Geographical variation in an ant–plant interaction correlates with domatia occupancy, local ant diversity, and interlopers

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Interactions between potentially mutualistic partners can vary over geographic areas. Myrmecophytes, which are plants harbouring ants, often do not exhibit sufficient intraspecific variability to permit comparative studies of myrmecophytic traits over space or time. *Humboldtia brunonis* (Fabaceae), a dominant, endemic myrmecophyte of the Indian Western Ghats, is unique in exhibiting considerable variability in myrmecophytic traits, e.g. domatia presence, as well as domatia occupancy and associated ant diversity throughout its geographic range. Although its caulinary domatia are occupied by at least 16 ant species throughout its distribution, young leaves and floral buds producing extrafloral nectar (EFN) are protected by ants from herbivory only in the southernmost region, where *Technomyrmex albipes* (Dolichoderinae) is the most abundant ant species. The extent of protection by ants was positively related to local species richness of ants and their occupancy of domatia. On the other hand, the highest abundance of interlopers in the domatia, including non-protective ants, the arboreal earthworm *Perionyx pullus*, and other invertebrates, occurred in sites with the least protection from herbivory by ants. Whereas domatia morphometry did not vary between sites, domatia occupied by protective ants and invertebrate interlopers were longer and broader than empty ones at all sites. The lowest percentage of empty domatia was found at the southernmost site. There was a progressive decline in ant species richness from that found at the sites, to that feeding on *H. brunonis* EFN, to that occupying domatia, possibly indicating constraints in the interactions with the plants at various levels. Our study of this dominant myrmecophyte emphasizes the impact of local factors such as the availability of suitable ant partners, domatia occupancy, and the presence of interlopers on the emergence of a protection mutualism between ants and plants. © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2010, **100**, 537–550.

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INTRODUCTION

Ants and plants interact in various ways, with the protection services afforded by ants to plants against herbivores being the most widely investigated (Heil & McKey, 2003; Rico-Gray & Oliveira, 2007). Ant–plant interactions also often exhibit a geographic mosaic (*sensu* Thompson, 2005) in which the nature of the interactions, whether positive, negative, or neutral, varies with location, and may depend on properties of the plants, the ant species at the local sites, or both of these factors (Barton, 1986; Mody & Linsenmair,

2004; Rudgers & Strauss, 2004; Léotard *et al*., 2009; Moraes & Vasconcelos, 2009). Thus the emergence of a mutualism between ants and plants is often conditional on a variety of parameters (Bronstein, 1994; Billick & Tonkel, 2003). Moreover, protective ant–plant mutualisms are more often the result of 'species sorting' (*sensu* Jordano, 1987) than species-specific interactions shaped by co-evolution (Davidson & McKey, 1993; Heil & McKey, 2003). These associations range from facultative interactions, usually mediated by liquid nutrients (extrafloral nectar or EFN), provisioned via extrafloral nectaries, and sometimes also by solid food bodies, to more specialized obligate symbioses based on housing for ants provided by specialized myrmecophytic plants (Fiala *et al*.,

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1999; Heil & McKey, 2003; Dejean *et al*., 2006; Webber, Abaloz & Woodrow, 2007a). Network analyses on communities of interacting ants and plants reveal that ant–nectar webs are more generalized, involving many ant species per plant, whereas ant–myrmecophyte webs are more restricted, with lower connectance (Fonseca & Ganade, 1996; Blüthgen *et al*., 2007). The positive nature of ant–plant interactions is therefore conditional on many factors, often resulting in a geographic mosaic of regions that differ in consequences for the interacting partners.

Examination of variability in protection by ants and of the quality of plant traits is therefore an important prerequisite for understanding the evolution of a protection mutualism, as was found for other service-oriented mutualisms (Schemske & Horvitz, 1984). However, myrmecophytes, which are plants harbouring ants (Davidson & McKey, 1993; Webber *et al*., 2007b), often do not offer sufficient intraspecific variability in their physiological or ecological traits to permit an investigation of geographical variation in these traits. Furthermore, spatial variation of ant communities and the availability of potential plant partners are rarely investigated at the geographical scale of the host plant. This is because the specialization of myrmecophytes often precludes an examination of potential partners, or potential geographical locations where co-evolutionary processes may occur, because plant and ant partners are already locked into a close association (but see Debout *et al*., 2009). Thus, exploring the potential for suitable partners in a myrmecophyte over its geographical distribution can offer important insights into the evolution of ant–plant mutualisms.

At a broader biogeographic scale, the species richness of myrmecophytes (and myrmecophiles), and consequently the potential for varied types of ant–plant interactions, is much higher in the Neotropics than in the Old World (Davidson & McKey, 1993; Rico-Gray & Oliveira, 2007). This diversity difference is reflected in the huge geographical bias in the ant-plant literature. Furthermore, although spatial variation in ant–plant mutualisms has been examined largely in the Neotropics, Africa, and south-east Asia, it remains completely uninvestigated in the Indian subcontinent (Rico-Gray & Oliveira, 2007). The extreme paucity of ant plants in India (compared with Africa and south-east Asia) makes the investigation of ant–plant interactions in this realm more relevant, because species sorting and opportunistic interactions are to be expected, rather than co-evolved mutualisms, in the strongly seasonal monsoonal forests of this area. Therefore, an examination of plant traits and those of interacting ant species, coupled with estimates of ant abundances in this area, are likely to provide insight into opportunities for the development of ant–plant mutualisms.

We therefore selected the Indian ant-plant *Humboldtia brunonis* to examine variation in ant–plant interactions over large spatial scales. We examined variation in identity and local abundance of the interacting ant partners, domatia morphometry, domatia occupancy by ants and other invertebrates, and ant species visiting real (EFN) and artifical nectar solutions, along with variation in the protective function of the ant community, to determine which of these factors are potentially important for the evolution of a protection mutualism in this myrmecophyte. We chose *H. brunonis* for this investigation because: (1) its distribution spans a north–south distance of > 300 km in the monsoonal Western Ghats of India (Ramesh & Pascal, 1997), and consequently different plant populations across this distribution are subjected to variation in climatic seasonality (Gadgil & Joshi, 1983; Gunnel, 1997), and therefore possibly exhibit a diversity of plant traits and ant partners, as in other ant–plant systems (Rico-Gray *et al*., 1998; Billick & Tonkel, 2003; Kersch & Fonseca, 2005); (2) *H. brunonis* populations are genetically isolated by distance (Dev, Shenoy & Borges, 2010), and consequently variation in plant traits such as domatia characteristics may exist between populations, with possible impacts on domatia occupancy (Brouat *et al*., 2001); (3) it is an unspecialized myrmecophyte, and at one site has been recorded to interact with diverse ants (Rickson *et al*., 2003; Gaume *et al*., 2005b) of variable protective function (Gaume, Zacharias & Borges, 2005a; Gaume *et al*., 2005b), besides harbouring a very large diversity and abundance of non-ant invertebrates within its caulinary domatia (Rickson *et al*., 2003; Gaume *et al*., 2006), and consequently the opportunity exists to examine the protection efficiency of potential ant partners across sites; and (4) it is a dominant tree growing in high-density patches (Ramesh & Pascal, 1997), and hence local effects on a measurable scale are expected, as with other dominant ant plants such as *Cecropia* (Janzen, 1969), *Acacia* (Heil, Rattke & Boland, 2005), and *Macaranga* (Fiala & Linsenmair, 1995).

MATERIAL AND METHODS

STUDY SYSTEM AND SITES

Humboldtia brunonis Wall. (Fabaceae: Caesalpinioideae) is a dominant understorey (10–15 m tall) ant plant in the low-elevation wet evergreen forests (11°10′–13°50′N) of the Indian Western Ghats (Ramesh & Pascal, 1997). It is unique among myrmecophytes because it is polymorphic for the presence of caulinary domatia: although all individuals produce

EFN, individual trees vary in the presence of domatia. The EFN is produced on young leaves, flower bud sepals, bracts, stipules, and bracteoles. Leaf nectaries are dotted along leaf margins, whereas floral nectaries occur at sepal bases. Peak floral-bud EFN secretion is between 1900 and 2100 h (Gaume *et al*., 2005a), whereas young leaf EFN secretion peaks between 1500 and 1700 h (Gaume *et al*., 2005b). Caulinary domatia are hollow stem internodes with a self-opening slit. Flowers and young leaves are available only in the dry season (December–April) (Ramesh & Pascal, 1997), when the abundance of terrestrial ants is also highest (Basu, 1997). EFN is absent on mature leaves.

Study sites spanned from the northern to nearly the southernmost part of the plant's distribution range. We determined protection from herbivory on floral buds and young leaves of *H. brunonis* by ants, ant diversity and abundance, and domatia occupancy at three sites: (1) Agumbe Reserve Forest (13°50′N, 75°09′E; 633 m a.s.l.); (2) Sampaji Reserve Forest (12°29′N, 75°35′E; 665 m a.s.l.); (3) Solaikolli: Brahmagiri Wildlife Sanctuary (12°4′N, 75°49′E; 651 m a.s.l.). We also investigated domatia occupancy and morphometry at three additional sites, situated along the same latitudinal gradient: (1) Bisle Reserve Forest (12°43′N, 75°40′E; 650 m a.s.l.); (2) Talacauveri, Padinalaknad Ghat Reserve Forest (12°21′N, 75°29′E; 650 m a.s.l.); (3) Mammankolli: Brahmagiri Wildlife Sanctuary (12°06′N, 75°47′E; 550 m a.s.l.). To minimize altitudinal effects, sites were matched for elevation as best as possible. The sites varied in dry-spell length in both the dry and wet (monsoon) seasons (Fig. 1).

GEOGRAPHICAL VARIATION IN PROTECTION BY ANTS

We performed ant-exclusion experiments using Tanglefoot® glue (Tanglefoot Company, now available from Contech, http://www.contech-inc.com) in the dry season at Agumbe, Sampaji, and Solaikolli, to determine the effect of ants on herbivory to young leaves and floral buds (sample sizes provided in the Results section). Ten days after applying Tanglefoot® to the petiole, we measured the percentage of leaf area consumed in ant-excluded and ant-patrolled treatments, using the image-analysis software ImageJ 1.36b and a Leica DM IRB microscope. We determined the percentage of buds (based on bud numbers) damaged or consumed on each inflorescence (percentage bud herbivory) in each treatment at the end of this period, as well as fruit set (proportion of flowers on each inflorescence forming fruit) 21 days after Tanglefoot® was applied to inflorescence stalks. All buds and young leaves were undamaged at the start of the experiment; all experimental and control plant
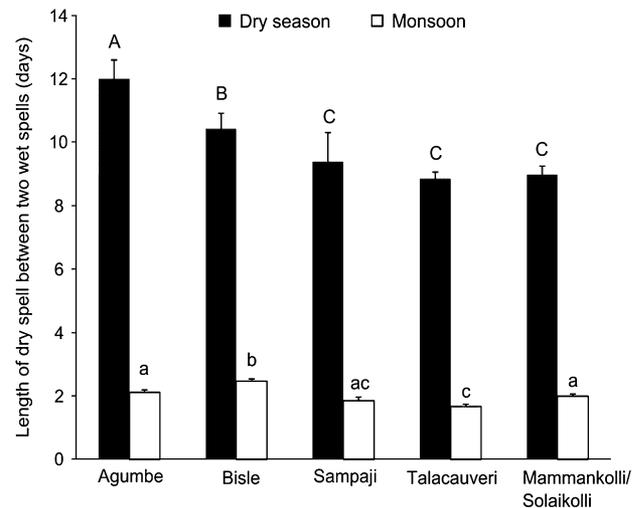

Figure 1. Length of the dry spell in the dry and monsoon season at five sites in the Western Ghats. Means ± SE followed by the same letter are not significantly different at the $P < 0.05$ level (Kruskal–Wallis ANOVA followed by Mann–Whitney *U*–tests after appropriate Bonferroni correction). A dry spell is the number of consecutive days between two wet days (wet day = day with rainfall of > 0.3 cm). Rainfall data obtained from http://caos.iisc.ernet.in/intro.html for a period of 100 years (1901–2000). As rainfall data were unavailable for all of the exact sites examined in this study, data were taken from sites situated ≤ 25 km from each site examined.

parts were matched for size. Herbivory was analysed using paired *t*-tests after angular transformation of the data. As fruit set in this species is very low (Shenoy & Borges, 2008), fruit set distribution could not be normalized by any transformation procedure; we therefore analysed these data using Wilcoxon matched-pairs signed ranks tests.

GEOGRAPHICAL VARIATION IN DOMATIA OCCUPANCY

We identified ants and other invertebrate domatia inhabitants at six sites (Agumbe, $N = 376$ domatia on 52 trees; Bisle, $N = 285$ on 25 trees; Sampaji, $N = 170$ on 17 trees; Talacauveri, $N = 196$ on 30 trees; Mammankolli, $N = 141$ on 30 trees; Solaikolli, $N = 151$ on ten trees). As the understorey *H. brunonis* has easily bendable branches, our sampling included almost all domatia on selected trees. All sampling of domatia was performed in the period between December and February. We divided domatia into those occupied by invertebrates and those left unoccupied. We subdivided the occupied domatia into those occupied by: (1) ants, (2) the arboreal earthworm *Perionyx pullus* Stephenson (Oligochaeta, Megascolecidae), and (3) other invertebrate species. We performed Kendall's tests for concordance on the frequency of domatia

occupied by these groups after Hellinger's transformation (square-root transformation of proportions). We performed Spearman's rank correlation tests between latitude and percentage of domatia in each group.

GEOGRAPHICAL VARIATION IN
DOMATIA MORPHOMETRICS

We measured domatia length and breadth at the widest part at three sites (Bisle, Talacauveri, and Mammankolli), prior to recording their contents. We compared domatia morphometrics (length and breadth) of: (1) empty and occupied domatia, and (2) those occupied by ants, arboreal earthworms (*P. pullus*), and other invertebrates. We applied square-root transformation on domatia lengths and analysed them using a two-way ANOVA followed by post-hoc Tukey's honestly significant difference (HSD) tests (for unequal sample size) and Bonferroni corrections. As domatia breadths could not be rendered normal by any transformation method, we analysed these data using a Kruskal–Wallis ANOVA, and post-hoc Mann–Whitney U-tests with Bonferroni corrections.

GEOGRAPHICAL VARIATION IN ANT DIVERSITY
AND ABUNDANCE

To determine the pool of ant species potentially available for forming interactions with *H. brunonis*, we used the standard ants of the leaf litter (ALL) protocol for the collection of ground dwelling and leaf litter ants (Agosti & Alonso, 2000) at three sites (Agumbe, Sampaji, and Solaikolli). At each site we demarcated a 1-hectare plot, where the protection of *H. brunonis* plants by resident ants was being monitored, for ant collections. This was divided into 20 quadrats (20 m × 25 m), from each of which ants were collected from a pitfall trap (open for 48 h) and a leaf litter sample (1 m²). Leaf litter was sorted manually. At each site, ant species were also manually collected by active search in five transects (20 m × 100 m) for 25 man-hours per hectare in one day. To represent the abundance of different ant species, we calculated percentage species occurrence (the percentage of quadrats in which a species occurred in either a pitfall trap or leaf litter sample) for each ant species at each site. The skewness of the percentage species occurrence at each site was also calculated.

At three sites (Agumbe, Sampaji, and Solaikolli) we sampled and identified the range of ants in the arboreal stratum (0.5–3 m) by observing the numbers of ant species feeding on *H. brunonis* at both EFN and vials containing artificial solutions. We collected ants feeding on floral-bud and young leaf EFN and recorded their presence three times a day (1000–1200,

1300–1500, and 1600–1830 h) over 21 days. We also collected and recorded the numbers of ant species feeding on two categories of artificial nectar solutions ('EFN mimics' and 'aqueous' solutions), presented in Eppendorf vials with cotton wicks, placed on the tree trunk at a height of about 1 m in *H. brunonis* trees. EFN-mimicking solutions approximated the average compositions of EFN (in terms of sugars and amino acids) produced by young leaves and floral buds of *H. brunonis*. Aqueous solutions contained: (1) sugars such as sucrose, glucose, and fructose in the standard concentration ranges (5–50%) used for testing ant sugar preferences, (2) equicaloric soutions of these sugars at two calorific values (high and low), or (3) standard sugar and amino acid mixtures. Compositions of these solutions are detailed in Shenoy *et al*. (unpubl. data). At each site, ten trees were used to present the artificial nectar solutions to arboreal ants following the methodology of Blüthgen & Fiedler (2004): these trees were separated by a minimum distance of 8 m to minimize the pseudoreplication of ant colonies (Blüthgen & Fiedler, 2004). We recorded the number of ant species feeding on these solutions at 1500, 1800, and 2100 h on the same day, and 1000 and 1200 h the next day.

All ants were collected in 70% ethanol, separated into different morphospecies, identified to species or genus level using standard identification keys (Bingham, 1903; Bolton, 1994, 1995), and were then stored in the collections being maintained at the Insect Museum of the Centre for Ecological Sciences, Indian Institute of Science, Bangalore, India. We compared the number of ant species found in the surveys, i.e. around *H. brunonis* trees, with those feeding on artificial solutions or real EFN, and also with the number of ant species that were recorded to inhabit *H. brunonis* domatia.

RESULTS

GEOGRAPHICAL VARIATION IN PROTECTION
FROM HERBIVORY

Lower levels of herbivory with ant-patrolled treatments were observed on young leaves and floral buds only at the southernmost site: Solaikolli (Agumbe, buds, $N = 72$ pairs on 25 trees, $P = 0.39$; leaves, $N = 36$ pairs on 22 trees, $P = 0.63$; Sampaji, buds, $N = 26$ pairs on 21 trees, $P = 0.56$; leaves, $N = 19$ pairs on 13 trees, $P = 0.92$; Solaikolli, buds, $t = 2.36$, $N = 31$ pairs on 14 trees, $P = 0.02$; leaves: $t = 3.41$, $N = 19$ pairs on 16 trees, $P = 0.003$; Fig. 2A). Furthermore, this reduced herbivory of floral buds translated into a significantly higher fruit set for ant-patrolled inflorescences (Agumbe, 0.03 ± 0.24 SD; Sampaji, 1.19 ± 2.42; Solaikolli, 0.32 ± 0.91) compared with ant-excluded
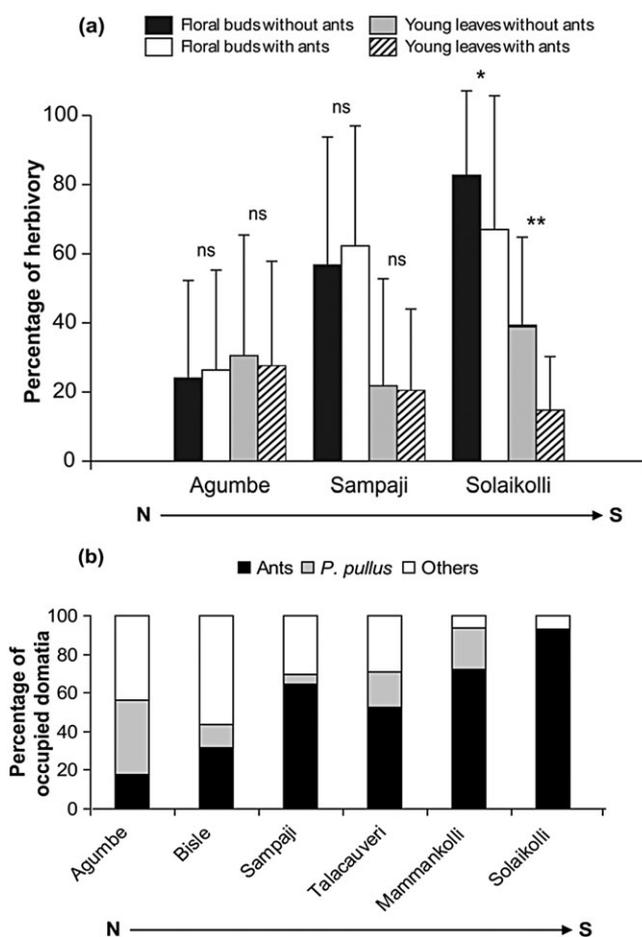
Figure 2. Spatial variation in protection from herbivory by ants and occupancy of domatia of *Humboldtia brunonis* by different invertebrate groups. A, protection from herbivory by ants on ant-patrolled and ant-free floral buds and young leaves (% herbivory ± SD) at three sites. B, percentage of domatia occupied by different invertebrates (within domatia occupied by a single species).

inflorescences (Agumbe, 0.11 ± 0.46; Sampaji, 0.15 ± 0.78; Solaikolli, 0.03 ± 0.18) only at Solaikolli (Agumbe, $N = 72$, $P = 0.25$; Sampaji, $N = 26$, $P = 0.07$; Solaikolli, $Z = 1.9917$, $N = 31$, $P = 0.046$). Thus, there was significant protection of *H. brunonis* by ants only at the southernmost site: Solaikolli. Yet there also appeared to be differences in basic herbivory levels between the sites, with Agumbe experiencing lower floral bud herbivory in the absence of ants (24%) compared with the other sites (57% in Sampaji and 83% in Solaikolli) (Fig. 2A).

GEOGRAPHICAL VARIATION IN DOMATIA OCCUPANCY

Earthworms were the most abundant occupants of the domatia in the north, whereas ant occupancy of

the domatia was highest in the southernmost site (Fig. 2B). Although a large proportion (more than 50%) of domatia also remained unoccupied at some sites, the lowest percentage of unoccupied domatia occurred in the southernmost site (Table 1). A considerable percentage of domatia was also occupied by other invertebrates at some sites (Fig. 2B). These included various invertebrates such as *Braunsapis* bees and centipedes (M. Shenoy & R.M. Borges, unpubl. data). There was a significant negative correlation between latitude and the percentage of domatia occupied by ants ($r_s = -0.829$, $N = 6$ sites, $P = 0.042$), indicating that relatively more domatia were occupied by ants towards the south. There was no significant concordance in the percentage of domatia occupied by ants, earthworms, and other invertebrates across the six sites ($W = 0.073$, $P = 0.955$), and no significant correlation between latitude and the percentage of domatia occupied by earthworms ($r_s = 0.486$, $N = 6$ sites, $P = 0.392$), or other invertebrates ($r_s = 0.771$, $N = 6$ sites, $P = 0.072$), despite a positive trend in both cases (Fig. 2B).

Sixteen ant species in nine genera belonging to three subfamilies (Dolichoderinae, Formicinae, and Myrmicinae) occupied domatia at the six sites (Table 1). This constituted only 9.6% of the ant species at all sites (20.6% in Agumbe, 12.3% in Sampaji, and 8.1% in Solaikolli; Table 2; Supporting Information, Table S1). Several ant species recorded in the terrestrial stratum were also found within the domatia (Supporting Information, Table S1). Only dolichoderine and myrmicine ants inhabited domatia at all sites (Table 1). Myrmicine ants occupied the highest percentage of domatia at all sites excepting the southernmost site, Solaikolli, where dolichoderine ants (represented only by *Technomyrmex albipes*) dominate (Table 1). Among the nine genera of ants inhabiting domatia, *Crematogaster* and *Vombisidris* species were found at five of the six sites. At the species level, *Vombisidris humboldticola* was found in domatia at five of six sites, whereas other ant species were found only at one to three of the sites, indicating considerable variation in the identity of domatia occupants across sites (Table 1).

GEOGRAPHICAL VARIATION IN DOMATIA MORPHOMETRICS

Empty domatia were shorter than occupied domatia (Table 3) ($F_{1, 616} = 11.901$, $P = 0.0006$), with no significant difference in this pattern across sites ($F_{2, 616} = 2.942$, $P = 0.053$) (although the value borders significance) and no significant interaction effect ($F_{2, 616} = 0.727$, $P = 0.484$). Empty domatia were narrower than occupied domatia in Bisle ($U_{212, 73} = 6589.5$, $P = 0.049$) and Talacauveri ($U_{141, 55} = 3153$,

Table 1. Percentage of domatia left unoccupied, and those occupied by different ant species, considering all of the domatia examined[a] and only those occupied by ants[b]

Site	Agumbe	Bisle	Sampaji	Talacauveri	Mammankolli	Solailkolli
	$N = 376$[a] (26)[b]	$N = 285$[a] (23)[b]	$N = 170$[a] (60)[b]	$N = 196$[a] (29)[b]	$N = 141$[a] (34)[b]	$N = 151$[a] (96)[b]
Unoccupied	53.9	74.4	45.3	71.9	66.7	30.5
Dolichoderinae	1.9 (26.9)	1.8 (21.7)	4.1 (11.7)	3.1 (20.7)	3.6 (14.7)	57.0 (89.6)
1 *Tapinoma indicum*	0	1.8 (21.7)	0	0	0	0
2 *Tapinoma melanocephalum*	0	0	3.5 (10.0)	0	0	0
3 *Tapinoma* sp. 1	0	0	0	3.1 (20.7)	3.6 (14.7)	57.0 (89.6)
4 *Technomyrmex albipes*	0	0	0.6 (1.7)	0	0	0
5 *Technomyrmex* sp. A	1.9 (26.9)	0	0	0	0	0
Formicinae	0.5 (7.7)	0	0.6 (1.7)	0	0.7 (2.9)	4.0 (6.2)
6 *Camponotus* sp. 1	0	0	0	0	0.7 (2.9)	0
7 *Plagiolepis* sp. A	0.5 (7.7)	0	0	0	0	3.3 (5.2)
8 *Plagiolepis exigua*	0	0	0	0	0	0.7 (1.0)
9 *Plagiolepis watsoni*	0	0	0.6 (1.7)	0	0	0
Myrmicinae	4.5 (65.4)	6.3 (78.2)	30.6 (86.7)	11.7 (79.3)	15.6 (64.7)	2.7 (4.1)
10 *Catalaucus taprobanae*	0.5 (7.7)	1.1 (13.0)	1.2 (3.3)	0	0	0.7 (1.0)
11 *Crematogaster dohrni*	1.6 (23.1)	0	15.9 (45)	0	0	0
12 *Crematogaster wroughtonii*	0	3.5 (43.5)	6.5 (18.4)	0	2.1 (8.8)	0
13 *Dilobocondyla* sp. 1	0	1.8 (21.7)	0	0	0	0
14 *Monomorium monomorium*	0.5 (7.7)	0	0	0	0	0
15 *Monomorium* sp. A	0.3 (3.8)	0	0	0	0	2.0 (3.1)
16 *Vombisidris humboldticola*	1.6 (23.1)	0	7.1 (20.0)	11.7 (79.3)	13.5 (55.9)	0
Unclassified	0 (0)	0 (0)	0 (0)	0 (0)	4.3 (17.6)	0 (0)
17 Winged single ant	0	0	0	0	4.3 (17.6)	0
TOTAL	6.9 (100)	8.1 (100)	35.3 (100)	14.8 (100)	24.2 (100)	63.7 (100)

Table 2. Number of ant species found in different strata and at different arboreal substrates at the study sites

Site	Terrestrial and arboreal strata	At aqueous solutions	At EFN mimics	At H. brunonis EFN	In H. brunonis domatia
Agumbe	34	15	7	11	7
Sampaji	57	12	10	7	7
Solaikolli	62	7	5	4	5
All three sites	125	25	16	13	12

Utilization of different resources by ant species at the study sites

Ant taxa	Agumbe Aqueous solutions	Agumbe EFN mimics	Agumbe EFN	Agumbe Domatia	Sampaji Aqueous solutions	Sampaji EFN mimics	Sampaji EFN	Sampaji Domatia	Solaikolli Aqueous solutions	Solaikolli EFN mimics	Solaikolli EFN	Solaikolli Domatia
Dolichoderinae												
Tapinoma melanocephalum	×		×		×	×	×	×				
Technomyrmex albipes					×		×	×	×	×	×	×
Technomyrmex sp. A	×		×	×								
Formicinae												
Camponotus angusticollis	×	×	×		×	×	×		×	×	×	
Camponotus oblongus					×	×						
Camponotus parius	×	×										
Paratrechina taylori					×	×						
Pheidole sp. A2					×	×						
Pheidole sp. S1					×	×						
Pheidole watsoni				×*								
Plagiolepis exigua												×
Plagiolepis sp. A				×								
Plagiolepis watsoni								×				×
Polyrachis illaudata	×		×									
Myrmicinae												
Catalaucus taprobanae	×	×	×	×			×	×				
Crematogaster dohrni	×	×	×	×	×	×	×	×	×	×	×	×
Crematogaster wroughtonii	×	×	×	×	×	×	×	×				
Monomorium floricola	×		×	×								
Monomorium monomorium	×		×	×								
Monomorium sp. A	×		×									
Myrmicaria brunnea					×	×	×		×	×	×	
Pheidole sp. A1	×	×										
Pheidole sp. A2	×	×										
Pheidole sp. M									×			
Pheidole sp. M1									×	×		
Pheidole sp. M2									×			
Strumigenys godeffroyi	×				×	×						
Tetramorium smithi					×	×						
Tetramorium sp. A	×		×	×								
Vombisidris humboldticola												×

*Found without brood.

Table 3. Morphometric measurements of *Humboldtia brunonis* domatia from three sites

Length and breadth (cm) of empty domatia and those occupied by a single species (mean ± SD, range)

Site	Empty domatia			Occupied domatia		
	N	Length	Breadth	N	Length	Breadth
Bisle	212	8.79 ± 2.31, 3.5–15.0	0.48 ± 0.13, 0.3–1.0	73	9.54 ± 2.78, 4.5–19.0	0.51 ± 0.10, 0.3–1.0
Talacauveri	141	8.96 ± 2.88, 3.4–16.5	0.58 ± 0.14, 0.3–1.0	55	10.15 ± 2.47, 5.5–17.0	0.61 ± 0.12, 0.3–0.9
Mammankolli	94	8.55 ± 2.75, 3.4–17.0	0.64 ± 0.22, 0.4–1.7	47	9.07 ± 2.85, 4.5–17.0	0.64 ± 0.16, 0.3–1.0

Length and breadth (cm) of domatia occupied by different groups of invertebrates (mean ± SD, range)

Site	Ants			Earthworms			Other invertebrates		
	N	Length	Breadth	N	Length	Breadth	N	Length	Breadth
Bisle	23	8.61 ± 2.51, 5.0–15.4	0.50 ± 0.10, 0.3–0.7	9	10.26 ± 3.85, 6.8–19.0	0.48 ± 0.07, 0.4–0.6	41	9.91 ± 2.59, 4.5–15	0.51 ± 0.11, 0.3–1.0
Talacauveri	29	10.39 ± 2.24, 5.8–17.0	0.61 ± 0.12, 0.4–0.9	10	8.61 ± 1.97, 5.5–11.0	0.62 ± 0.15, 0.3–0.8	16	10.67 ± 2.88, 5.7–16.0	0.61 ± 0.11, 0.4–0.8
Mammankolli	34	9.19 ± 2.86, 4.5–17.0	0.63 ± 0.15, 0.3–1.0	10	7.86 ± 2.24, 5.5–12.0	0.63 ± 0.14, 0.5–0.9	3	11.30 ± 3.86, 7.9–15.5	0.87 ± 0.15, 0.7–1.0

$P = 0.036$), but this was not so in the southern site Mammankolli ($U_{94, 47} = 1972.5$, $P = 0.291$).

The length of domatia occupied by ants, earthworms, and other invertebrates (Table 3) was not different ($F_{2, 166} = 2.633$, $P = 0.075$) across sites ($F_{2, 166} = 0.323$, $P = 0.725$), with no significant interaction effect ($F_{4, 166} = 1.826$, $P = 0.126$). Moreover, the breadth of domatia occupied by these invertebrate groups was also not different at all sites (Table 3) (Bisle, $H_{2, 73} = 0.833$, $P = 0.659$; Talacauveri, $H_{2, 55} = 0.274$, $P = 0.872$; Mammankolli, $H_{2, 47} = 4.962$, $P = 0.084$).

These data could mean that only a subset of domatia can be occupied by plant-dwelling invertebrates, including potentially mutualistic ants and interlopers, or that there is a preference for larger domatia.

SPATIAL VARIATION IN ANT DIVERSITY AND ABUNDANCE

A total of 125 ant species (40 genera and six subfamilies) were collected from the three sites with the arboreal and terrestrial sampling employed (Agumbe, 34 species, 18 genera, five subfamilies; Sampaji, 57 species, 30 genera, five subfamilies; Solaikolli, 62 species, 31 genera, six subfamilies; Table 2, Supporting Information, Table S1). In the terrestrial strata, most species occurred in only one of the 20 quadrats sampled, i.e. 5% occurrence, indicating extremely localized distributions (Fig. 3) that were similarly skewed across sites (skewness: Agumbe, 3.2 ± 0.5; Sampaji, 3.4 ± 0.5; Solaikolli, 3.4 ± 0.5). In the northernmost site Agumbe, no species occurred in more than 20% ($N = 4$ quadrats by *Anoplolepis gracilipes*) of the quadrats sampled. In Sampaji the maximum percentage of quadrats in which a species occurred was 70% ($N = 14$ by *Pheidole* sp. S2), whereas in the southernmost site, Solaikolli, it was 85% ($N = 17$ by *T. albipes*). These data suggest that the northernmost site, Agumbe, is considerably impoverished in terms of ant species in the sampled terrestrial and arboreal strata, compared with Sampaji and Solaikolli (Fig. 3), and that the highest ant diversity in these strata occurred in the southernmost site.

As *H. brunonis* is an understorey tree, the ALL protocol, along with manual sampling of ants in the arboreal stratum, was sufficient to detect a progressive decline in ant species richness from those found at the site to those feeding on *H. brunonis* at different types of sugar solutions to those found in *H. brunonis* domatia (Table 2). In fact, 90.5% of ants that were found in the arboreal stratum ($N = 42$) were also found in the terrestrial stratum ($N = 38$) (Supporting Information, Table S1). Furthermore, several ant species recorded in the terrestrial stratum were
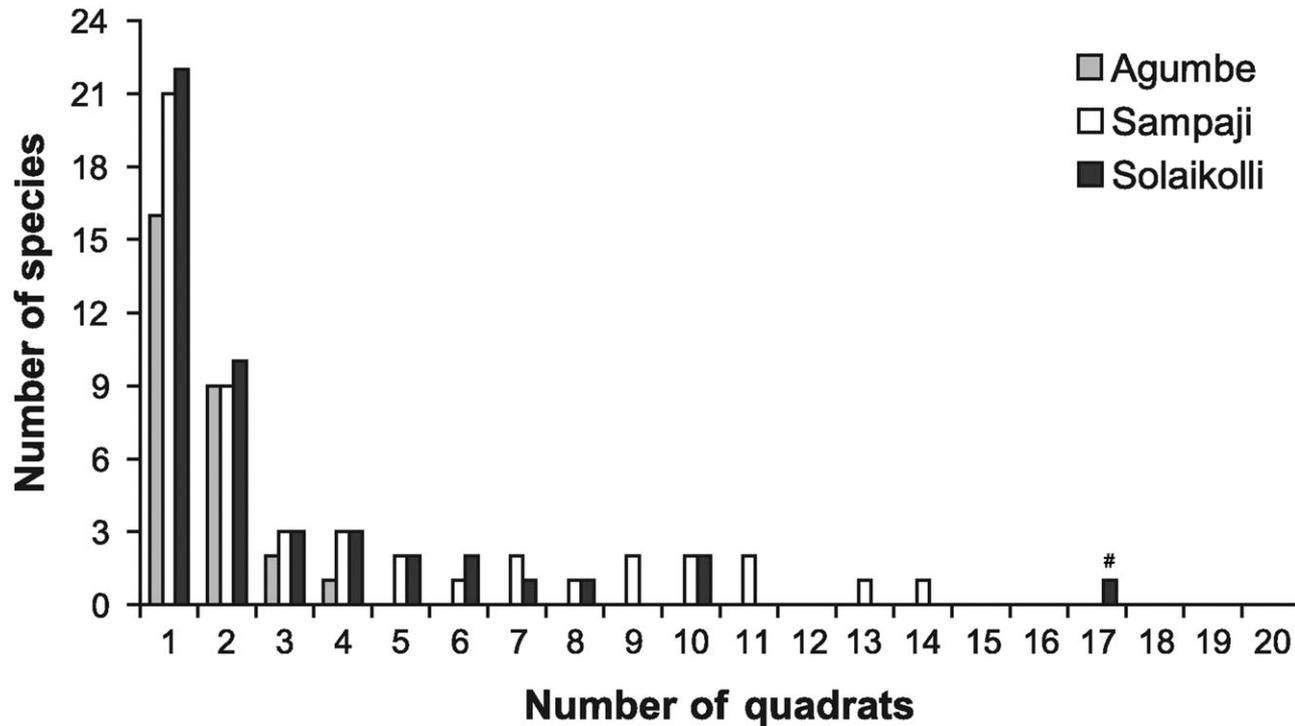
Figure 3. Percentage of species occurrence of ants at the three sites. #Number of quadrats occupied by *Technomyrmex albipes* in Solaikolli.

also found feeding on EFN (Supporting Information, Table S1). This progressive decline in numbers of ant species suggests a progressive specialization of ant interactions with *H. brunonis*.

DISCUSSION

The present study has shown that the extent of ant protection from herbivory received by a myrmecophyte is positively related to the local richness of ants, and extent of ant occupancy of domatia, and that too over a geographical scale tat exhibits considerable climatic variation. The highest proportion of interlopers in the domatia occurred in sites with the least protection from herbivory by ants. Domatia morphometry did not vary across sites: the same sizes of domatia were occupied by ants and interlopers across the geographic range. However, domatia harbouring ants and interlopers were longer and broader than empty ones. The protection received from ants against herbivory was significant only at the southernmost site, where one potentially mutualistic ant species *T. albipes* was most abundant. The study therefore emphasizes the impact of local factors such as the availability of suitable ant partners, domatia occupancy, and the presence of interlopers on the possible emergence of a protection mutualism between ants and plants.

ANT TRAITS THAT FACILITATE THE ESTABLISHMENT OF PROTECTION MUTUALISM

With studies on ant-plants from diverse continents, ant and plant traits that facilitate the establishment of protection mutualisms will be better understood, and may provide insights into partner-choice mechanisms (Bull & Rice, 1991; Foster & Wenseleers, 2006). In our investigation of the myrmecophyte *H. brunonis* over its geographic distribution, only the dolichoderine ant *T. albipes* appeared to be a suitable partner. At one southern site (Makut, separated by only 10 km from the southernmost site in this study, Solaikolli) where the interaction between ants and *H. brunonis* has previously been studied, only *T. albipes* was found to provide significant protection against herbivory on young leaves, compared with other ants (Gaume *et al.*, 2005b, 2006); trees occupied by *T. albipes* had higher fruit set compared with those inhabited by other ant species. The significant protection against herbivory by ants observed only at the southernmost site, Solaikalli, in the present study is therefore attributed to the abundant *T. albipes* at this site, although this was not specifically investigated.

The following ant traits may contribute to the success of the *T. albipes–H. brunonis* interaction.

Polydomy (Debout et al., 2007): Technomyrmex can also nest opportunistically (Philpott & Foster, 2005),

and may be immune to nest-site limitation (Fonseca, 1999). Consequently, non-domatia-bearing individuals of *H. brunonis* also obtain protection from ants that construct carton nests on them (Gaume *et al*., 2005b).

Dominance: Its ubiquity within the southernmost site coupled with its large colony size contributed to its effectiveness as a protective ant (Meunier *et al*., 1999). Yet not all species of *Technomyrmex* are dominant, as we found for *Technomyrmex* sp. A in Agumbe, and was observed in Peninsular Malaysia and Borneo (Pfeiffer, Tuck & Lay, 2008). *Technomyrmex albipes* is also regarded as a tramp and invasive species elsewhere (Holway *et al*., 2002; Oliver *et al*., 2008b); such ants are superior at establishing EFN-mediated mutualisms with plants (Ness & Bronstein, 2004).

Circumdiel activity rhythms: Technomyrmex albipes displays circumdiel activity, unlike sympatric and syntopic *Crematogaster dohrni* or *Camponotus infuscus* (Gaume *et al*., 2005a). As *H. brunonis* is attacked by diurnal and nocturnal herbivores (Gaume *et al*., 2005a), circumdiel activity is an important trait for an ant partner, as observed in other ant–plant systems (Gaume & McKey, 1999; Debout, Schatz & McKey, 2005).

Aggression and recruitment: The aggressive *T. albipes* responded to herbivores with quicker recruitment compared with other arboreal ants (Gaume *et al*., 2006).

Although *T. albipes* possesses several traits that make it suitable for establishing a protection mutualism with *H. brunonis*, the extreme site-specific positive interaction between these ants and *H. brunonis* is yet to be explained. Although *T. albipes* was recorded at Sampaji (Table 2; see Supporting Information, Table S1), it was not a dominant ant there. Therefore, local site effects influencing the competitiveness of the species must be considered. However, the occurrence of *T. albipes* as the major domatia-inhabiting ant in *Humboldtia laurifolia*, a true myrmecophyte, in Sri Lanka (Krombein *et al*., 1999), lends support to the suitability of the dolichoderine *T. albipes* as a partner of *Humboldtia*. Worldwide, the Dolichoderinae had the highest percentage of genera developing mutualistic interactions with domatia-bearing plants (Oliver, Leather & Cook 2008a).

CONSTRAINTS ON ANT–PLANT INTERACTIONS

Various traits such as domatia or petiole characters (Brouat *et al*., 2001; Djiéto-Lordon *et al*., 2005; Webber *et al*., 2007b; Grangier *et al*., 2009), stem surface characters (Davidson, Snelling & Longino,

1989; Federle & Rheindt, 2005), and EFN composition (Heil *et al*., 2005) can constrain ant–plant interactions. That at every site the number of ant species found in the litter and arboreal strata was much higher than that found in the domatia of *H. brunonis* (Table 2), as also found elsewhere (Hossaert-McKey *et al*., 2001), suggests selection mechanisms at the level of the plant or ants. Presence in the domatia may also be influenced by nesting habit (Fonseca, 1999). Thus, for example, although ponerine ants and *Strumigenys* sp. inhabit domatia of other tropical ant plants (Davidson & McKey, 1993), they were absent from *H. brunonis* domatia, but were present in the local ant community. On the other hand, the small and timid *V. humboldticola* has only been found in the domatia of *H. brunonis* (Zacharias & Rajan, 2004; Gaume *et al*., 2006) and of the congeneric ant-plant *Humboldtia decurrens* (Zacharias & Rajan, 2004). This ant appears to be the most specialized occupant of *Humboldtia* domatia, although the benefits, if any, that it provides to its host are unknown.

The ant species associated with the EFN of *H. brunonis* at each site (Table 2) was close to the worldwide average of 9.4 ant species per EFN-producing plant species (Oliveira & Brandão, 1991). Our study therefore also reaffirms that although the connectance between ants and plants in an EFN-based network is larger, that between ants and plants in a myrmecophytic network is much lower (Guimarães *et al*., 2006, 2007; Blüthgen *et al*., 2007). Such networks are also affected by ant body size (Chamberlain & Holland, 2009) and species abundance, with interactions between pairs of abundant species being stronger and more symmetric (Vázquez *et al*., 2007), as would be expected, therefore, for the abundant *H. brunonis* and the dominant ant *T. albipes* at some sites. At our Western Ghats sites, the number of EFN-producing species is very low (M. Shenoy & R.M. Borges, pers. observ.): hence the dominant EFN-producing *H. brunonis* is predicted to be a very important though ephemeral node in any ant–plant interaction network in this area. A larger number of ants was recorded at standard sugar solutions relative to EFN-mimicking solutions at each site (Table 2), suggesting another constraint in operation, i.e. one based on nutrients in liquid form (Heil *et al*., 2005). Whereas generalist ants may be found at standard sugar solutions, only more specialized plant ants such as *T. albipes* were found at EFN-mimicking solutions, which contain more dilute sugars and high concentrations of amino acids (M. Shenoy, V. Radhika, S. Satish & R.M. Borges, unpubl. data; Shenoy, 2008).

INTERLOPERS IN ANT–PLANT SYSTEMS

Some types of domatia may be more accessible and susceptible to opportunistic dwellers. Interlopers

such as earthworms, arthropods, and even gastropods have been recorded within leaf pouch domatia (Dejean & Djiéto-Lordon, 1996), suggesting easy access. The self-opening slit in the domatia of *H. brunonis* may make it more susceptible to interlopers, and may explain their large numbers in the domatia of both *H. brunonis* (Rickson *et al.*, 2003; Gaume *et al.*, 2006; Shenoy, 2008; Shenoy & Borges, 2008) and *Humboldtia laurifolia* (Krombein *et al.*, 1999). Consequently, domatia barriers may not be as effective in this genus as in other ant–plant associations (e.g. Djiéto-Lordon *et al.*, 2005). Yet, domatia morphometry could matter, as many domatia were found to be unoccupied, and the size of the unoccupied domatia was found to be significantly smaller than that of the occupied domatia (Table 3). The minimum breadth of occupied domatia was 3 mm (Table 3), similar to the minimum observed in another case (Moog, Drude & Maschwitz, 1998). Furthermore, across the six sites, several domatia were sometimes occupied by more than a single invertebrate species (Gaume *et al.*, 2006; Shenoy, 2008), corroborating the possible shortage of domatia suitable for occupancy. Also, there was no significant difference in the morphometry of domatia occupied by different groups of invertebrates (ants, arboreal earthworm, and other invertebrates). This may indicate competition for particular size classes of these specialized housing spaces, as predicted by the nesting limitation hypothesis (Davidson *et al.*, 1989; Fiala & Maschwitz, 1992; Fonseca, 1999).

The dominance of earthworms (*P. pullus*) in the northern site (Agumbe) and the reversal of this pattern in the southern site (Solaikolli), where *T. albipes* is dominant in the domatia, may be related to differences in climate. Whereas Agumbe has the highest annual rainfall in the Western Ghats, viz. 900 cm (Rai, 2000), it also has the longest length of dry spells between consecutive rainy spells during the dry season (Fig. 1). Earthworms are found within domatia only in the dry season, during which they attain sexual maturity; earthworms leave the domatia to mate only in the monsoon (Gaume *et al.*, 2006). Therefore, the latitudinal pattern in the length of dry spells during the dry season (Fig. 1) may explain why such earthworms, which are sensitive to drying on exposure to air outside the domatia (Gaume *et al.*, 2006), dominate the domatia in the northern site at Agumbe. Whether the negative effect of the earthworm mucus on ants (Gaume *et al.*, 2006) also has a role to play in this pattern remains to be determined. Furthermore, although the role of some interlopers as parasites of ants is known in some systems (Stanton, Palmer & Young, 2005), the roles of many of the alleged interlopers in our system remain to be discovered.

GEOGRAPHICAL VARIATION IN PROTECTION MUTUALISMS IN SEASONAL MONSOONAL FORESTS

Seasonal and monsoonal forests in the Paleotropics with fewer ant-plant species compared with the Neotropics may have interactions that are more often shaped by species sorting (*sensu* Jordano, 1987) than by pair-wise co-evolution (Davidson & McKey, 1993; Heil & McKey, 2003). The generalist nature of interactions between *H. brunonis* and its network of EFN feeders and domatia inhabitants along with the restricted distribution of its potentially protective mutualistic ant species *T. albipes* in *H. brunonis*-dominated forests suggest the existence of such sorting mechanisms. The ephemeral, abundant nectar resources provided by the EFN of the dominant *H. brunonis* are largely exploited by opportunistic ants. The identities of partners as well as the types and strengths of ant–plant interactions could vary because of spatiotemporal fluctuations (Feldhaar *et al.*, 2003; Bruna, Lapola & Vasconcelos, 2004; Izzo & Vasconcelos, 2005; Gaume *et al.*, 2005a, b). Although this variation can also be exhibited at the intraspecific level (Maschwitz *et al.*, 1996; McKey, 2000; Murase *et al.*, 2003; Dalecky *et al.*, 2005, 2007; Léotard *et al.*, 2009), our study is the first to find latitudinal relationships with protective ants and interlopers. It is also possible that sites may vary in their basic herbivore pressure, in the absence of ants, as we found for floral buds in our study, and thus the need for ant protection could also vary with site, and with time.

Domatia of *H. laurifolia* in Sri Lanka, which was connected to the Western Ghats in geological time (Munasinghe & Dissanayke, 1982), were occupied by ants to an even greater extent than that found for *H. brunonis* at the southern site Solaikolli, with *T. albipes* occupying up to 80% of domatia in the former species (Krombein *et al.*, 1999). This suggests prima facie evidence for the gradual increase in domatia occupancy by *T. albipes* for this endemic plant genus from the north to the south of the Indian subcontinent (including Sri Lanka). Although the causes of this pattern are not yet understood, preliminary observations suggest a correlation with the proportion of individual trees of *H. brunonis* that bear domatia at each site increasing towards the south (J. Chanam & R.M. Borges, unpubl. data). In *H. laurifolia* all trees bear domatia, and it is a typical myrmecophyte. Studies on polymorphic myrmecophytes such as *H. brunonis* therefore provide excellent opportunities to understand the evolutionary impetus for the development of ant–plant protection mutualisms.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Resource utilization, habitat, and number of quadrats occupied by ants found at three sites in the Western Ghats using the ALL protocol, including manual sampling.

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