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Ants inhabiting myrmecophytic ferns regulate the distribution of lianas on emergent trees in a Bornean tropical rainforest

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Little is known about the spatial distribution of lianas on emergent trees in tropical rainforests and the factors affecting this distribution. The present study investigated the effects of an arboreal ant species, *Crematogaster difformis*, which forms myrmecophytic symbioses with two epiphytic ferns, *Lecanopteris* sp. and *Platycerium* sp., on the spatial distribution of lianas associated with emergent trees. Living lianas were placed onto trunk surfaces inside and outside the territories of the ants in the canopy, to examine their ability to remove them. The number of leaves pruned by the ants was significantly higher on lianas inside than outside their territories. The spatial overlap of the distributions of lianas and the two ferns on emergent trees were then examined. The frequency of liana colonization of tree crowns was found to be significantly lower on trees with than without ferns. Under the natural conditions, *C. difformis* workers were observed biting and pruning the lianas. These results suggest that *C. difformis* regulates the distribution of lianas on emergent trees.

Keywords: ant–plant interactions; *Crematogaster difformis*; liana-pruning behaviour; epiphytes; *Lecanopteris*; *Platycerium*

1. INTRODUCTION

As symbionts, ants greatly influence the survival and distribution of plants [1–3]. Among ant species exhibiting mutualistic symbioses with myrmecophytic plants, some prune the lianas that encroach upon these partners [4–7], thus providing them with advantages in interspecific competition for light [5]. In tropical rainforest canopies, where lianas are present in high abundance and biomass [8], various ant species maintain mutualistic symbioses with specific epiphytes [9,10]. Although some of these species are known to prune lianas climbing their host myrmecophytes, whether ants likewise protect their host epiphytes have yet to be determined.

Crematogaster difformis F. Smith (Formicidae, Hymenoptera) is a symbiotic ant species that nests in the hollow rhizomes of the myrmecophytic epiphyte *Lecanopteris* sp. (Pteridophyta: Polypodiaceae) and in the enclosed layers of old leaves of another myrmecophytic epiphyte, *Platycerium* sp. (Pteridophyta: Polypodiaceae) [11,12]. Both epiphytic ferns establish themselves in the crown of emergent trees in Bornean lowland forests. All mature plants of the two fern species harbour *C. difformis*, and all the nests of this ant species are located within the ferns [12]. The ants tend to establish territories encompassing most of the surface areas of the tree crowns where they live, defending not only their host ferns, but also host emergent trees from herbivores inside the territories [12,13]. The present study examined the hypothesis that workers of *C. difformis* prune lianas climbing the emergent trees on which their partner myrmecophytic ferns grow, and that the distribution of the lianas is reduced by the presence of *C. difformis* nests inside the ferns. Accordingly, living lianas were experimentally placed in *C. difformis* territories, after which the distributions of lianas and epiphytic fern species on the emergent trees were examined.

2. MATERIAL AND METHODS

This research was conducted in a lowland mixed dipterocarp forest in Lambir Hills National Park, Sarawak, Malaysia (4°20′ N, 113°50′ E; 60 (m) a.s.l.). Some emergent trees reach 70 m in the canopy there. We defined a tree whose lowest branch of the crown was greater than 10 m above the tops of crowns of neighbouring trees and not linked with any branches of other trees as an emergent tree. A canopy access system is in place, which consists of observation towers and aerial walk-ways, allowing close observation of the organisms inhabiting the emergent trees [14].

(a) *Field experiment*

The liana *Tetrastigma leucostaphylum* (Vitaceae: Vitoideae) develops on trunks of canopy trees growing at the study site. In September 2006 and August 2010, we used the canopy access system and single-rope techniques to experimentally place stem pieces of the liana on eight emergent trees which ranged from 55 to 65 m in height and were territorialized by *C. difformis*. The eight emergent trees belong to five tree species, which have been found to harbour the two ferns in their crowns in the study site (table 1). Sixteen stem pieces 1 m long, 9–11 mm in diameter and bearing five or six leaves were collected from a randomly selected individual of *T. leucostaphylum*. Liana pieces with obvious herbivory damage were excluded. Immediately after their collection, each stem piece was placed in a bottle of water to keep it fresh. The bottle was then set on each of the eight target trees within the boundary of a *C. difformis* territory, with nylon tapes used to place the stem pieces closely onto the surfaces of the trees trunks at 30–40 m above the ground (liana-introducing treatment; figure 1*a*). As a control treatment, a liana piece in the bottle was set on each of the eight target trees outside a *C. difformis* territory but near the territory boundary in the same way. The distance between the two treatment sites was more than 2 m for each tree. The behaviours of *C. difformis* worker ants in response to each experimentally placed stem piece were observed for 30 min immediately after stem placement.

Our preliminary observation of leaves and stems bitten by *C. difformis* workers enabled us to recognize bite marks made by the ants on the experimental stem pieces. Subsequently, each liana piece was checked for such bite marks 1 and 3 days after the liana-introducing placement. One week after the placement, the number of pruned leaves on each liana piece was counted. Differences in the number of pruned leaves between the two treatments were statistically tested using the non-parametric paired Wilcoxon signed-rank test.

(b) *Census*

In September 2009, we conducted a field census on epiphytic ferns and lianas associated with the crowns of 30 emergent trees of the five species (table 1). Using binoculars from several viewpoints on the canopy access system and on the ground, we verified the presence (or absence) of ferns and lianas on each emergent tree. We defined the presence of at least one liana climbed on the main trunk and extended onto first branches of the tree as the presence of a liana

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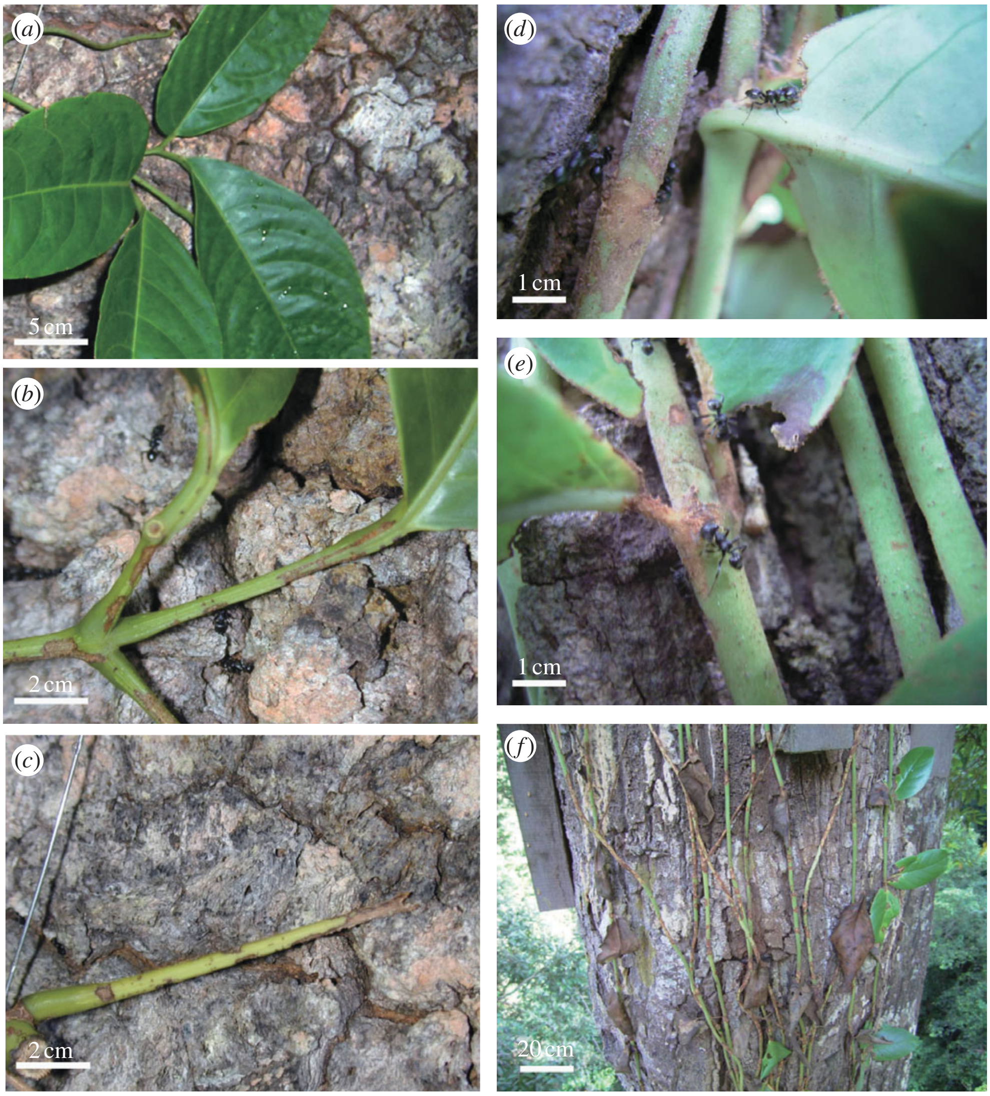

Figure 1. Control of liana growth as imposed by *C. difformis*: (*a*) a leaf from a 1 m-long liana piece experimentally placed on a trunk surface inside the territory of *C. difformis* immediately, (*b*) 1 day and (*c*) 3 days after placement. The (*d*,*e*) pruning behaviours of *C. difformis* workers: and (*f*) pruned lianas clinging to the trunk of an emergent tree inhabited by *C. difformis*.

Table 1. Number of emergent trees with (left column) and without (right column) lianas in the crowns of five tree species and trees in a group of unidentified species. Separate counts were conducted of trees with and without two epiphytic ferns, *Lecanopteris* sp. and *Platycerium* sp.

tree species	ferns	with lianas	without lianas
D. lanceolata	present	0	4
	absent	7	1
Shorea ferruginea	present	0	1
	absent	3	0
Shorea parvifolia	present	0	1
	absent	0	0
Shorea pilosa	present	0	1
	absent	2	0
S. smithiana	present	0	4
	absent	6	0
unidentified species	present	0	14
	absent	43	16

in the crown. Fisher's exact test was then applied to examine the association between the lianas and the myrmecophytic ferns for *Dryobalanops lanceolata* and *Shorea smithiana*, two tree species that had sufficient replicates in the study site. The same association test was performed for the other three tree species.

In addition, to examine the association between lianas and the ferns on a broader spatial scale (i.e. at community-wide levels), the distributions of the lianas and the two species of epiphytes were recorded for 73 emergent trees whose canopies were inaccessible but nonetheless visible from several viewpoints within the canopy access system. While the species of these 73 emergent trees could not be determined, we used binoculars to record the absence or presence of lianas and ferns. The association between liana and fern presence was statistically tested using Fisher's exact test.

3. RESULTS

(a) *Field experiment*

The number of pruned leaves was significantly higher inside than outside *C. difformis* territories ($p < 0.05$; figure 2). Thirty minutes after the stem pieces had been placed on trees within the territory, 6.3 ± 0.8 *C. difformis* workers were observed aggregating at the stems, which they began to bite. One day later, 23.8 ± 1.6 bite marks were recorded on leaves and petioles (figure 1*b*). After 3 days, at least one leaf had been removed from half the lianas and by one week from all of them (figure 1*c*). Conversely, none of the lianas outside the ant territories was damaged.

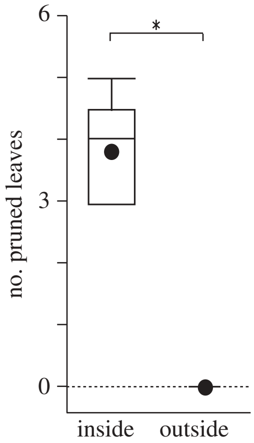

Figure 2. The average numbers of leaves pruned (closed circles) one week after the experimental placement of 1 m-long liana pieces on the trunk surface inside ($n = 8$) and outside ($n = 8$) the territories of *Crematogaster difformis* on eight emergent trees. The three horizontal lines of the box plot represent the 25th (upper), 50th (middle) and 75th (lower) percentiles, respectively. The vertical whisker shows the highest value. *$p < 0.05$, as determined by Wilcoxon's signed-rank test.

(b) *Census*

The proportion of emergent *D. lanceolata* and *S. smithiana* trees with a liana present was significantly higher on trees with neither of the two ferns present than on those with the ferns ($p < 0.01$ for *D. lanceolata*; $p < 0.05$ for *S. smithiana*; table 1). Similarly, for the other three emergent tree species associated with the ferns, the proportion of trees with a liana present was significantly higher in trees with neither of the ferns present than in those with the ferns ($p < 0.05$). For all target tree species, no liana reached the crown of an emergent tree that harboured the ferns. On the trunks of four of the emergent trees bearing ferns, the tips of the lianas were seen to have reached the farthest boundaries of *C. difformis* territories; the leaves and stems were heavily damaged in the vicinities of the trees and *C. difformis* workers were observed biting and pruning the lianas (figure 1*d–f*).

Of the 73 emergent trees of unidentified species, 14 harboured at least one fern while the others harboured none (table 1). The proportion of trees with at least one liana climbing to the crown was significantly lower on the 14 trees with the ferns than on the 59 trees with neither of the ferns ($p < 0.001$).

4. DISCUSSION

The spatial distribution of lianas in tropical rainforest canopies has been explained mainly by abiotic factors and by the morphological features of the host trees [15,16]. Our results suggest that the ant species *C. difformis*, which maintains symbioses with two species of epiphytic ferns, *Lecanopteris* sp. and *Platycerium* sp., regulates the spatial distribution of lianas climbing towards the crowns of emergent trees. The liana-regulating effect of *C. difformis* is also supported by our observations that lianas have been kept out of the crowns of four emergent trees harbouring the ferns for at least 16 years, since we started to observe the emergent trees in 1994. Moreover, lianas began to climb to the crown of an emergent tree only after all individuals of the two fern species that had previously grown on the tree died (owing to unknown causes) and their symbiont, *C. difformis*, had disappeared.

In tropical rainforests, some ant species that exhibit mutualistic symbioses with myrmecophytic plants, referred to as plant–ant species, prune lianas encroaching on their host [4–7]. However, the effect of *C. difformis* on the spatial distribution of lianas may be larger than expected, as suggested by the results obtained from previously studied plant–ant species. Although in those studies, workers of the plant–ant species patrolled only their host plants and pruned lianas clinging to them, *C. difformis* workers appear to patrol not only their host ferns but also the emergent trees on which the ferns have settled, thus pruning lianas clinging to their host ferns and to the host emergent trees. In general, lianas have negative effects on the growth and survival of host trees through competition for light, nutrients and water [16]. Thus, by reducing the presence of lianas, the presence of the ferns and their symbiotic partner *C. difformis* is probably to benefit the host emergent trees.

All *C. difformis* territories are limited to an area near their nests, which are usually formed in the cavity of their partner ferns [13]. Therefore, the survival and the growth of *C. difformis* colonies probably depend wholly on the two ferns. In turn, the activities of *C. difformis* may be indirectly affected by factors determining the distribution of the ferns. These factors remain to be explored in future studies.

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