these already EFN-rich clades will prove to contain the bulk of as yet unreported EFN taxa. Inconspicuous EFN morphologies are also likely to be underrepresented in the literature, and studies that systematically check for specific EFN structures or locations (such as formless or small laminar EFN, Fig. 1) are likely to be successful in uncovering additional taxa.

Whereas the overall number of plants reported to have EFNs will undoubtedly increase with additional surveys, some reports of EFNs may eventually prove to be false positives. Not all extrafloral plant glands are nectaries (hydathodes and laticifers, in particular, have been confused with EFNs) and tests for sugar secretion were not always performed before reporting a gland as an EFN (for an example of sugar testing, see Pemberton, 1998). Thus, additional studies documenting sugar secretion will be necessary. Further, because the functions of the majority of EFNs have not been studied, most current discussions (including this one) cannot distinguish between EFNs that function in plant defence and those that reward pollinators (e.g. in Euphorbia and Australian acacias; Knox *et al.*, 1985) or attract prey (e.g. on carnivorous plants such as Droseraceae, Nepenthaceae and Sarraceniaceae). Future analyses should consider how the phylogenetic distribution of EFNs relates to variation in their functions.

Studies that pair clade-specific surveys of EFN presence and absence with phylogenetic comparative analyses hold particular promise for revealing the drivers of EFN evolution (Table 3). For example, testing for phylogenetic correlations between EFNs and ecological factors hypothesized to influence EFN evolution (such as nutrient availability, ant abundance or aggressiveness; Bentley, 1977; Schupp and Feener, 1991; Heil and McKey, 2003) can evaluate whether evolutionary patterns are consistent with ecological adaptation hypotheses (e.g. Weber *et al.*, 2012). Similarly, phylogenetic model testing can be used to ask whether EFNs are more likely to evolve in clades with certain plant traits than clades without those traits. This approach can be applied to the investigation of trade-off hypotheses (e.g. indirect defensive traits; Rudgers

TABLE 4. *Examples of genera with EFNs and published phylogenies, good candidates to include in replicated phylogenetic comparative studies of EFN evolution*

Genus	Family	Most recent published phylogeny
Ruellia	Acanthaceae	Tripp *et al.* (2008)
Viburnum	Adoxaceae	Clement and Donoghue (2011)
Philodendron	Araceae	Gauthier *et al.* (2008)
Impatiens	Balsaminaceae	Janssens *et al.* (2006)
Catalpa	Bignoniaceae	Li (2008)
Centaurea	Compositae	Garcia-Jacas *et al.* (2001)
Helianthus	Compositae	Timme *et al.* (2007)
Dioscorea	Dioscoreaceae	Wilkin *et al.* (2005)
Shorea	Dipterocarpaceae	Kamiya *et al.* (2005)
Croton	Euphorbiaceae	Berry *et al.* (2005)
Mallotus	Euphorbiaceae	Sierra *et al.* (2010)
Manihot	Euphorbiaceae	Chacun *et al.* (2008)
Acacia	Fabaceae	Miller and Bayer (2001)
Senna	Fabaceae	Marazzi and Sanderson (2010)
Byttneria	Malvaceae	Whitlock and Hale (2011)
Gossypium	Malvaceae	Cronn *et al.* (2002)
Hibiscus	Malvaceae	Pfeil *et al.* (2002)
Ficus	Moraceae	Jousselin *et al.* (2003)
Adenia	Passifloraceae	Hearn (2006)
Turnera	Passifloraceae	Truyens *et al.* (2005)
Prunus	Rosaceae	Shaw and Small (2004)
Populus	Salicaceae	Cervera *et al.* (2005)

et al., 2004) or tests of predispositions (e.g. EFNs evolve more frequently in vines than in other growth habits; Bentley, 1977). Studies that test for these patterns across multiple independent clades will be particularly influential in revealing general drivers of EFN evolution. Indeed, many plant clades with species reported to have EFNs already have published phylogenies available that could be used in comparative analyses of this sort (Table 4).

Finally, an understanding of the drivers of macroevolutionary patterns in EFNs will require assessments of trait homology across variable EFN forms. Most research to date on this topic has focused on orthologues of the *CRABS CLAW* gene, which is necessary for nectary development in arabidopsis (Bowman and Smyth, 1999; Baum *et al.*, 2001; Lee *et al.*, 2005*b*). Indeed, *CRABS CLAW* holds promise for unravelling the genetic control of EFNs across the core eudicots. Lee *et al.* (2005*a*) found that *CRABS CLAW* is conserved across species of rosids and asterids, despite their having morphologically different nectary structures (floral and extrafloral). This pattern suggests that EFN in core eudicots may share a common ontology despite being highly modified over evolutionary time. However, there is no evidence of *CRABS CLAW* activity in the EFNs of early eudicots (Lee *et al.*, 2005*a*), and we are unaware of studies that examine this relationship in ferns or monocots. Furthermore, while *CRABS CLAW* is necessary and can be sufficient for floral nectary formation in core eudicots, nectary formation on non-floral tissue requires the modification of several genes other than *CRABS CLAW* (Lee *et al.*, 2005*a*). Thus, an understanding of the genetic drivers of the origin, loss, and modification of EFN across the plant-tree of life will require more detailed evolutionary developmental genetic studies that incorporate non-eudicot clades (Table 3).

Conclusions

EFNs are relatively common and broadly distributed plant traits that often function to mediate ecologically widespread mutualistic interactions. They have originated and been lost a great number of times across vascular plants. They have evolved in ferns, monocotyledons and many eudicots, and repeatedly make evolutionary shifts onto similar locations (e.g. leaves, stipules, petioles) in disparate plant clades. Because of their widespread phylogenetic distribution and their ability to mediate mutualistic interactions between plants and arthropods, EFNs are a powerful trait for inclusion in comparative studies linking phylogenetic patterns to ecological hypotheses.

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LITERATURE CITED

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.

Baum SF, Eshed Y, Bowman JL. 2001. The *Arabidopsis* nectary is an ABC-independent floral structure. *Development* **128**: 4657–4667.

Becerra JXI, Venable DL. 1989. Extrafloral nectaries: a defense against ant–homoptera mutualisms? *Oikos* **55**: 276–280.

Belt T. 1874. *The naturalist in Nicaragua*. London: John Murry.

Bentley BL. 1976. Plants bearing extrafloral nectaries and the associated ant community: interhabitat differences in the reduction of herbivore damage. *Ecology* **57**: 815–820.

Bentley BL. 1977. Extrafloral nectaries and protection by pugnacious bodyguards. *Annual Review of Ecology and Systematics* **8**: 407–427.

Berry PE, Hipp AL, Wurdack KJ, Van Ee B, Riina R. 2005. Molecular phylogenetics of the giant genus *Croton* and tribe Crotoneae (Euphorbiaceae *sensu stricto*) using ITS and TRNL-TRNF DNA sequence data. *American Journal of Botany* **92**: 1520–1534.

Bollback JP. 2006. SIMMAP: stochastic character mapping of discrete traits on phylogenies. *BMC Bioinformatics* **7**: 88. http://dx.doi.org/10.1186/1471-2105-7-88.

Bonnier G. 1879. Les nectaries: étude critique, anatomique et physiologique. *Annales des Sciences Naturelles; Botanique* **VI**: 5–212.

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-domains. *Development* **126**: 2387–2396.

Bronstein JL, Alarcon R, Geber M. 2006. The evolution of plant–insect mutualisms. *New Phytologist* **172**: 412–428.

Bunge J. 2011. Estimating the number of species with CatchAll. In: Altman RB, Dunker AK, Hunter L. eds. *Proceedings of the Pacific Symposium on Biocomputing*. Singapore: World Scientific Publishing, 121–130

Bunge J, Woodard L, Böhning D, Foster JA, Connolly S, Allen HK. 2012. Estimating population diversity with CatchAll. *Bioinformatics* **28**:1045–1047.

Caspary R. 1848. De nectariis. Adolphum Marcum. *Botanische Zeitung* **6**: 628–630.

Cervera MT, Storme V, Soto A, *et al*. 2005. Intraspecific and interspecific genetic and phylogenetic relationships in the genus *Populus* based on AFLP markers. *Theoretical and Applied Genetics* **111**: 1440–1456.

Chacun J, Madrion S, Debouck D, Rodriguez F, Tohme J. 2008. Phylogenetic patterns in the genus *Manihot* (Euphorbiaceae) inferred

from analyses of nuclear and chloroplast DNA regions. *Molecular Phylogenetics and Evolution* **49**: 260–267.

Clement WL, Donoghue MJ. 2011. Dissolution of *Viburnum* section Megalotinus (Adoxaceae) of Southeast Asia and its implications for morphological evolution and biogeography. *International Journal of Plant Sciences* **172**: 559–573.

Cohen O, Aschkenazy H, Belinky F, Huchon D, Pupko T. 2010. GLOOME: gain loss mapping engine. *Bioinformatics* **26**: 2914–2915.

Cronn RC, Small RL, Haselkorn T, Wendel JF. 2002. Rapid diversification of the cotton genus (*Gossypium:* Malvaceae) revealed by analysis of sixteen nuclear and chloroplast genes. *American Journal of Botany* **89**: 707–725.

Darwin F. 1877. On the nectar glands of the common brake fern. *Journal of the Linnean Society of London (Botany)* **15**: 398–409.

Delpino F. 1886. Funzione mirmecofila nel regno vegetale. In: Memoire della R. Accademia delle Scienze dell'Istituto di Bologna, *Serie 4 **VII***: 215–392.

Elias TS. 1983. Extrafloral nectaries: their structure and distribution. In: Bentley B, Elias TS. eds. *The biology of nectaries*. New York, NY: Columbia University Press.

Elias TS, Gelband H. 1977. Morphology, anatomy, and relationship of extrafloral nectaries and hydathodes in two species of *Impatiens* (Balsaminaceae). *Botanical Gazette* **138**: 206–212.

Fiala B, Maschwitz U. 1991. Extrafloral nectaries in the genus *Macaranga* (Euphorbiaceae) in Malaysia: comparative studies of their possible significance as predispositions for myrmecophytism. *Biological Journal of the Linnean Society* **44**: 287–305.

Fritz SA, Purvis A. 2010. Selectivity in mammalian extinction risk and threat types: a new measure of phylogenetic signal strength in binary traits. *Conservation Biology* **24**: 1042–1051.

Fryxell PA. 1978. The natural history of the cotton tribe (Malvaceae, Tribe Gossypieae). College Station, TX: Texas A & M University Press.

Garcia-Jacas N, Susanna A, Garnatje T, Vilatersana R. 2001. Generic delimitation and phylogeny of the subtribe *Centaureinae* (Asteraceae): a combined nuclear and chloroplast DNA analysis. *Annals of Botany* **87**: 503–515.

Gauthier M-PL, Barabe D, Bruneau A. 2008. Molecular phylogeny of the genus *Philodendron* (Araceae): delimitation and infrageneric classification. *Botanical Journal of the Linnean Society* **156**: 13–27.

Govaerts R. 2001. How many species of seed plants are there? *Taxon* **50**: 1085–1090.

Gustavo HC, Cianciaruso MV, Batalha MA. 2010. Plantminer: a web tool for checking and gathering plant species taxonomic information. *Environmental Modelling & Software* **25**: 815–816.

Hearn DJ. 2006. *Adenia* (Passifloraceae) and its adaptive radiation: phylogeny and growth form diversification. *Systematic Botany* **31**: 805–821.

Heil M, McKey D. 2003. Protective ant–plant interactions as model systems in ecological and evolutionary research. *Annual Review of Ecology Evolution and Systematics* **34**: 425–453.

Huelsenbeck JP, Nielsen R, Bollback JP. 2003. Stochastic mapping of morphological characters. *Systematic Biology* **52**: 131–158.

Janssens S, Geuten K, Yuan Y, Song Y, Küpfer P, Smets E. 2006. Phylogenetics of *Impatiens* and *Hydrocera* (Balsaminaceae) using chloroplast atpB-rbcL spacer sequences. *Systematic Botany* **31**: 171–180.

Jousselin E, Rasplus J-Y, Kjellberg F. 2003. Convergence and coevolution in a mutualism: evidence from a molecular phylogeny of *Ficus*. *Evolution* **57**: 1255–1269.

Kamiya K, Harada K, Tachida H, Ashton PS. 2005. Phylogeny of *PgiC* gene in *Shorea* and its closely related genera (Dipterocarpaceae), the dominant trees in Southeast Asian tropical rain forests. *American Journal of Botany* **92**: 775–788.

Kato M, Inoue T, Negamitshu T. 1995. Pollination biology of *Gnetum* (Genetaceae) in a lowland mixed dipterocarp forest in Sarawak. *American Journal of Botany* **82**: 862–868.

Keeler KH. 1980. Distribution of plants with extrafloral nectaries in temperate communities. *American Midland Naturalist* **104**: 274–280.

Keeler KH. 1985. Plants with extrafloral nectaries in ecosystems without ants: Hawaii. *Oikos* **44**: 407–414.

Keeler KH. 2008. *World list of plants with extrafloral nectaries*. http://biosci-labs.unl.edu/Emeriti/keeler/extrafloral/Cover.htm.

Killip EP. 1938. The American species of Passifloraceae. *Field Meuseum of Natural History Botanical Series* **19**: 1–613.

Knox RB, Kenrick J, Bernhardt P, *et al*. 1985. Extrafloral nectaries as adaptations for bird pollination in *Acacia terminalis*. *American Journal of Botany* **72**: 1185–1196.

Koptur S. 1992. Extrafloral nectary-mediated interactions between insects and plants. In: Bernays E. ed. *Insect–plant interactions*. Boca Raton, FL: CRC Press, 81–129

Lee J-Y, Baum SF, Oh S-H, Jiang C-Z, Chen J-C, Bowman JL. 2005*a*. Recruitment of *CRABS CLAW* to promote nectary development within the eudicot clade. *Development* **132**: 5021–5032.

Lee JY, Baum SF, Alvarez J, Patel A, Chitwood DH, Bowman JL. 2005*b*. Activation of CRABS CLAW in the nectaries and carpels of *Arabidopsis*. *The Plant Cell* **17**: 25–36.

Letunic I, Bork P. 2007. Interactive Tree of Life (iTOL): an online tool for phylogenetic tree display and annotation. *Bioinformatics* **23**: 127–128.

Levin DA. 1976. The chemical defenses of plants to pathogens and herbivores. *Annual Review of Ecology and Systematics* **7**: 121–159.

Li J. 2008. Phylogeny of *Catalpa* (Bignoniaceae) inferred from sequences of chloroplast ndhF and cuclear ribosomal DNA. *Journal of Systematics and Evolution* **46**: 341–348.

Lüttge U. 1971. Structure and function of plant glands. *Annual Review of Plant Physiology* **22**: 23–44.

McKey D. 1989. Interactions between ants and leguminous plants. In: Stirton CH, Zarucchi JL. eds. *Advances in legume biology*. St Louis, MO: Missouri Botanical Garden.

Machado SR, Morellato 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, Endress PK, De Queiroz LP, Conti E. 2006. Phylogenetic relationships within *Senna* (Leguminosae, Cassiinae) based on three chloroplast DNA regions: patterns in the evolution of floral symmetry and extrafloral nectaries. *American Journal of Botany* **93**: 288–303.

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.

Metcalf CJ, Chalk L. 1971. *Anatomy of the dicotyledons*, 2nd edn. Oxford: Charedon Press.

Miller JT, Bayer RJ. 2001. Molecular phylogenetics of *Acacia* (Fabaceae: Mimosoideae) based on the chloroplast MATK coding sequence and flanking TRNK intron spacer regions. *American Journal of Botany* **88**: 697–705.

Nepi M, von Aderkas P, Wagner R, Mugnaini S, Coulter A, Pacini E. 2009. Nectar and pollination drops: how different are they? *Annals of Botany* **104**: 205–219.

Oliveira PS, Leitao-Filho HF. 1987. Extrafloral nectaries: their taxonomic distribution and abundance in the woody flora of cerrado vegetation in southeast Brazil. *Biotropica* **19**: 140–148.

Orme CDL, Freckleton RP, Thomas GH, Petzold T, Fritz SA. 2011. *caper: comparative analyses of phylogenetics and evolution in R*. http://R-Forge.R-project.org/projects/caper/.

Pagel M. 1994. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proceedings of the Royal Society of London Series B: Biological Sciences* **255**: 37–45.

Paradis E, Claude J, Strimmer K. 2004. APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics* **20**: 289–290.

Pemberton R. 1998. The occurrence and abundance of plants with extrafloral nectaries, the basis for antiherbivore defense mutualisms, along a latitudinal gradient in east Asia. *Journal of Biogeography* **25**: 661–668.

Pfeil BE, Brubaker CL, Craven LA, Crisp MD. 2002. Phylogeny of *Hibiscus* and the tribe Hibisceae (Malvaceae) using chloroplast DNA sequences of ndhF and the rpl16 intron. *Systematic Botany* **27**: 333–350.

Poulsen VA. 1877. Das extraflorale Nectarium bei *Batatas edulis*. *Botanische Zeitung* **35**: 780–782.

R Development Core Team. 2010. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. www.r-project.org.

Rehr SS, Feeny PP, Janzen DH. 1973. Chemical defence in central American non-ant-acacias. *Journal of Animal Ecology* **42**: 405–416.

Rudgers JA, Strauss SY, Wendel JE. 2004. Trade-offs among anti-herbivore resistance traits: insights from *Gossypieae* (Malvaceae). *American Journal of Botany* **91**: 871–880.

Sage RF, Christin P-A, Edwards EJ. 2011. The C4 plant lineages of planet Earth. *Journal of Experimental Botany* **62**: 3155–3169.

Sanchez A, Schuster Tanja M, Kron KA. 2009. A large-scale phylogeny of polygonaceae based on molecular data. *International Journal of Plant Sciences* **170**: 1044–1055.

Schnell R, Cusset G, Quenum M. 1963. Contribution a l'etude des glandes extra-florales chez quelques groupes de plantes tropicales. *Revue générale de botanique* **70**: 269–342.

Schupp EW, Feener DH. 1991. Phylogeny, lifeform, and habitat dependence of ant-defended plants in a Panamanian forest. In: Huxley CR, Cutler DC. eds. *Ant–plant interactions*. Oxford: Oxford University Press, 175–197.

Scotland RW, Wortley AH. 2003. How many species of seed plants are there? *Taxon* **52**: 101–104.

Shaw J, Small RL. 2004. Addressing the 'hardest puzzle in American pomology': phylogeny of *Prunus* sect. *Prunocerasus* (Rosaceae) based on seven noncoding chloroplast DNA regions. *American Journal of Botany* **91**: 985–996.

Sierra SEC, Kuiju KKM, Fišer Z, Aparicio M, van Welzen PC. 2010. The phylogeny of *Mallotus* s.str. (Euphorbiaceae) inferred from DNA sequence and morphological data. *Taxon* **59**: 101–116.

Smith SA, Beaulieu JM, Donoghue M. 2009. Mega-phylogeny approach for comparative biology: an alternative to supertree and supermatrix approaches. *BMC Evolutionary Biology* **9**: 37. http://dx.doi.org/10.1186/1471-2148-9-37.

Stevens PF. 2001 onwards. Angiosperm Phylogeny Website. Version 12, July 2012 [and more or less continuously updated since].

Stewart JL, Keeler KH. 1988. Are there tradeoffs among antiherbivore defenses in *Ipomoea* (Convulvalaceae) *Oikos* **53**: 79–86.

Timme RE, Simpson BB, Linder CR. 2007. High-resolution phylogeny for *Helianthus* (Asteraceae) using the 18S-26S ribosomal DNA external transcribed spacer. *American Journal of Botany* **94**: 1837–1852.

Tripp EA, Manos PS, Shykoff J. 2008. Is floral specialization an evolutionary dead-end? Pollination system transitions in *Ruellia* (Acanthaceae). *Evolution* **62**: 1712–1737.

Truyens S, Arbo MM, Shore JS. 2005. Phylogenetic relationships, chromosome and breeding system evolution in *Turnera* (Turneraceae): inferences from its sequence data. *American Journal of Botany* **92**: 1749–1758.

Vesque J. 1886. Caracteres des principales familles gamopetales. Tires de l'anatomie de la feuille. *Annales des Sciences Naturelles Botanique. Ser. 7*, **1**: 183–360.

Watson L, Dallwitz MJ. 1992 onwards. *The families of flowering plants retrieval*, version: 18 May 2012. http://delta-intkey.com

Webb CO, Ackerly DD, Kembel SW. 2008. Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics* **24**: 2098–2100.

Weber MG, Agrawal AA. 2012. Phylogeny, ecology, and the coupling of comparative and experimental approaches *Trends in Ecology & Evolution* **27**: 394–403.

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.

Whitlock BA, Hale AM. 2011. The phylogeny of *Ayenia, Byttneria*, and *Rayleya* (Malvaceae s. l.) and its implications for the evolution of growth forms. *Systematic Botany* **36**: 129–136.

Wikström N, Savolainen V, Chase MW. 2001. Evolution of the angiosperms: calibrating the family tree. *Proceedings of the Royal Society of London Series B: Biological Sciences* **268**: 2211–2220.

Wilkin P, Schols P, Chase MW, *et al*. 2005. A plastid gene phylogeny of the yam genus, *Dioscorea*: roots, fruits and Madagascar. *Systematic Botany* **30**: 736–749.

Zimmermann JG. 1932. Über die extraflorale Nectarien der Angiospermen. *Beihefte zum Botanishes Centralblatt* **49**: 99–196.