Context dependency of rewards and services in an Indian ant–plant interaction: southern sites favour the mutualism between plants and ants

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Abstract: Protection-based ant–plant mutualisms may vary in strength due to differences in ant rewards, abundance of protective ants and herbivory pressure. We investigated geographical and temporal variation in host plant traits and herbivory pressure at five sites spanning the distribution range of the myrmecophyte *Humboldtia brunonis* (Fabaceae) in the Indian Western Ghats. Southern sites had, on average, 2.4 times greater abundance of domatia-bearing individuals, 1.6 times greater extrafloral nectary numbers per leaf, 1.2 times larger extrafloral nectary sizes, 2.2 times greater extrafloral nectar (EFN) volumes and a two-fold increase in total amino acid and total sugar concentrations in EFN compared with northern sites. A strong protection-based mutualism with ants occurred at only one southern site where herbivory was highest, suggesting that investments in attracting ants correlate with anti-herbivore benefits gained from the presence of protective ants. Our results confirm a temporally stable north–south gradient in myrmecophytic traits in this ant-plant as several of these traits were re-sampled after a 5-y interval. However, the chemical composition of EFN varied at both spatial and short-term temporal scales suggesting that only repeated measurements of rewards such as EFN can reveal the real spectrum of trait variation in an ant–plant mutualistic system.

Key Words: amino acids, domatia, extrafloral nectar, geographical variation, *Humboldtia brunonis*, nectary, sugars

INTRODUCTION

Outcomes of ant–plant mutualisms span a dynamic spectrum of possibilities, determined by spatiotemporal variation in biotic and abiotic factors (Bronstein 1994, Díaz-Castelazo *et al.* 2013, Kersch & Fonseca 2005, Rico-Gray *et al.* 2012). Thus, protection benefits received by ant-plants could vary with herbivory pressure, and availability of protective ants (Barton 1986, Rudgers & Strauss 2004, Shenoy & Borges 2010); availability of protective ant partners may also be determined by quality and quantity of rewards, viz. extrafloral nectar (EFN) and nesting shelters (domatia) (González-Teuber *et al.* 2012, Shenoy *et al.* 2012).

Few studies have investigated the actual spectrum of outcomes in ant–plant interactions resulting from geographical variation in plant traits, viz. domatia (Fonseca 1999) and extrafloral nectaries (Rico-Gray *et al.* 1998, Rios *et al.* 2008). Furthermore, with few

exceptions (Rios *et al.* 2008, Rudgers & Strauss 2004), most research on geographical variation in ant–plant interactions has focused on multi-species communities (Rico-Gray & Oliveira 2007), possibly because, being usually spread over similar habitats, most ant-plants show little intraspecific variation in characters. Fewer studies have examined temporal variation in such systems (Díaz-Castelazo *et al.* 2013). However, since costs and benefits in mutualistic interactions are finely balanced, with context-dependent dynamics (Baker-Méio & Marquis 2012, Chamberlain & Holland 2008), investigating spatiotemporal intraspecific variation in ant-related host-plant traits in the light of contexts (e.g. herbivory pressure, presence of protective ants at the sites) is important to our understanding of such mutualisms.

We investigate spatial and temporal variation in protection mutualism in a unique myrmecophytic plant *Humboldtia brunonis* Wall. (Fabaceae), in which every individual produces EFN on young leaves and bracts of floral buds, but only some individuals produce domatia. The domatia are occupied by 16 species of ants and myriad other invertebrates, most notably an arboreal earthworm

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Perionyx pullus Stephenson (Megascolecidae). However, only one ant species, *Technomyrmex albipes* (Smith) (Dolichoderinae), provides significant anti-herbivore protection (Gaume *et al.* 2005, Shenoy & Borges 2010). In an earlier study, done at three sites approximately 5 y before the present one, the geographical variation in protection indicated stronger ant–plant interaction towards the southern portion of the plant's distribution range in the Indian Western Ghats which exhibit a north–south gradient of seasonality in rainfall (Shenoy & Borges 2010). We hypothesize that the dynamics of the interaction between a host plant species and its associated ants varies over space and time, such that stronger mutualism, characterized by increased rewards to ants by the host plant and greater protection by the partner ant, would occur under conditions of greater herbivory pressure and at sites where protective ants are present. In the present study, we recorded five ant-related traits of *H. brunonis*, viz. (1) abundance of domatia-bearing trees, (2) number of extrafloral nectaries per leaf, (3) size of foliar nectaries, (4) volume of EFN per leaf and (5) composition of foliar EFN. We sampled foliar EFN since *T. albipes* prefers foliar EFN rich in essential amino acids and low sugar : amino acid ratios (Shenoy *et al.* 2012). We also recorded herbivory pressure and ant protection to leaves and floral buds at the five sites. Of these, data on volume and composition of EFN were also available from the earlier study at three sites, as were data on herbivory pressure and ant protection. Our study therefore re-sampled these site-specific factors to examine temporal stability in them, and additionally investigated spatial variation in three new ant-related plant traits, viz. abundance of domatia-bearing trees, number of nectaries per leaf, and nectary size, to confirm an earlier suggested north–south gradient in myrmecophytism in this species.

METHODS

Study system and sites

Humboldtia brunonis is an understorey tree, endemic to the low-elevation, tropical wet evergreen forests of the Western Ghats of India (Pascal 1988). It is polymorphic for the presence of domatia – modified swollen hollow internodes – which are inhabited by ants and various other invertebrates, including an arboreal earthworm. Domatia in southern sites are occupied mostly by ants; those in the north are dominated by earthworms (Shenoy & Borges 2010). Foliar nectaries are present on each of the two pairs of leaflets per leaf. *Humboldtia brunonis* is distributed between 11°10′N and 13°45′N in the Western Ghats within a narrow north–south strip. To sample the entire distribution, we divided it into five sections at intervals of 0.5° latitude, from
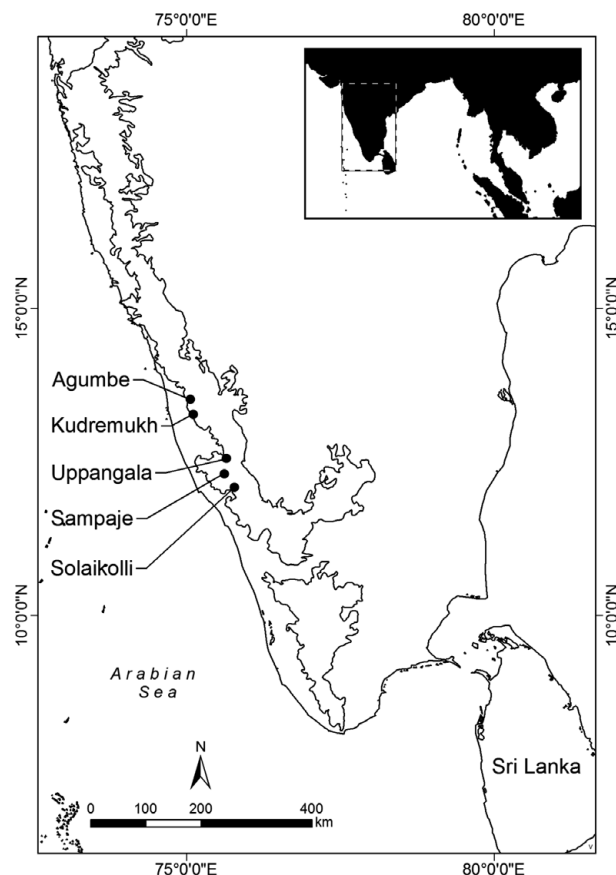

Figure 1. Map of Peninsular India indicating the *Humboldtia brunonis* study sites within the Western Ghats. The sites span the extant distribution of the species.

which we chose sites within the altitudinal range 200–600 m asl: Agumbe (13°31′N, 75°04′E), Kudremukh (13°16′N, 75°07′E), Uppangala (12°33′N, 75°39′E), Sampaje (12°27′N, 75°37′E) and Solaikolli (12°05′N, 75°47′E) (Figure 1). These sites span a rainfall seasonality gradient in the Ghats with longer dry seasons (8 mo) towards the north compared with the south (6 mo) since the southern Western Ghats receive rain from both the south-west monsoon (June–September) as well as the north-east or retreating monsoon (October–November) (Davidar *et al.* 2005, Gadgil & Joshi 1983, Pascal 1988, Shenoy & Borges 2010) and also suggested a natural geographical grouping between northern sites: Agumbe and Kudremukh, and southern sites: Uppangala, Sampaje and Solaikolli. *Humboldtia brunonis* occurs in clonal clumps (Dev *et al.* 2010) and is so abundant that it features within two forest type associations: (1) *Dipterocarpus indicus–Humboldtia brunonis–Poeciloneuron indicum* type, and (2) *Dipterocarpus indicus–Kingiodendron pinnatum–Humboldtia brunonis* type (Pascal 1988, Rai 2000). Agumbe and Kudremukh occur within the first, and the other three sites within the second forest type.

Our research was conducted during the dry season (December–April), during which flowers and young leaves are available (Shenoy & Borges 2010). The abundance of domatia-bearing trees and number of nectaries per leaflet were recorded from December 2007–March 2008. Leaf herbivory was measured from December 2009–March 2010. EFN collection and additional sampling of nectaries occurred between December 2010–March 2011.

Abundance of domatia-bearing *Humboldtia brunonis* trees

Within each site, three transects each of 300 m length were sampled. Each transect was divided into five equal sections, on which five points were randomly chosen. From each such point, one sampling point at randomly determined distances (between 10–60 m) from left and right sides alternately was selected (discounting 10 m on either side of foot-trails to avoid edge effects). Presence or absence of domatia was noted on the *H. brunonis* plant at each of the sampling points and five of its nearest conspecific neighbours. Thus we sampled 30 trees in each transect, and with three transects in each of the five sites, we sampled a total of 450 trees. Plants less than 1 m tall were avoided, since domatia in *H. brunonis* are formed only at heights $\geq$70 cm (Brouat & McKey 2000).

Number of extrafloral nectaries per leaf

From each tree sampled for domatia abundance, five intact leaflets, independent of age, were haphazardly collected ($n = 90$ trees per site), and the number of nectaries per leaflet was counted. Average values of nectary numbers per leaflet per tree were obtained which were then used in a multivariate analysis to examine the effect of domatia presence on nectary numbers per leaflet ($n = 450$ tree-average nectary values from five sites). Subsequent sampling revealed that the leaflets of the distal pair had greater numbers of nectaries than the proximal pair, and this was true for all sites. Therefore, whole intact leaves (with four leaflets) were collected haphazardly from the transects, independent of domatia presence/absence on the trees (Agumbe: $n = 34$ whole leaves, Kudremukh: $n = 30$, Uppangala: $n = 25$, Sampaje: $n = 40$, Solaikolli: $n = 30$), and their nectaries counted.

Size of foliar nectaries

Young leaves were collected in 75% alcohol. Nectary size was estimated as the area of the circular nectary disc (Rudgers 2004). The diameter (and radius r) of each nectary was measured with a stereomicroscope using a micrometer on hand-cut, safranin-stained, transverse sections; nectary area was calculated as πr^2 (Agumbe: $n = 39$ samples, Kudremukh: $n = 39$, Uppangala: $n = 40$, Sampaje: $n = 39$, Solaikolli: $n = 40$). Since there was no significant difference between the nectary sizes of very young red and expanding young green leaves (Chanam & Borges, unpubl. data), nectaries of such leaves were pooled for each site.

Volume and composition of EFN per leaf

Young leaves were enclosed in cloth bags to prevent nectar consumption. EFN volume (pooled across four leaflets) was measured the next morning (after 24 h) between 06h00 and 09h00 using micropipettes (Agumbe: $n = 21$ leaves; Kudremukh: $n = 43$; Uppangala: $n = 23$; Sampaje: $n = 21$; Solaikolli: $n = 46$). EFN was collected in sterile gas chromatography vials, using micropipettes with sterile tips (Agumbe: $n = 9$ EFN samples; Kudremukh: $n = 10$; Uppangala: $n = 9$; Sampaje: $n = 7$; Solaikolli: $n = 5$) in the same way. Up to 50 μL of HPLC-grade methanol was added to each vial to prevent microbial growth; vials were stored on ice, and their contents later lyophilized.

The composition of sugars and amino acids in EFN was determined using gas chromatography-mass spectrometry (GC-MS). A mixture of 1 mg each of several sugars (sucrose, glucose, fructose, inositol, galactose, mannose, arabinose) and each of the 20 naturally occurring amino acids (Sigma-Aldrich, Germany) was used as reference. Each component was converted to its trimethyl silylated derivative by treating the mixture with 1 ml of N-methyl-N-(trimethyl silyl) trifluoro acetamide and 2 ml pyridine (adapted from Kost & Heil 2005). The standard solution was diluted $1:10$ using dichloromethane, and $1\,\mu$L of it was injected into the GC-MS instrument (Agilent-HP GC model 6890N, MS model 5973N) fitted with an HP-5 MS column (30 m $\times$ 0.25 mm $\times$ 0.25 mm; J and W Scientific, USA). The GC was operated with a split-ratio of $10:1$ with a temperature program adapted from Shenoy *et al.* (2012). EFN samples were analysed as above. The concentration of each component was estimated and compared across sites as were the sugar : amino acid mass ratios. Amino acids were grouped into essential or non-essential categories using standard human criteria since standard insect criteria are not available.

Herbivory and protection to young leaves and floral buds

At each site, one pair of young leaves per tree was tagged (Agumbe: $n = 18$ pairs; Kudremukh: $n = 26$; Uppangala: $n = 27$; Sampaje: $n = 21$; Solaikolli: $n = 30$). Ants, irrespective of the species present on the trees at each

site, were allowed access to one leaf (control) of each pair, while they were excluded from the other using Tanglefoot® glue. After 10 d the leaves were collected, photocopied and later scanned. Herbivory, measured as percentage leaf area consumed using the software ImageJ 1.36b (http://rsb.info.nih.gov/ij), was compared between ant-excluded and control leaves. Pairs of control and ant-excluded inflorescence primordia were tagged (Agumbe: $n = 36$ pairs; Kudremukh: $n = 29$; Uppangala: $n = 30$; Sampaje: $n = 31$; Solaikolli: $n = 27$) and the numbers of floral buds on each inflorescence counted. After 10 d, the proportion of floral buds damaged or removed by herbivory was determined, and compared between ant-excluded and control inflorescences. Herbivory pressure was defined as herbivory on ant-excluded leaves or floral buds. Ant protection was estimated as reduction in herbivory by the presence of ants, by comparing the herbivory on ant-excluded and control samples of each pair.

Statistical analysis

Non-parametric statistics were used for all inter-site comparisons. Kruskal–Wallis ANOVAs (denoted by the χ^2 test statistic) were employed, followed by post hoc Wilcoxon rank sum tests (denoted by the W test statistic) after appropriate Bonferroni corrections. Wilcoxon rank sum tests were also used for comparison between northern and southern groups of sites. To determine the effect of domatia and site, and their interaction on the number of nectaries per leaflet, we used a linear modelling framework, and simplified the model by sequentially dropping terms (Crawley 2012) to find the most parsimonious model. Wilcoxon matched-pairs signed ranks tests were used for comparing herbivory on ant-excluded versus control pairs of leaves or floral buds. All statistical analyses were performed using R software (version 2.14.1). All values mentioned in the results are mean ± SD.

RESULTS

Geographical variation in morphological ant-plant traits

The relative abundance of domatia-bearing trees varied across the five sites (Kruskal–Wallis test: $\chi^2 = 22.5$, P = 0.0002, $n = 450$; Agumbe: 8.9%; Kudremukh: 22.2%; Uppangala: 33.3%; Sampaje: 48.9%; Solaikolli: 31.1%; Figure 2). The southern group of sites had a significantly greater relative abundance (an average of 2.4 times higher abundance) of domatia-bearing trees than the northern group (Wilcoxon rank sum test: W = 308, P < 0.001) (Figure 2).

Site identity was the only factor to affect the nectary number per leaflet significantly ($F_{4,445} = 31.9$, P ≪
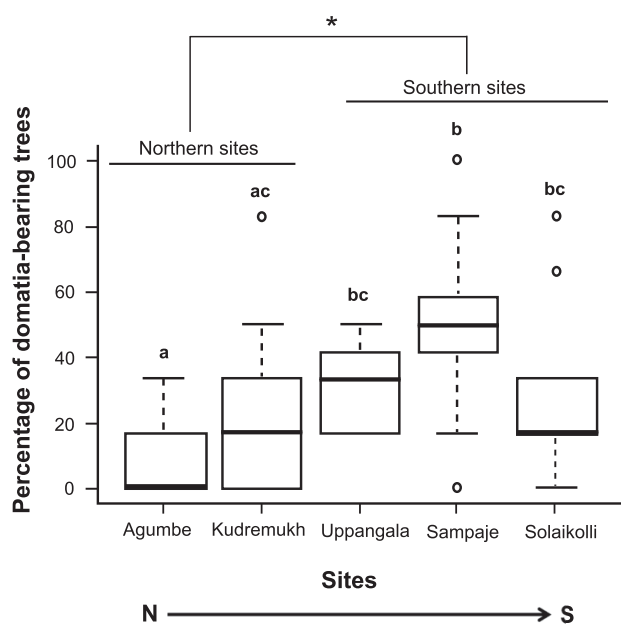

Figure 2. Abundance of domatia-bearing trees of *Humboldtia brunonis* across sites spanning its distribution in the Indian Western Ghats ($n = 90$ trees per site). The lower and upper boundaries of each box indicate lower and upper quartile values; the bar in the box indicates the median. Error bars indicate minimum and maximum values, excluding outliers which are indicated by open circles. Different letters above the boxes indicate significant differences (Kruskal–Wallis test followed by post hoc Wilcoxon rank sum tests after Bonferroni correction). ∗ P < 0.05, Wilcoxon rank sum test between northern and southern sites.

0.001), while domatia presence ($F_{1,444} = 0.001$, P = 0.97) and the interaction term ($F_{4,440} = 0.95$, P = 0.43) had no effect. The average number (± SD) of nectaries per leaflet was lowest in the northern sites, viz. Agumbe (5.8 ± 2.2) and Kudremukh (5.7 ± 2.4), and higher in the southern sites, being highest in Sampaje (8.3 ± 2.7), while there was no significant difference between Uppangala (6.7 ± 2.7) and Solaikolli (6.7 ± 2.5) (Figure 3a). At each site, the number of nectaries per leaflet varied amongst the four leaflets of a leaf (Kruskal–Wallis tests: Agumbe: $\chi^2 = 18.2$, df = 3, P < 0.01; Kudremukh: $\chi^2 = 29.2$, df = 3, P < 0.01; Uppangala: $\chi^2 = 15.3$, df = 3, P < 0.01; Sampaje: $\chi^2 = 29.8$, df = 3, P < 0.01; Solaikolli: $\chi^2 = 42.6$, df = 3, P < 0.01), with the two distal leaflets bearing more nectaries that the two proximal leaflets (Figure 3b). The number of nectaries per whole leaf varied significantly across the five sites ($\chi^2 = 50.0$, df = 4, P < 0.0001), being higher in southern (Uppangala: 32.2 ± 9.6, Sampaje: 30.7 ± 11.1 and Solaikolli: 29.2 ± 9.8) than northern sites (Agumbe: 17.3 ± 5.4, Kudremukh: 21.8 ± 6.7) (W = 972, P < 0.001) (Figure 3b) with the average nectary number per leaf in the southern sites being 1.6 times higher than that of the northern sites.

The area of foliar nectaries varied across the five sites ($\chi^2 = 26.7$, df = 4, P < 0.05), with a trend of
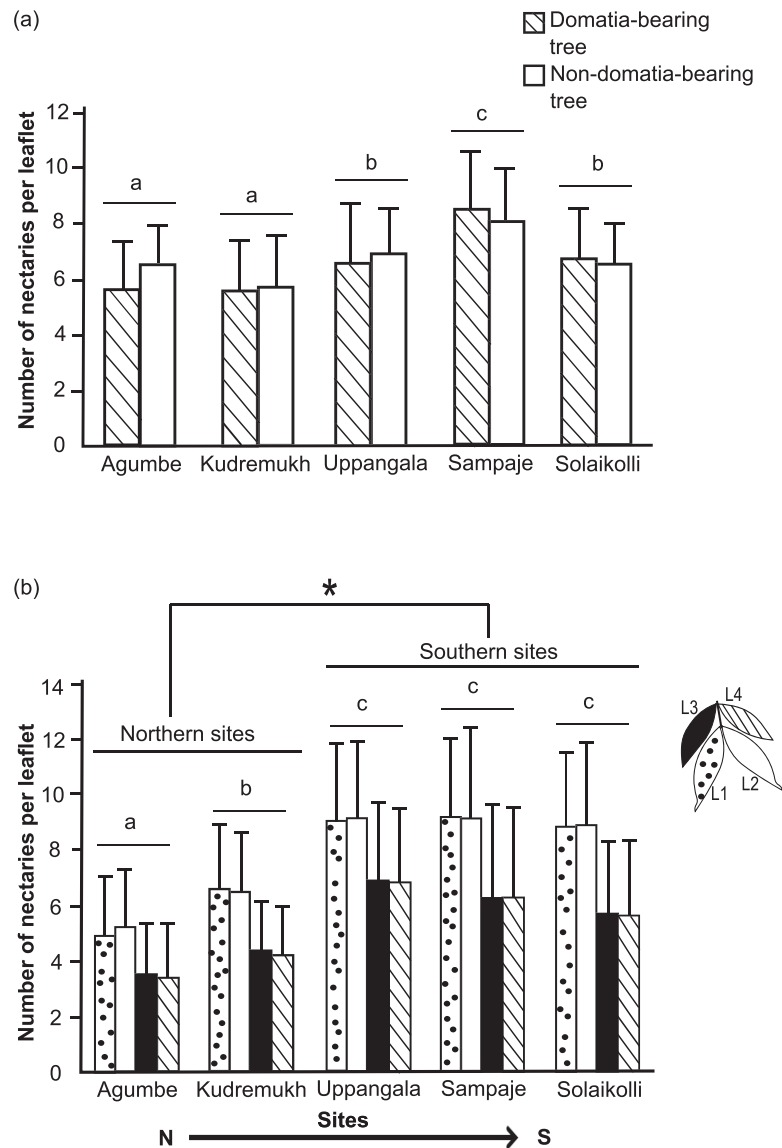
Figure 3. Abundance of foliar extrafloral nectaries in *Humboldtia brunonis* across sites spanning its distribution in the Indian Western Ghats. Number of extrafloral nectaries per leaflet ($n = 90$ trees per site and 450 leaflets in total) in domatia- and non-domatia bearing trees (a). Numbers of extrafloral nectaries per leaf (summed across four leaflets of each leaf) across sites (b). Bar plots depict mean $\pm$ SD. Letter notation and symbols as in Figure 2. Agumbe: $n = 34$ whole leaves, Kudremukh: $n = 30$, Uppangala: $n = 25$, Sampaje: $n = 40$, Solaikolli: $n = 30$.

increases in area from north to south (Agumbe: 0.14 ± 0.08 mm^2; Kudremukh: 0.18 ± 0.06 mm^2; Uppangala: 0.21 ± 0.06 mm^2; Sampaje: 0.20 ± 0.06 mm^2; Solaikolli: 0.19 ± 0.06 mm^2) (Figure 4a). The southern group of sites had significantly larger nectaries (1.2 times larger on average) than the northern group (W = 3022, P < 0.001) (Figure 4a).

Geographical variation in foliar nectar traits

The volume of EFN per leaf varied across the five sites ($\chi^2 = 24.0$, df = 4, P < 0.0001; Agumbe: 5.6 ± 4.3 μl;

Kudremukh: 3.4 ± 2.7 μl; Uppangala: 11.7 ± 9.3 μl; Sampaje: 5.9 ± 7.6 μl; Solaikolli: 9.6 ± 11.4 μl). EFN volume was significantly greater in the southern group (2.24 times greater on average) compared with the northern group (W = 1870, P < 0.001) (Figure 4b). Sugars had higher EFN concentrations than amino acids (Figure 5); however, the sugar:amino acid ratios (per cent weight/volume) varied across the five sites, ranging from 17.2 (Uppangala) to 48.6 (Agumbe); ratios for the other sites were 47.8 (Kudremukh), 32.0 (Sampaje) and 35.5 (Solaikolli). Overall, seven sugars (sucrose, glucose, fructose, inositol, galactose, mannose and altrose) were detected (Figure 4a). The total concentration of sugars
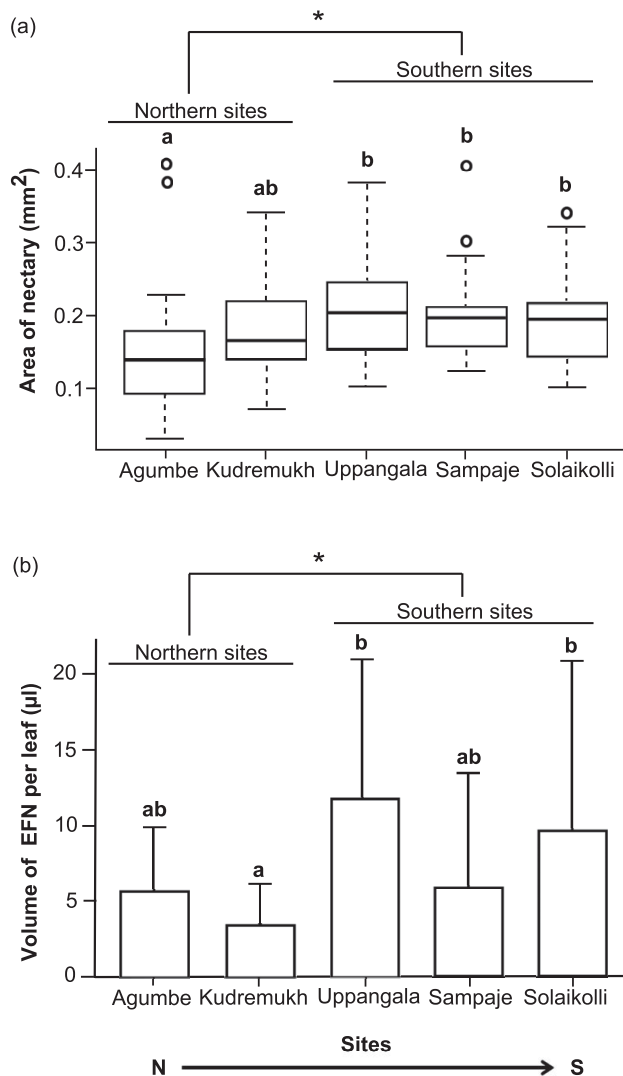
Figure 4. Size of foliar extrafloral nectaries of *Humboldtia brunonis* across sites spanning its distribution in the Indian Western Ghats. Size of nectaries of young leaves (a), and volume of extrafloral nectar (EFN) per leaf (b). For nectaries: Agumbe: $n = 39$ nectaries, Kudremukh: $n = 39$, Uppangala: $n = 40$, Sampaje: $n = 39$, Solaikolli: $n = 40$. For EFN volume per leaf: Agumbe: 21 leaves; Kudremukh: 43; Uppangala: 23; Sampaje: 21; Solaikolli: 46. Box-and-whisker plots, letter notation and symbols as in Figure 2.

(g per 100 ml volume) in EFN varied across the five sites ($\chi^2 = 14.7$, df = 4, P = 0.005), being significantly higher in the southern group (2.1 times higher on average) than the northern group (W = 88.0, P = 0.002) (Figure 5a, inset).

The total concentration of the major sugars (sucrose, glucose and fructose) varied across the five sites ($\chi^2 = 9.45$, df = 1, P = 0.002). The southern group had significantly greater concentration of total major sugars (W = 86.0, P = 0.002) than the northern group. Minor sugars included galactose, mannose and altrose

(Appendix 1). Interestingly, there was no significant difference in total minor sugars between the northern and southern groups. Inositol was present in much lower proportion compared with major sugars in all sites (0.3 g per 100 ml in Kudremukh to 1.6 g per 100 ml in Sampaje), with variation across the five sites ($\chi^2 = 10.2$, df = 4, P = 0.037); post hoc pairwise tests revealed significant difference in inositol concentrations only between Kudremukh and Uppangala (W = 11, P < 0.001).

Twelve amino acids were detected, comprising five essential (isoleucine, leucine, phenylalanine, threonine and valine) and seven non-essential (alanine, aspartic acid, glutamine, glycine, proline, serine and tyrosine) amino acids (Figure 5b). The average total concentration of the amino acids ranged between 1.08 ± 1.13 g per 100 ml (Agumbe) and 2.99 ± 0.76 g per 100 ml (Uppangala) (Table 1), with significant variation across the five sites in concentrations of total amino acids ($\chi^2 = 13.8$, df = 4, P = 0.008), total essential amino acids ($\chi^2 = 14.6$, df = 4, P = 0.006), and total non-essential amino acids ($\chi^2 = 13.0$, df = 4, P = 0.01). The southern group of sites had higher concentration of essential (W = 79.0, P = 0.001), as well as non-essential amino acids (W = 109, P = 0.01) than the northern group (Figure 5b, inset) with average concentration of total amino acids in the southern sites being 2.2 times higher than the northern sites.

Geographical variation in herbivory pressure

Herbivory pressure varied significantly across the five sites for both young leaves ($\chi^2 = 21.0$, df = 4, P = 0.0003) and floral buds ($\chi^2 = 12.7$, df = 4, P = 0.01), with the highest herbivory occurring in the southern site Uppangala (approximately 60% and 50% herbivory for leaves and floral buds respectively). The control leaves had significantly lower herbivory than ant-excluded leaves only at Uppangala (Wilcoxon matched pairs test: V = 88, P = 0.045, $n = 27$ pairs). There was no significant reduction in herbivory in the control floral buds at any site. In Kudremukh, ant-excluded floral buds demonstrated lower herbivory levels (Wilcoxon matched pairs test: V = 74, P = 0.006, $n = 29$ pairs).

DISCUSSION

Context dependency in ant–plant mutualisms has usually been demonstrated in relation to the identity of protective ants, types of herbivore and abiotic factors (Barton 1986, Kersch & Fonseca 2005, Pringle & Gordon 2013). We found that conditionality in an ant–plant mutualism was linked to myrmecophytic traits which
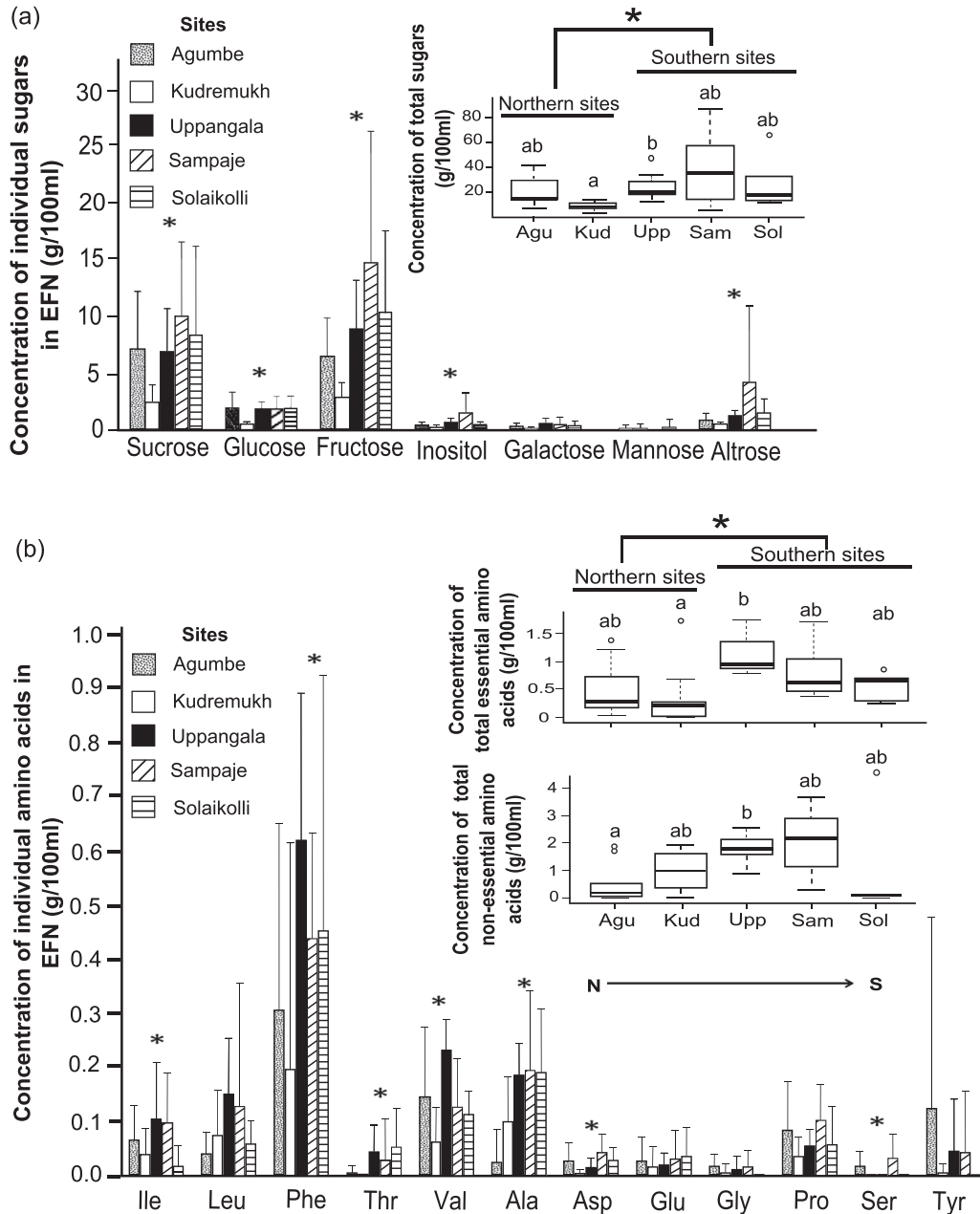
Figure 5. Geographical variation in concentrations of sugars and amino acids in foliar extrafloral nectar (EFN) of *Humboldtia brunonis* at study sites in the Indian Western Ghats. Concentrations of individual sugars (a) and amino acids (b) are compared across sites. Concentrations of total sugars (inset of a), and total essential and non-essential amino acids (inset of b) are compared across sites as well as between northern and southern groups of sites. Bar plots depict mean ± SD. Box-and-whisker plots, letter notation and symbols as in Figure 2. Agu (Agumbe): $n = 9$ EFN samples; Kud (Kudremukh): $n = 10$; Upp (Uppangala): $n = 9$; Sam (Sampaje): $n = 7$; Sol (Solaikolli): $n = 5$. Ile: Isoleucine, Leu: Leucine, Phe: Phenylalanine, Thr: Threonine, Val: Valine, Ala: Alanine, Asp: Aspartic acid, Glu: Glutamic acid, Gly: Glycine, Pro: Proline, Ser: Serine, Tyr: Tyrosine.

varied with geography and demonstrated a clear north–south pattern. Herbivory pressure in the absence of ants was high towards the south, especially at the Uppangala site, but its reduction in the presence of ants suggests that greater ant attraction by suitable myrmecophytic traits could have led to stronger protection services by ants at this site.

Context dependency of rewards to ants in relation to protection services

Nesting space availability can affect ant colony size and consequently protection benefits (Fonseca 1999). In *H. brunonis*, greater availability of domatia could promote ant protection services since the sole protective

Table 1. Concentration (mean ± SD, g per 100 ml) of important sugars and total essential and non-essential amino acids in foliar EFN of *Humboldtia brunonis* at study sites in the Indian Western Ghats.

	Agumbe (n = 9)	Kudremukh (n = 10)	Uppangala (n = 9)	Sampaje (n = 7)	Solaikolli (n = 5)
Total sugars	21.0 ± 12.0	9.16 ± 3.64	24.8 ± 10.4	39.1 ± 30.0	28.8 ± 22.6
Sucrose	7.26 ± 5.11	2.56 ± 1.46	7.04 ± 3.8	10.2 ± 6.6	8.52 ± 7.86
Glucose	2.02 ± 1.36	0.58 ± 0.2	1.93 ± 0.59	1.9 ± 1.1	1.99 ± 1.07
Fructose	6.64 ± 3.34	2.96 ± 1.26	9.06 ± 4.32	14.99 ± 11.71	10.59 ± 7.21
Inositol	0.47 ± 0.23	0.31 ± 0.13	0.74 ± 0.31	1.58 ± 1.75	0.54 ± 0.2
Total amino acids	1.08 ± 1.13	1.34 ± 1.18	2.99 ± 0.76	1.69 ± 2.57	2.87 ± 1.65
Total essential amino acids	0.56 ± 0.54	0.37 ± 0.58	1.16 ± 0.36	0.82 ± 0.48	0.7 ± 0.52
Total non-essential amino acids	0.52 ± 0.69	0.97 ± 0.78	1.84 ± 0.54	2.05 ± 1.34	0.99 ± 2.09

ant, *T. albipes*, being polydomous, is likely attracted by abundant nesting spaces (domatia) (Shenoy & Borges 2010). Greater abundance of domatia-bearing trees and of the protective ant in the southern sites (Shenoy & Borges 2010) supports the facilitation of ant protection by increased availability of domatia as also suggested by Fiala & Maschwitz (1992). However, the southernmost site Solaikolli had relatively fewer domatia-bearing trees for reasons that cannot currently be explained.

In *H. brunonis*, there may be greater selective advantage to increased foliar nectary numbers in the southern sites due to site-specific effects, e.g. herbivory pressure and abundance of the protective ant. However, whether in *H. brunonis*, as in other ant-plants, nectary number is genetically determined (Rudgers 2004, Rudgers & Strauss 2004) or modulated by herbivory (Mondor *et al.* 2006, Pulice & Packer 2008) is still unknown. The greater EFN volume in the southern sites could result from the larger nectary size (Baker-Méio & Marquis 2012) characteristic of the southern plants. These results differ from those of Shenoy *et al.* (2012) who reported similar volumes at three sites (northern: Agumbe; southern: Sampaje and Solaikolli). This spatiotemporal variation in EFN volume, in relation to differing herbivory pressure and protection, suggests that EFN volume is a plastic trait as found in other ant-plants (Bixenmann *et al.* 2011, Escalante-Pérez *et al.* 2012, Heil *et al.* 2000, 2001). We recorded only seven of the 12 sugars reported in Shenoy *et al.* (2012), while the rest (maltose, arabinose, xylose, ribose and arabinoic acid) were either undetected or present in trace amounts. Of the amino acids, methionine and tryptophan, reported by Shenoy *et al.* (2012), were undetected in the present study. The sugar : amino acid ratio of Uppangala (17.2) was lower than the lowest ratio value reported by Shenoy *et al.* (2012) (Agumbe: 50, Sampaje: 323 and Solaikolli: 34); therefore Uppangala had the richest amino acid concentration recorded in any site at any time for this plant. In the two studies separated by 5 y, Agumbe and Solaikolli showed little variation in sugar : amino acid ratios, while the ratio in Sampaje changed considerably. The absence of a north–south gradient in minor sugars

and in inositol, which may not be important to ants (Rudgers & Gardener 2004) or whose effect on ants is equivocal (Shenoy 2008), alludes to the fact that the latitudinal trend in EFN composition is also likely under context-based selection such that EFN is rich in sugars important to ants at sites where protective ants need to be rewarded for their services. Our results also emphasize that repeated sampling of EFN volume and composition is necessary to uncover the range of variation expressed within an ant–plant mutualism, and that data from a single study at a single point in time may be inadequate.

Increased investment in EFN volume and composition can increase ant protective efficacy (González-Teuber *et al.* 2012, Shenoy *et al.* 2012). Since *T. albipes* is a tramp ant and may shift between populations of *H. brunonis* (while remaining 'faithful at the meta-population scale', Gaume *et al.* 2005), and also prefers 'costly' EFN with higher essential amino acid content, it would be beneficial to the host plant to express context dependency in the production of attractive rewards, producing them only as and when it would most benefit the plant. Our findings of higher EFN volumes and attractive EFN compositions only at the site most threatened by herbivory in the south, demonstrates such context dependency in the relationship of this myrmecophyte with its protective ant in the southern sites. Therefore, plants invest in ant protection when herbivory is high and when protective ants are available. Furthermore, plants appear to be able to modulate some ant rewards to suit the physiological requirements of their protective ants.

A gradient conducive to the evolution of myrmecophytism

Northern Western Ghat sites receive only the south-west monsoon (June–September) while southern sites also receive the north-east monsoon (October–December) (Gadgil & Joshi 1983). Furthermore, the southern and northern sites occur within two distinct forest types which probably result from differences in rainfall and other geophysical factors (Pascal 1988, Rai 2000). The

dry season is shorter towards the southern sites while the interval between dry spells is longer towards the north (Shenoy & Borges 2010). This long dry period in the north may explain the greater dominance of interlopers such as arboreal earthworms in the domatia of the northern sites (Shenoy & Borges 2010). These dominant interloping earthworms that cannot survive outside the domatia in the dry season, coupled with the lower relative abundance of trees bearing domatia in the northern sites, further reduces the availability of domatia for ants in the northern sites. The earthworms have a negative association with ants and may displace them from domatia (Gaume *et al.* 2006). Since caulinary woody-stem domatia are costly to produce (Blatrix *et al.* 2012), the domatium trait may be favoured when the net benefits of domatia production outweigh their costs. The domatia inhabitants of *H. brunonis* also provide nitrogen to the plant that is incorporated into plant tissue (Chanam *et al.* 2014). How the benefits of domatia accrued from protective ants nesting within domatia interact with the nutritional benefits obtained from miscellaneous and even interloping domatia inhabitants and affect selection on the domatium trait is still unknown.

Invoking the geographic mosaic model (Gomulkiewicz *et al.* 2000, Thompson 1999), it appears that the south zone is a 'hot spot' of protection mutualism for the *H. brunonis* system, whereas the north zone is a 'cold spot'. Unlike the semi-myrmecophyte *H. brunonis*, *Humboldtia laurifolia* Vahl is a true myrmecophyte, one in which all individuals bear stem domatia. The occurrence of this true myrmecophyte, *H. laurifolia*, in Sri Lanka (Krombein *et al.* 1999) which lies to the south of the Western Ghats (where it is mostly associated with the protective dolichoderine *T. albipes*), further supports the north–south trend in protection mutualism in this region. Sri Lanka also receives two monsoons (Suppiah 1996) as does the southern Western Ghats. Latitudinal effects on ant-plant distributions are known in Asia (Pemberton 1988) and may be dictated by temperature, rainfall and rainfall seasonality gradients. Similar latitudinal effects in India resulting from the length and timing of the monsoons have been demonstrated for other plant–animal dependent interactions such as the dispersal phenology of plants (Aravind *et al.* 2013). It is therefore entirely possible that only the less seasonal southern sites in the Western Ghats are most conducive to the development of ant–plant mutualisms. However, some southern sites such as Solaikolli did not follow the north–south trend in all ant-related traits; greater attention in future research must be paid to factors that could have caused such deviations. Our findings of an overall stable north–south gradient of myrmecophytism in the Western Ghats of India have implications for the existence of such gradients in several other plant–animal interactions that merit exploration in this region.

LITERATURE CITED

ARAVIND, N. A., GANESHAIAH, K. N. & UMA SHAANKER, R. 2013. Indian monsoons shape dispersal phenology of plants. *Biology Letters* 9:20130675.

BAKER-MÉIO, & MARQUIS, R. J. 2012. Context-dependent benefits from ant–plant mutualism in three sympatric varieties of *Chamaecrista desvauxii*. *Journal of Ecology* 100:242–252.

BARTON, A. M. 1986. Spatial variation in the effect of ants on an extrafloral nectary plant. *Ecology* 67:495–504.

BIXENMANN, R. J., COLEY, P. D. & KURSAR, T. A. 2011. Is extrafloral nectar production induced by herbivores or ants in a tropical facultative ant–plant mutualism? *Oecologia* 165:417–425.

BLATRIX, R., RENARD, D., DJIETO-LORDON, C. & McKEY, D. 2012. The cost of myrmecophytism: insights from allometry of stem secondary growth. *Annals of Botany* 110: 943–951.

BRONSTEIN, J. L. 1994. Conditional outcomes in mutualistic interactions. *Trends in Ecology and Evolution* 9:214–217.

BROUAT, C. & MCKEY, D. 2000. Origin of caulinary ant domatia and timing of their onset in plant ontogeny: evolution of a key trait in horizontally transmitted ant–plant symbioses. *Biological Journal of the Linnean Society* 71:801–819.

CHAMBERLAIN, S. A. & HOLLAND, N. J. 2008. Density-mediated, context-dependent consumer-resource interactions between ants and extrafloral nectar plants. *Ecology* 89:1364–1374.

CHANAM, J., SHESHSHAYEE, M. S., KASINATHAN, S., JAGDEESH, A., JOSHI, K. A. & BORGES, R. M. 2014. Nutritional benefits from domatia inhabitants in an ant–plant interaction: interlopers do pay the rent. *Functional Ecology* doi: 10.1111/1365–2435.12251

CRAWLEY, M. J. 2012. *The R book.* John Wiley & Sons. 942 pp.

DAVIDAR, P., PUYRAVAUD, J. P. & LEIGH, E. G. JR. 2005. Changes in rain forest tree diversity, dominance and rarity across a seasonality gradient in the Western Ghats, India. *Journal of Biogeography* 32:493–501.

DEV, S. A., SHENOY, M. & BORGES, R. M. 2010. Genetic and clonal diversity of the endemic ant-plant *Humboldtia brunonis* (Fabaceae) in the Western Ghats of India. *Journal of Biosciences* 35:267–279.

DÍAZ-CASTELAZO, C., SÁNCHEZ-GALVÁN, I. R., GUIMARÃES, P. R., RAIMUNDO, R. L. G. & RICO-GRAY, R. 2013. Long-term temporal

variation in the organization of an ant–plant network. *Annals of Botany* 111:1285–1293.

ESCALANTE-PÉREZ, M., JABORSKY, M., LAUTNER, S., FROMM, J., MÜLLER, T., DITTRICH, M., KUNERT, M., BOLAND, W., HEDRICH, R. & ACHE, P. 2012. Poplar extrafloral nectaries: two types, two strategies of indirect defenses against herbivores. *Plant Physiology* 159:1176–1191.

FIALA, B. & MASCHWITZ, U. 1992. Domatia as most important adaptations in the evolution of myrmecophytes in the paleotropical tree genus *Macaranga* (Euphorbiaceae). *Plant Systematics and Evolution* 180:53–64.

FONSECA, C. R. 1999. Amazonian ant-plant interactions and the nesting space limitation hypothesis. *Journal of Tropical Ecology* 15:807–825.

GADGIL, S. & JOSHI, N. V. 1983. Climatic clusters of the Indian region. *Journal of Climatology* 3:47–53.

GAUME, L., ZACHARIAS, M., GROSBOIS, V. & BORGES, R. M. 2005. The fitness consequences of bearing domatia and having the right ant partner: experiments with protective and non-protective ants in a semi-myrmecophyte. *Oecologia* 145:76–86.

GAUME, L., SHENOY, M., ZACHARIAS, M. & BORGES, R. M. 2006. Co-existence of ants and an arboreal earthworm in a myrmecophyte of the Indian Western Ghats: anti-predation effects of the earthworm mucus. *Journal of Tropical Ecology* 22:341–344.

GOMULKIEWICZ, R., THOMPSON, J. N., HOLT, R. D., NUISMER, S. L. & HOCHBERG, M. E. 2000. Hot spots, cold spots, and the geographic mosaic theory of coevolution. *American Naturalist* 156:156–174.

GONZÁLEZ-TEUBER, M., SILVA BUENO, J. C., HEIL, M. & BOLAND, W. 2012. Increased host investment in extrafloral nectar (EFN) improves the efficiency of a mutualistic defensive service. *PLoS One* 7(10): e46598.

HEIL, M., FIALA, B., BAUMANN, B. & LINSENMAIR, K. E. 2000. Temporal, spatial and biotic variations in extrafloral nectar secretion by *Macaranga tanarius*. *Functional Ecology* 14:749–757.

HEIL, M., KOCH, T., HILPERT, A., FIALA, B., BOLAND, W. & LINSENMAIR, K. E. 2001. Extrafloral nectar production of the ant-associated plant, *Macaranga tanarius*, is an induced, indirect, defensive response elicited by jasmonic acid. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 98:1083–1088.

KERSCH, M. F. & FONSECA, C. R. 2005. Abiotic factors and the conditional outcome of an ant–plant mutualism. *Ecology* 86:2117–2126.

KOST, C. & HEIL, M. 2005. Increased availability of extrafloral nectar reduces herbivory in Lima bean plants (*Phaseolus lunatus*, Fabaceae). *Basic and Applied Ecology* 6:237–248.

KROMBEIN, K. V., NORDEN, B. B., RICKSON, M. M. & RICKSON, F.R. 1999. Biodiversity of the domatia occupants (ants, wasps, bees, and others) of the Sri Lankan myrmecophyte *Humboldtia laurifolia* Vahl (Fabaceae). *Smithsonian Contributions to Zoology* 603:1–34.

MONDOR, E. B., TREMBLAY, M. N. & MESSING, R. H. 2006. Extrafloral nectary phenotypic plasticity is damage-and resource-dependent in *Vicia faba*. *Biology Letters* 2:583–585.

PASCAL, J. P. 1988. *Wet evergreen forests of the Western Ghats of India. Ecology, structure, floristic composition and succession*. French Institute, Pondicherry. 342 pp.

PEMBERTON, R. W. 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.

PRINGLE, E. G. & GORDON, D. M. 2013. Protection mutualism and the community: geographic variation in an ant-plant symbiosis and the consequences for herbivores. *Sociobiology* 60:242–251.

PULICE, C. E. & PACKER, A. A. 2008. Simulated herbivory induces extrafloral nectary production in *Prunus avium*. *Functional Ecology* 22:801–807.

RAI, S. N. 2000. *Productivity of tropical rain forests of Karnataka*. Eastern Press, Bangalore. 128 pp.

RICO-GRAY, V. & OLIVEIRA, P. S. 2007. *The ecology and evolution of ant–plant interactions*. University of Chicago Press, Chicago. 200 pp.

RICO-GRAY, V., GARCIA-FRANCO, J. G., PALACIOS-RIOS, M., DÍAZ-CASTELAZO, C., PARRA-TABLA, V. & NAVARRO, J. A. 1998. Geographical and seasonal variation in the richness of ant-plant interactions in Mexico. *Biotropica* 30:190–200.

RICO-GRAY, V., DÍAZ-CASTELAZO, C., RAMÍREZ-HERNÁNDEZ, A., GUIMARÃES, P. R. & HOLLAND, J. N. 2012. Abiotic factors shape temporal variation in the structure of an ant–plant network. *Arthropod–Plant Interactions* 6:289–295.

RIOS, R. S., MARQUIS, R. J. & FLUNKER, J. C. 2008. Population variation in plant traits associated with ant attraction and herbivory in *Chamaecrista fasciculata* (Fabaceae). *Oecologia* 156:577–588.

RUDGERS, J. A. 2004. Enemies of herbivores can shape plant traits: selection in a facultative ant–plant mutualism. *Ecology* 85:192–205.

RUDGERS, J. A. & GARDENER, M. C. 2004. Extrafloral nectar as a resource mediating multispecies interactions. *Ecology* 85:1495–1502.

RUDGERS, J. A. & STRAUSS, S. Y. 2004. A selection mosaic in the facultative mutualism between ants and wild cotton. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 271:2481–2488.

SHENOY, M. 2008. *Spatial variation in interactions of the semi-myrmecophyte* Humboldtia brunonis *(Fabaceae) with ants and other invertebrates*. PhD dissertation, Indian Institute of Science, Bangalore, India.

SHENOY, M. & BORGES, R. M. 2010. Geographical variation in an ant–plant interaction correlates with domatia occupancy, local ant diversity, and interlopers. *Biological Journal of the Linnean Society* 100:538–551.

SHENOY, M., RADHIKA, V., SATISH, S. & BORGES, R. M. 2012. Composition of extrafloral nectar influences interactions between the myrmecophyte *Humboldtia brunonis* and its ant associates. *Journal of Chemical Ecology* 38:88–99.

SUPPIAH, R. 1996. Spatial and temporal variations in the relationships between the Southern Oscillation phenomenon and the rainfall of Sri Lanka. *International Journal of Climatology* 16:1391–1407.

THOMPSON, J. N. 1999. Specific hypotheses on the geographic mosaic of coevolution. *American Naturalist* 153:S1–S14.

APPENDIX 1. Concentrations (mean ± SD, g per 100 ml) of minor sugars, and individual amino acids in foliar EFN of *Humboldtia brunonis* at study sites in the Indian Western Ghats.

	Agumbe (n = 9)	Kudremukh (n = 10)	Uppangala (n = 9)	Sampaje (n = 7)	Solaikolli (n = 5)
Sugars					
Galactose	3.74 ± 2.37	2.01 ± 0.78	4.45 ± 3.75	6.16 ± 4.36	5.26 ± 6.09
Mannose	0	0.17 ± 0.27	0.21 ± 0.33	0	0.30 ± 0.67
Altrose	0.91 ± 0.59	0.57 ± 0.16	1.32 ± 0.41	4.3 ± 6.8	1.6 ± 1.18
Essential amino acids					
Isoleucine	0.07 ± 0.06	0.04 ± 0.05	0.10 ± 0.1	0.1 ± 0.09	0.02 ± 0.04
Leucine	0.04 ± 0.04	0.07 ± 0.08	0.15 ± 0.1	0.13 ± 0.23	0.06 ± 0.04
Pheylalanine	0.30 ± 0.35	0.2 ± 0.42	0.62 ± 0.27	0.44 ± 0.2	0.45 ± 0.47
Threonine	0.004 ± 0.01	0	0.04 ± 0.05	0.03 ± 0.08	0.05 ± 0.47
Valine	0.15 ± 0.13	0.06 ± 0.06	0.23 ± 0.05	0.13 ± 0.09	0.11 ± 0.04
Non-essential amino acids					
Alanine	0.02 ± 0.06	0.1 ± 0.08	0.19 ± 0.06	0.2 ± 0.15	0.1 ± 0.21
Aspartic acid	0.03 ± 0.03	0.002 ± 0.01	0.01 ± 0.02	0.04 ± 0.03	0.03 ± 0.02
Glutamine	0.03 ± 0.04	0.02 ± 0.04	0.019 ± 0.02	0.03 ± 0.02	0.03 ± 0.05
Glycine	0.02 ± 0.02	0.005 ± 0.02	0.01 ± 0.02	0.02 ± 0.03	0
Proline	0.08 ± 0.09	0.035 ± 0.04	0.05 ± 0.03	0.1 ± 0.06	0.056 ± 0.07
Serine	0.02 ± 0.03	0	0	0.03 ± 0.04	0
Tyrosine	0.12 ± 0.36	0.005 ± 0.01	0.04 ± 0.1	0.04 ± 0.11	0