

# Setting the trap: cleaning behaviour of *Camponotus schmitzi* ants increases long-term capture efficiency of their pitcher plant host, *Nepenthes bicalcarata*

Daniel G. Thornham<sup>\*1</sup>, Joanna M. Smith<sup>2</sup>, T. Ulmar Grafe<sup>3</sup> and Walter Federle<sup>1</sup>

<sup>1</sup>Insect Biomechanics, Zoology Department, University of Cambridge, Cambridge CB2 3EJ, UK; <sup>2</sup>Animal Biology Department, Anglia Ruskin University, Cambridge CB1 1PT, UK; and <sup>3</sup>Department of Biology, Universiti Brunei Darussalam, Tungku Link, Gadong 1410, Brunei Darussalam

## Summary

1. Beneficial ‘services’ in mutualistic interactions have often not evolved *de novo*, but from ancestral traits that had a function before the emergence of the association. These traits can then acquire novel functions in a mutualism. Even in many close insect–plant relationships, the services provided by each partner are still unclear.

2. In the well-known association between the carnivorous pitcher plant, *Nepenthes bicalcarata*, and *Camponotus schmitzi* ants in Borneo, the ants benefit by receiving food and nesting space in swollen, hollow pitcher tendrils, but the benefits to the plant are less clear. Previous studies have suggested that the ants protect against herbivory, prevent putrefaction of pitchers by removing prey items from them or increase the pitcher’s prey retention rate by attacking pitcher visitors.

3. Field observations showed that *C. schmitzi* ants regularly clean the pitcher rim (peristome), which is the main surface responsible for prey capture. We found that this behaviour increases the carnivorous plant’s prey capture efficiency and helps to maintain it over the pitcher’s life span. Running tests with *Oecophylla smaragdina* ants on colonised and ant-free older pitchers demonstrated 45.8% higher capture efficiency in pitchers inhabited by *C. schmitzi*. Head counts of trapped ants showed that *C. schmitzi*-colonised pitchers indeed captured significantly (45.2%) more prey than uncolonised pitchers of the same age.

4. The peristomes of ant-free older pitchers were strongly contaminated by fungal hyphae and other particles. Experimental contamination of clean pitcher peristomes with starch also strongly reduced capture efficiency. While the peristome was cleaned rapidly in ant-colonised pitchers and capture efficiency returned to the previous level within approximately 1 week, no cleaning and recovery were observed in ant-free pitchers.

5. Within the genus *Nepenthes*, *N. bicalcarata* has exceptionally long-lived pitchers. We propose that *C. schmitzi* ants benefit *N. bicalcarata* by keeping the pitchers effective over long periods of time, likely allowing the plant to acquire more nutrients per pitcher and thereby minimise pitcher construction costs.

6. Our findings demonstrate that the cleaning behaviour typical of many plant–ants has acquired a novel function in this ant–plant association, giving rise to a new type of myrmecotrophic mutualism.

**Key-words:** ant–plant interaction, carnivorous plants, cleaning behaviour, mutualism, myrmecotrophy

\*Correspondence author. E-mail: d.g.thornham@hotmail.com

## Introduction

Almost all terrestrial ecosystems are strongly shaped by interactions between insects and plants. While plants are usually eaten by animals, this trophic order is reversed in some plants which grow in particularly nutrient-poor environments. Plants of this kind either derive food from ants that live in special hollows provided by the plant (myrmecotrophic ant–plants) or they directly capture and digest insects (carnivorous plants) (Thompson 1981). Although the nutritional benefits for the plant are similar in myrmecotrophic and carnivorous plants, one is a mutualistic and the other an antagonistic interaction.

The association between the Bornean pitcher plant, *Nepenthes bicalcarata* Hook f., and the ant, *Camponotus schmitzi* Stärke (Fig. 1), is a very special case in this context, because it is both a carnivorous plant and an ant–plant (Beccari 1904). Like other *Nepenthes* pitcher plants, *N. bicalcarata* possesses specialised leaves to attract, capture, retain and digest arthropod prey. At the same time, however, its pitcher tendrils are swollen and hollow, providing nesting space for the highly unusual ant partner *C. schmitzi* (Schuitemaker & Staercke 1933). In striking contrast to other insects, *C. schmitzi* ants are able to run safely across the slippery trapping surfaces of the pitcher and never become trapped (Clarke & Kitching 1995). Moreover, the ants not only feed on extrafloral nectar, but they are able to dive and swim in the pitcher fluid, where they hunt for prey and retrieve food from the pitcher (Clarke & Kitching 1995; Merbach *et al.* 2007).

While it is obvious that *C. schmitzi* ants gain both food and nesting space from this association, the benefits for *N. bicalcarata* have been less clear. The ants' 'stealing' of prey items from the pitcher has led some to consider this relationship as nutritional parasitism (Adlassnig, Peroutka & Lendl 2010), but the presence of the swollen hollow pitcher tendrils suggests that there is some benefit to *N. bicalcarata*. A variety of hypotheses have been proposed to explain this benefit, focusing on different aspects of the relationship. First, it has been shown that although *C. schmitzi* ants are relatively non-aggressive, they provide some protection against herbivory, in particular against a weevil feeding on the shoot tips of *N. bicalcarata* (Merbach *et al.* 2007). Second, it was proposed that *C. schmitzi* helps to prevent putrefaction of *N. bicalcarata* pitchers by removing large prey items (Clarke & Kitching 1995). Third, it was recently observed that *C. schmitzi* sometimes attack pitcher visitors, thereby indirectly feeding its host plant by increasing the pitcher's prey retention efficiency (Bonhomme *et al.* 2011).

In this study we show that one important ecological benefit of *C. schmitzi* ants for *N. bicalcarata* has so far remained overlooked. It is based on the ants' ability to keep the surface of their host plant clean. Many plant–ants remove insect eggs and fungal pathogens from their host plants (Letourneau 1998; Heil & McKey 2003). Fiala *et al.* (1989) demonstrated that the *Crematogaster* (*Decacrema*) ant partners of *Macaranga* trees exhibit a cleaning behaviour towards all kinds of

foreign objects, which are collected with the legs and mouthparts and are often dropped off the plant. Similar cleaning behaviours have been reported from a variety of other ant–plant associations (Janzen 1972; Stout 1979; Risch 1982; Letourneau 1983; Federle, Maschwitz & Fiala 1998).

While the removal of herbivore eggs and pathogens may benefit *N. bicalcarata* in a similar way as in other ant–plants, surface cleanliness has special implications for *Nepenthes* pitcher plants. In most *Nepenthes*, insects are trapped by 'aquaplaning' when stepping on the rim of the pitcher (peristome) (Bohn & Federle 2004). The peristome has a highly regular microstructure formed by smooth, overlapping epidermal cells, which provide grip for insect claws only when the insect is running towards the pitcher inside, but not on the way out (Bohn & Federle 2004). Moreover, the peristome's microstructure, in combination with its hydrophilicity, makes the surface completely wettable, allowing a thin layer of water to be held (Bohn & Federle 2004; Bauer, Bohn & Federle 2008). Arthropods stepping onto the peristome surface 'aquaplane' and fall into the pitcher fluid, where they are retained and subsequently digested. As both surface chemistry and microtopography are essential for the slipperiness of the peristome, it is likely that the pitchers' trapping function will degrade by contaminating particles, fungi or epiphylls on the peristome over a pitcher's lifetime. However, the pitchers of many *Nepenthes* species, and of *N. bicalcarata* in particular, can be very long-lived (Osunkoya, Daud & Wimmer 2008).

Here we investigate (i) whether natural contamination of the peristome reduces the trapping efficiency of *N. bicalcarata* and (ii) whether the presence of *C. schmitzi* ants prevents or slows down this loss of efficiency.

## Materials and methods

Observations and field experiments on *N. bicalcarata* were performed at various stands of degraded peat swamp forests in Brunei Darussalam, Northwest Borneo (N 4°34', E 114°30'). We took video recordings of the behaviour of *C. schmitzi* ants on *N. bicalcarata* pitchers (a total of 746 h on 27 pitchers; Sony DCR-SR35E camera; Sony Corp., Tokyo, Japan), some of which had been artificially contaminated with starch. Recording times were chosen so as to ensure approximately equal coverage of all day and night times. However, as *C. schmitzi* are predominantly nocturnal (unpublished data), the cleaning behaviour was almost exclusively seen during the night. Therefore, much of the filming was performed using infrared light.

### ASSESSMENT OF PITCHER AGE

To determine the approximate age of each pitcher, we counted the number of leaf nodes from the apex of the plant to that bearing the pitcher such that the newest leaf was #1, the second newest was #2 and so on. Leaf production rate was estimated by tagging 26 emerging leaves in two study sites on plants of varying sizes. After 82 days (site 1,  $n = 14$ ) and 212 days (site 2,  $n = 12$ ), we determined the number of new leaves developed above the tagged leaf. We found that a new leaf was produced every  $31.9 \pm 1.76$  days (unless otherwise stated, mean  $\pm$  SEM are used throughout).

## ASSESSMENT OF NATURAL PREY CAPTURE SUCCESS

To compare natural prey capture rates between ant-colonised and uncolonised pitchers, the entire contents of 11 pitcher pairs matched for pitcher type (aerial or ground) and leaf node number were collected and examined under a stereomicroscope. As ants are the key taxon represented in the prey spectrum of *N. bicalcarata* (Adam 1997; Bonhomme *et al.* 2011), we counted the numbers of ant head capsules per pitcher; numbers were corrected for pitcher size (peristome perimeter length) and compared.

Exocuticle appears to be digested within pitchers very slowly so that the fused sclerites of insect heads remain intact and identifiable over long periods of time. Therefore, the number of head capsules can be used as a measure of the long-term prey capture success of *N. bicalcarata*. To check whether *C. schmitzi* ants remove any ant heads from the pitcher, we selected three densely ant-inhabited pitchers (two ground pitchers, one aerial), from which we removed the fluid, filtered it and returned it to the pitchers. Each pitcher was then 'fed' with 25 freshly killed *Crematogaster* sp. ants (collected from the growing tips of *N. bicalcarata*). The number of *Crematogaster* heads remaining after one week was counted. In all three pitchers, all 25 ants were still in the fluid one week later.

PERISTOME CLEANING EFFICIENCY OF *CAMPONOTUS SCHMITZI* ANTS

To determine whether the presence of *C. schmitzi* ants increases the cleaning rate of contaminated *N. bicalcarata* peristomes, pitchers were contaminated with starch and cleaning rates were compared between three different pitcher types: (i) ant-inhabited pitchers, (ii) previously ant-inhabited pitchers, but with ants removed and (iii) naturally ant-free pitchers. All experimental pitchers ( $n = 36$ ) were from different plants. For each pitcher, the numbers of *C. schmitzi* ants resting under the peristome were recorded using a dentist's mirror at the start of the experiment and on each observation day. For treatment 2, ants were removed from under pitchers' peristomes using a pooter. Additionally, two small openings were cut into the pitcher tendril at both ends of the domatium and the ants were sucked out. Tanglefoot® sticky resin (Tanglefoot Corp., Grand Rapids, MI, USA) was applied to the tendril, and any access points to the pitcher were removed to prevent re-colonisation.

Experimental contamination was performed using corn starch and potato starch (mean particle diameter =  $20.1 \pm 1.1 \mu\text{m}$ ,  $n = 79$ ). The peristome was generously coated with dry starch so that the first-order grooves were completely filled to the level of the ridges which remained visible. In an estimated 25% of cases, the ants responded immediately by running rapidly over the pitcher. This alarm behaviour was localised to the individual pitcher, it rapidly dissipated (within 1 min) and no subsequent behavioural changes were observed in response to the contamination. Following contamination, a small  $10 \times 10 \text{ mm}$  surface sample of the peristome was cut out and pinned into a box containing silica gel for drying and later microscope examination to determine contaminating particle density. To exclude rainfall (which can potentially decrease or increase contamination) as a confounding factor, pitchers were roofed using transparent plastic foil. Subsequent peristome samples were taken after 3, 7, 14, 20 and 28 days. The numbers of ants under the peristome were counted each time a sample was taken. Occasionally, individual *C. schmitzi* workers or queens were found in 'ants removed' or 'naturally ant-free' pitchers (19 of 216 observations). In such instances, these ants were removed without touching the peristome, and Tanglefoot was re-applied where appropriate.

On dry peristomes, the white starch was visible on the darker surface of the peristome. Relative contamination was estimated from the collected surface samples by placing them under a compound microscope (Leica DMR-HC upright microscope; Leica Microsystems, Wetzlar, Germany) using a  $5\times$  objective lens and epi-illumination. Images were recorded on a digital camera (Nikon Coolpix 4500; Nikon UK Ltd., Kingston upon Thames, UK) and analysed in ImageJ (National Institutes of Health, Bethesda, USA). To quantify the peristome area covered by particles, we first measured the greyscale levels of areas within the image (*c.*  $1500 \mu\text{m}^2$  or  $2.5 \times 10^3$  pixels) that were completely covered or dirt-free. We then defined an intensity threshold as the value halfway between the mean intensities of 'covered' and 'dirt-free'. The threshold range was 95–110 (of 256). Comparing the numbers of pixels on either side of the intensity threshold in a large area of each image (*c.*  $0.65 \text{ mm}^2$  or  $10^6$  pixels) yielded an estimate for the proportion of the peristome that was covered by starch ('contamination level'). Using an image-specific threshold corrected for variation of lighting between images and colour variation between peristomes.

The decrease of contamination level appeared to follow a sigmoidal pattern; data were therefore log-transformed using  $y_i = \ln\left(\frac{1}{y} - 1\right)$  to linearise and normalise them. A mixed model ANCOVA was carried out on the transformed data. Samples taken over successive weeks from individual pitchers were coded as repeated measures with time as a covariate and ant presence as the between-groups factor.

## RUNNING TESTS

We tested the slipperiness and trapping efficiency of *N. bicalcarata* pitchers by observing *Oecophylla smaragdina* Fabr. (weaver ants) walking on the pitcher surface. These ants often occur sympatrically with *N. bicalcarata* plants and are part of their natural prey spectrum; they are renowned for their extreme adhesive abilities (Wojtusiak, Godzinska & Dejean 1995; Federle *et al.* 2002b). To bring large numbers of ants into contact with each tested pitcher, we collected 50 workers and placed them in a plastic box coated inside with slippery Fluon (Blades Biological Ltd., Edenbridge, Kent) to prevent ants from escaping.

Water was sprayed onto the peristome of live *N. bicalcarata* pitchers (*in situ*), using a plant mister until it was visibly wet. The pitchers were then placed upright inside the container so that the ants had access. We recorded the ants' behaviour for 5 min using a video camera (Sony DCR-SR35E, Tokyo, Japan). We measured trapping efficiency as the proportion of ants walking on the peristome (with all six legs) that fell into the pitcher. To prevent the ants from habituating, a fresh box of ants was used for each pitcher and repetition.

We used these running tests to compare the trapping efficiency of *N. bicalcarata* pitchers under different experimental conditions.

CAPTURE EFFICIENCY OF *NEPENTHES BICALCARATA* FOLLOWING EXPERIMENTAL CONTAMINATION

To examine whether *C. schmitzi* ants accelerate the recovery of capture efficiency following severe contamination, running tests with *O. smaragdina* were carried out *in situ* on 14 young pitchers inhabited by *C. schmitzi* and 14 young, uninhabited pitchers. (mean leaf node =  $5.08 \pm 0.7$ ). As young pitchers have not yet accumulated any significant contamination, there is still no difference in contamination and capture efficiency between ant-inhabited and ant-free

pitchers. It was therefore not necessary to include an 'ants removed' control in this experiment. The capture efficiency of each peristome was recorded three times: prior to cornflour contamination, immediately after contamination and approximately one week later (4–9 days). Equal numbers of occupied and unoccupied pitchers were set up on any given day to control for the effect of weather on decontamination rates. Capture efficiencies of *C. schmitzi*-occupied pitchers were compared to those of vacant pitchers using repeated measures ANOVA. Thereafter, *t*-tests with Bonferroni adjustments were carried out, ignoring the paired experimental design.

#### EFFECT OF NATURAL CONTAMINATION AND *CAMPONOTUS SCHMITZI* ANTS ON TRAPPING EFFICIENCY

To determine whether natural contamination reduces the slipperiness of pitcher peristomes and whether the presence of *C. schmitzi* ants can slow down or prevent this process, 12 'older' pitchers (mean leaf node =  $18 \pm 1$ ) were selected, each from a different plant. Six of these were colonised by *C. schmitzi* and six were uninhabited (vacant). All pitchers possessed domatia entrances, thus even the vacant pitchers had been occupied previously. Running tests were carried out for occupied vs. vacant pitchers. Arcsine-transformed results were compared using a Student's *t*-test. The effect of relative pitcher age was tested by using a log-linear regression of capture efficiency against leaf node number. For this analysis, we combined the data from these older pitchers with the 28 pre-contamination results from the previous experiment (which used the same methods) to provide a dataset ranging from leaf node 1–24.

Following the tests, samples of the peristomes were excised and dried for scanning electron microscopy. Electron micrographs were taken to compare surfaces of *C. schmitzi*-colonised to uncolonised pitcher peristomes (XL30 FEG SEM; FEI Philips, Eindhoven, the Netherlands).



**Fig. 1.** *Camponotus schmitzi* ants on the peristome of a *Nepenthes bicalcarata* pitcher.

## Results

#### CLEANING BEHAVIOUR OF *CAMPONOTUS SCHMITZI*

*Camponotus schmitzi* ants were regularly observed to perform a stereotyped cleaning behaviour on the peristome. Workers walked round the peristome with the body oriented perpendicular to the ridges whilst moving their heads from side to side, apparently cleaning the grooves of the peristome with their mouthparts (Video S1, Supporting information). The behaviour occurred almost exclusively on the peristome and the large thorns ('fangs') and was observed only rarely on other parts of the pitcher (on the underside of the lid and on the outer wall). On peristomes heavily contaminated with starch, the ants occasionally removed coagulated lumps by picking them up with the mandibles and depositing them below the peristome on the outside of the pitcher. In addition to these cleaning behaviours, we observed that most of the ants that walked over the peristome (122 of 145 ants in  $4 \times 5$  min videos) briefly paused to 'lick' the substrate, possibly removing contamination.

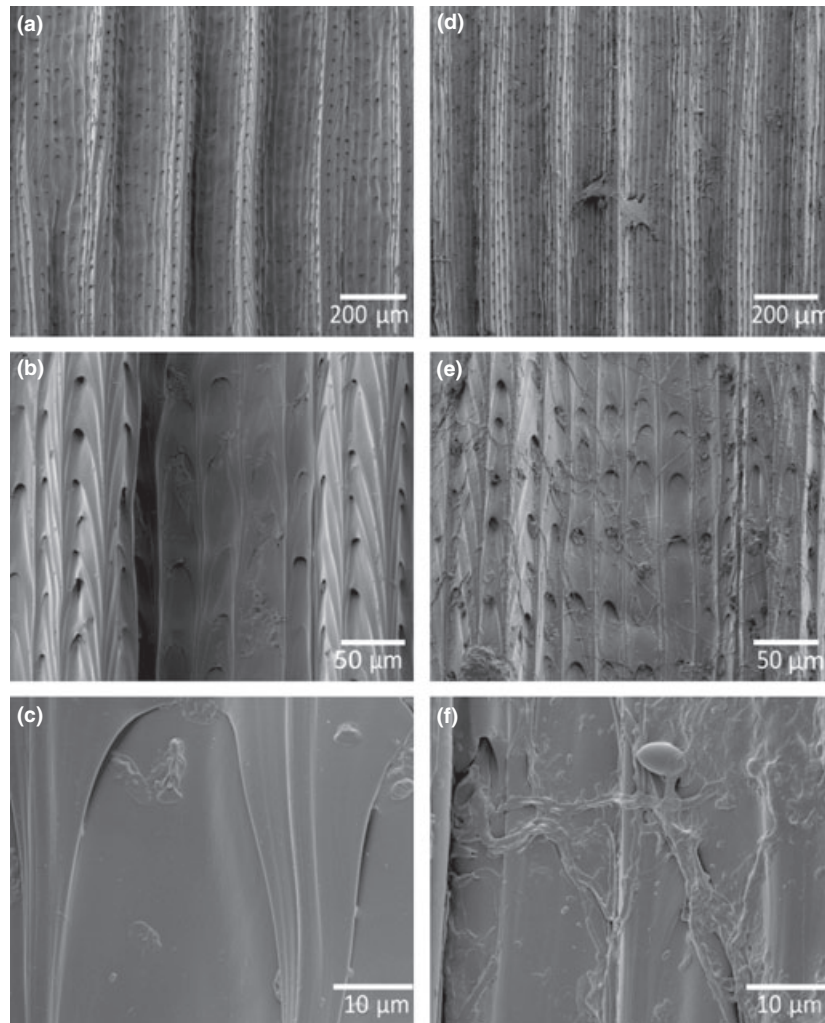
#### EFFECT OF *CAMPONOTUS SCHMITZI* ANTS ON NATURAL CONTAMINATION OF *NEPENTHES BICALCARATA* PITCHERS

Scanning electron micrographs of *N. bicalcarata* peristome samples highlighted the much greater level of contamination in unoccupied pitchers than in *C. schmitzi*-colonised pitchers (Fig. 2). Natural contamination of peristomes appears to be dominated by fungal hyphae. Analysis of the non-fungal particles revealed no difference in particle size between colonised and uncolonised pitchers (median diameter =  $13.2 \mu\text{m}$ ; *t*-test of log-transformed data,  $t_{90} = 0.57$ ,  $P = 0.57$ ) but a significant difference in their abundance (median particle density  $46.9 \text{ mm}^{-2}$  compared to  $0.00 \text{ mm}^{-2}$ ; *t*-test of log-transformed data,  $t_{22} = 2.65$ ,  $P = 0.015$ ).

#### CAPTURE EFFICIENCY OF *NEPENTHES BICALCARATA* FOLLOWING EXPERIMENTAL CONTAMINATION

Four days after the experimental contamination, there was visibly less starch remaining on the peristomes of *C. schmitzi*-occupied pitchers than on those of unoccupied pitchers (Fig. 3). Analysing contamination level, there was a significant interaction between ant presence and time (repeated measures ANOVA;  $F_{8,116} = 9.7$ ,  $P < 0.001$ ): Contamination level decreased more rapidly in ant-inhabited than ant-free pitchers (Fig. 4). The two ant-free treatments were not significantly different from each other ( $t_{20} = 0.975$ ,  $P_{\text{Bonferroni}} > 0.5$ ). Initially, the three groups (ant-inhabited pitchers, pitchers deprived of ants and naturally ant-free pitchers) had similar levels of experimental contamination.

Analysing capture efficiency, there was again a significant interaction between ant presence and time (repeated measures



**Fig. 2.** Scanning electron micrographs of *Nepenthes bicalcarata* peristomes from four older pitchers (leaf node numbers 19 and 20). (A–C) peristomes from pitchers colonised by *Camponotus schmitzi* ants; (D–F) peristomes from unoccupied pitchers.

ANOVA,  $F_{2,52} = 11.8$ ,  $P < 0.001$ ; Fig. 5). Whereas ant presence in such newly opened pitchers had no effect on capture efficiency for ‘uncontaminated’ and ‘contaminated’ ( $t < 0.67$ ,  $P > 0.50$ ), *C. schmitzi*-colonised pitchers were more efficient at capturing *O. smaragdina* than uncolonised pitchers 1 week after contamination ( $t_{26} = 5.31$ ,  $P_{\text{Bonferroni}} < 0.001$ ).

#### EFFECT OF *CAMPONOTUS SCHMITZI* ON PITCHER TRAPPING EFFICIENCY AND NATURAL PREY CAPTURE SUCCESS

Older pitchers (mean leaf node =  $18 \pm 1$ ) occupied by *C. schmitzi* ants were much more efficient at capturing *O. smaragdina* ants than unoccupied pitchers ( $t_{10} = 6.07$ ,  $P < 0.001$ ). Capture efficiency in young, uncolonised *N. bicalcarata* pitchers was similar to that of young, colonised pitchers. However, capture efficiency of uncolonised pitchers rapidly decreased with age (vacant pitchers,  $F_{1,18} = 35.0$ ,  $P < 0.001$ ; Fig. 6). By contrast, in ant-colonised pitchers, capture efficiency showed no significant dependence on

pitcher age (occupied pitchers,  $F_{1,18} = 0.025$ ,  $P = 0.88$ ; slope comparison,  $t_{35} = 4.08$ ,  $P < 0.001$ ).

To assess the overall effect of the presence of *C. schmitzi* on natural prey capture, we counted ant head capsules in the pitcher detritus. *Camponotus schmitzi*-colonised pitchers contained significantly more ants ( $241.2 \pm 55.5$ ) than uncolonised pitchers ( $166.2 \pm 40.4$ ; paired  $t$ -test;  $t_{10} = 2.87$ ,  $P = 0.017$ ), demonstrating that although pitchers were of the same age, those colonised by *C. schmitzi* had captured more insects.

#### Discussion

Our results provide evidence that the cleaning behaviour of *C. schmitzi* has acquired a new function in the context of the association with *N. bicalcarata*. In many mutualisms, beneficial ‘services’ may have evolved from ancestral traits that had other functions before the emergence of the relationship with the partner organism (Maynard Smith 1989). Several behaviours of plant–ants beneficial to the host plant (e.g. pruning of encroaching vegetation,

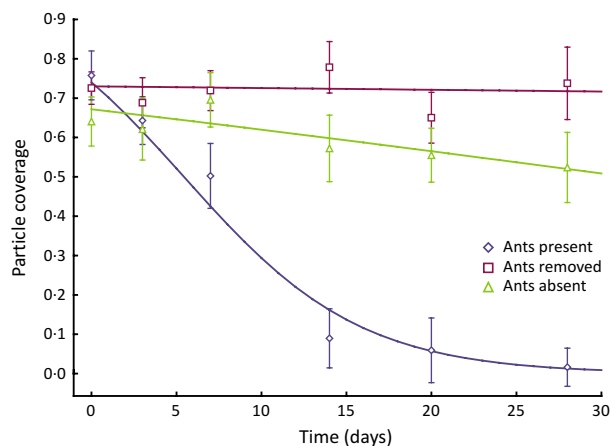


**Fig. 3.** Photographs of two *Nepenthes bicalcarata* pitchers (A, B and C, D) immediately following peristome contamination with starch (A, C) and 4 days after contamination (B, D). The pitcher in A and B was not occupied by *Camponotus schmitzi* ants, whereas the pitcher in C and D was. Scale bar: 20 mm.

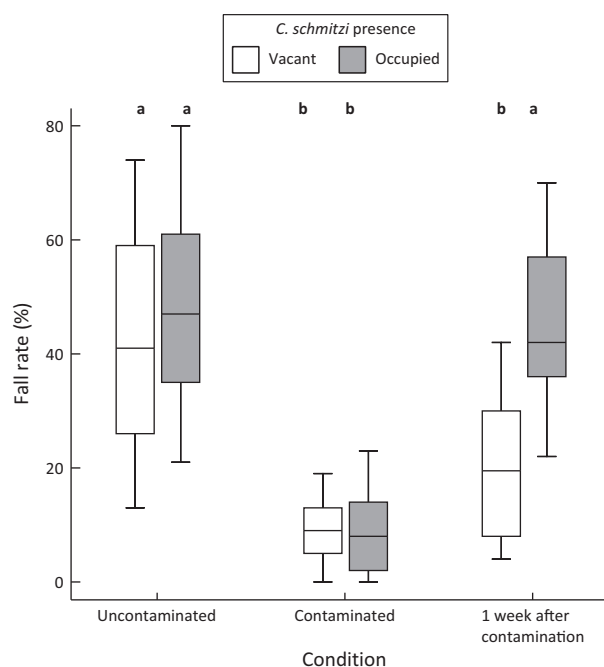
aggressiveness against herbivores) may originate from behaviours that had a direct benefit to the ant colony itself, and in many cases, this immediate benefit is still present (Davidson, Longino & Snelling 1988; Federle, Maschwitz & Hölldobler 2002a).

*Camponotus schmitzi* ants living on *N. bicalcarata* exhibited a stereotyped, repetitive cleaning behaviour on the pitcher peristomes, with their mouthparts moving along the peristome grooves. This behaviour almost exclusively occurred on the peristome, and other parts of the pitcher were apparently not cleaned. Starch was not removed from the outer pitcher wall in the contamination tests, and in older pitchers colonised by *C. schmitzi* ants, lid and outer wall were often visibly dirty. We cannot exclude that when performing the stereotyped behaviour, *C. schmitzi* also harvested nectar from the peristome, but the successful removal of particles and the observed deposition on the pitcher outside suggest that the function of this behaviour is to clean the peristome surface.

Cleaning behaviour has been recorded in other plant–ants, where herbivores, herbivore eggs and epiphyte propagules are removed from the surface of the host plant (Letourneau 1998; Heil & McKey 2003; Gaume *et al.* 2005). This behaviour may originate from generalised nest-cleaning activities found in other ants (Hölldobler & Wilson 1990). Whilst the cleaning behaviour of some plant–ants may be directed towards specific agents detrimental to the health of the host, Fiala *et al.* (1989) report that *Crematogaster* plant–ants remove ‘all kinds of foreign objects’ from their *Macaranga* hosts. Examples of this more generalist cleaning include another *Camponotus* plant–ant species (Federle, Maschwitz & Fiala 1998). The cleaning behaviour of plant–ants may occur primarily where the ants’ foraging efforts are concentrated, e.g. on young leaves. *Camponotus schmitzi* ants do not patrol the surface of their host plants as much as other plant–ants do (Bonhomme *et al.* 2011). Probably related to this restricted foraging pattern, the ants’ cleaning activity appears to target the peristome. Our results show that in the association between



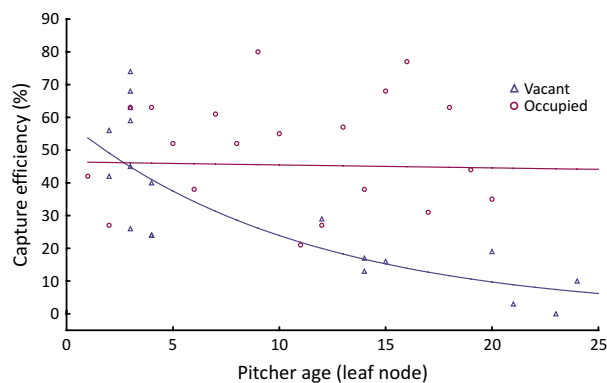
**Fig. 4.** Change in contamination level  $y$  with time  $t$  (in days) for the three treatments: ants present (diamonds and blue solid line);  $y = 1/(1 + 0.351 \cdot e^{-0.193 \cdot t})$ , ants removed (squares and red dotted line);  $y = 1/(1 + 0.37 \cdot e^{-0.0020 \cdot t})$  and uncolonised (triangles and green dashed line);  $y = 1/(1 + 0.489 \cdot e^{-0.023 \cdot t})$ . Means are plotted with their standard errors. Regression equations were calculated from mixed model ANCOVA.



**Fig. 5.** Boxplot showing the capture efficiencies of young *Nepenthes bicalcarata* pitchers (fall rates of *Oecophylla smaragdina* ants) prior to contamination with starch, immediately following contamination and 1 week later. Filled boxes represent pitchers occupied by *Campylopus schmitzi* and open boxes represent unoccupied pitchers. Bars sharing a letter were not significantly different ( $t$ -tests on unpaired data).

*N. bicalcarata* and *C. schmitzi*, the cleaning behaviour has acquired a new function: it maintains the slipperiness of the peristome and thereby facilitates prey capture by the host plant.

Pitchers not colonised by *C. schmitzi* were much more contaminated and infested by fungus. We found significantly



**Fig. 6.** Capture efficiency as a function of leaf node (pitcher age) for vacant and ant-inhabited *Nepenthes bicalcarata* pitchers. Capture efficiency decreased exponentially with pitcher age for vacant pitchers (triangles;  $y = e^{-0.090x + 4.07}$ ) but not for inhabited pitchers (circles). Statistics are presented in the text.

more particles on uncolonised peristomes, and many of these particles were embedded in a network of fungal hyphae. We cannot exclude that in addition to mechanically cleaning the peristome, the ants produce anti-fungal secretions similar to those identified from other ant species (Beattie *et al.* 1985; Letourneau 1998), controlling fungal growth on the peristome. Further work should establish whether such anti-fungal secretions play a role in *C. schmitzi*. The exact source of the contamination is not known, but rainfall may play a significant role both in contaminating pitchers through transfer of fungal spores and rain splash onto pitchers from the ground or from other vegetation (Fitt, McCartney & Walklate 1989; Madden 1997). At the same time, rain can wash contaminants from pitchers. Whatever the net effect of rainfall, or indeed other potential contamination vectors, our results show that contamination accumulated in the absence of *C. schmitzi* ants but not in their presence.

The trapping efficiency of *N. bicalcarata* peristomes was clearly lower in older pitchers that were not colonised by *C. schmitzi* ants. This trend was not evident in colonised pitchers. The lower trapping efficiency in older pitchers can be explained by the contamination of the peristome. The ability of *C. schmitzi* ants to clean and therefore increase the slipperiness of the peristome was confirmed by experimental contamination of the peristomes. Only when the *C. schmitzi* ants were present did the starch get removed and peristomes become cleaner. Furthermore, the contamination initially led to a large decrease in capture efficiency, which was subsequently mitigated in the pitchers that were inhabited by ants. Thus, our findings demonstrate that contamination reduces capture efficiency of pitchers and that colonisation by *C. schmitzi* ants maintains contamination at a low level. Moreover, the significant difference in the number of naturally captured prey (ant heads counted) confirms that *C. schmitzi* ants' presence correlates with an increase in the prey capture success of *N. bicalcarata*.

An interesting implication of our results is that they might provide an adaptive explanation for the high longevity of

*N. bicalcarata* pitchers. Assuming that leaves develop at a constant rate (of one leaf in 31.9 days) and that pitchers are usually open from leaf node #3 onwards, a pitcher at leaf node 18 has been open for 510 days ( $16 \times 31.9$ ). While such long-lived pitchers are typical for *N. bicalcarata* (Clarke 1997; Osunkoya, Daud & Wimmer 2008), pitchers of other *Nepenthes* species last much shorter (mean operational half-life time of 1.97 months for seven other *Nepenthes* forms vs. 6.01 months for *N. bicalcarata*; Osunkoya, Daud & Wimmer 2008). Our results show that ant-free pitchers of *N. bicalcarata* (and presumably other *Nepenthes* species) can rapidly become inefficient owing to the contamination of the peristome. Yet, if colonised by *C. schmitzi* ants, 16-month-old pitchers were still able to trap ants as efficiently as newly opened pitchers. The contribution of *C. schmitzi* to the plant's nutrition is therefore likely to be highly significant. This conclusion is supported by the significantly higher numbers of ants caught in *C. schmitzi*-colonised pitchers. While we compared the number of captured ants for pitchers of the same age, it is possible that *N. bicalcarata* pitchers also survive longer as a result of the ants' activity, either by limiting putrefaction of the pitcher contents (Clarke & Kitching 1995) or by removing particles and fungi from the peristome (we never recorded uncolonised pitchers with leaf nodes > 23, yet we found colonised pitchers with leaf nodes > 30). This would further increase the nutrient input from a single pitcher. The service provided by *C. schmitzi* ants allows *N. bicalcarata* pitchers to capture prey over a much longer time period than pitchers of other *Nepenthes* species. Osunkoya *et al.* (2007) found the construction costs per unit area of *N. bicalcarata* pitchers to be significantly higher than those of seven other *Nepenthes* forms, implying a trade-off between pitcher longevity and construction costs. However, while *N. bicalcarata* pitchers have 1.8 times higher area-specific construction costs (23.76 vs. 13.25 mg cm<sup>-2</sup>), they have a 3.1 longer half-life time than other pitchers (Osunkoya *et al.* 2007; Osunkoya, Daud & Wimmer 2008). Thus, more long-lived pitchers may be beneficial for the plant, if the pitcher is able to maintain a high trapping efficiency over its lifetime. The association with ants that maintain the peristome's function may therefore represent a significant economic benefit to the plant.

The detailed benefits of *C. schmitzi* ants for their carnivorous host plant, *N. bicalcarata*, have remained unclear since the association was first discovered. Clarke & Kitching (1995) showed that *C. schmitzi* remove and break down large prey which reduces putrefaction of the pitcher contents. However, there is no direct evidence to suggest that putrefaction of the pitcher contents harms the pitcher. In fact, *N. bicalcarata* readily absorbs dissolved ammonia (Clarke & Kitching 1995; Moran *et al.* 2010), one of the measures used in the earlier study to quantify putrefaction levels. A second benefit ascribed to *C. schmitzi* is that it protects *N. bicalcarata* against herbivory. Aggression towards herbivores is a common behaviour amongst plant-ants and this has also been shown to a limited extent for *C. schmitzi*. Although the ants appear relatively non-aggressive, Merbach *et al.* (2007) demonstrated that *C. schmitzi* do attack a curculionid that specifi-

cally targets *Nepenthes* and found reduced levels of pitcher damage in ant-colonised plants. A third possible benefit of *C. schmitzi* ants has been proposed recently. Bonhomme *et al.* (2011) showed that *C. schmitzi* ants can actively attack prey that has fallen into the *N. bicalcarata* pitcher and subsequently attempts to escape. It was suggested that this behaviour increases the pitcher's prey retention rate, and that for this reason, the ant-plant association represents a nutritional mutualism. However, many insects are retained without direct interference of *C. schmitzi* ants (Bonhomme *et al.* 2011), and our own observations on *Polyrhachis pruinosa* did not indicate any increase of prey retention caused by *C. schmitzi* (see Appendix S1, Supporting information).

Our findings show that the association between *C. schmitzi* and *N. bicalcarata* is indeed a nutritional mutualism, but for a different reason. The ants clean the trapping surfaces and maintain a high capture efficiency over the lifetime of *N. bicalcarata* pitchers. The recorded higher capture success confirms that this 'janitorial' service provides a considerable benefit to the host plant. Further work should attempt to quantify the costs and benefits of this fascinating ant-plant interaction in the field.

## Acknowledgements

The authors wish to thank the family of Abdul Hazdid Harith Tinggal and Iney Daud for their help and support in all logistical matters during our stay in Brunei. Particular thanks are attributed to Akilah Syafina b. Abd. Hazdid, James M. R. Bullock and Mathias Scharmann, who assisted with much of the fieldwork, and Thomas Endlein for providing Fig. 1. The work was conducted in Brunei Darussalam under the research permit UBD/PSR/5(a), and *Nepenthes* samples were brought to the UK for SEM under CITES permit BA/MAP/128/0911. The project was funded by a grant from the Leverhulme Trust (F/09 364/G).

## References

- Adam, J.H. (1997) Prey spectra of Bornean *Nepenthes* species (Nepenthaceae) in relation to their habitat. *Pertanika Journal of Tropical Agricultural Science*, **20**, 121–134.
- Adlassnig, W., Peroutka, M. & Lendl, T. (2010) Traps of carnivorous pitcher plants as a habitat: composition of the fluid, biodiversity and mutualistic activities. *Annals of Botany*, **107**, 181–194.
- Bauer, U., Bohn, H.F. & Federle, W. (2008) Harmless nectar source or deadly trap: *Nepenthes* pitchers are activated by rain, condensation and nectar. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 259–265.
- Beattie, A.J., Turnbull, C., Hough, T., Jobson, S. & Knox, R.B. (1985) The vulnerability of pollen and fungal spores to ant secretions: evidence and some evolutionary implications. *American Journal of Botany*, **72**, 606–614.
- Beccari, O. (1904) *Wanderings in the Great Forests of Borneo*. Archibald and Constable, London.
- Bohn, H.F. & Federle, W. (2004) Insect aquaplaning: *Nepenthes* pitcher plants capture prey with the peristome, a fully wettable water-lubricated anisotropic surface. *Proceedings of the National Academy of Sciences of the USA*, **101**, 14138–14143.
- Bonhomme, V., Gounand, I., Alaux, C., Jusselin, E., Barthélémy, D. & Gaume, L. (2011) The plant-ant *Camponotus schmitzi* helps its carnivorous host-plant *Nepenthes bicalcarata* to catch its prey. *Journal of Tropical Ecology*, **27**, 15–24.
- Clarke, C. (1997) *Nepenthes of Borneo*. Natural History Publications, Kota Kinabalu.



- Clarke, C.M. & Kitching, R.L. (1995) Swimming ants and pitcher plants: a unique ant–plant interaction from Borneo. *Journal of Tropical Ecology*, **11**, 589–602.
- Davidson, D.W., Longino, J.T. & Snelling, R.R. (1988) Pruning of host plant neighbors by ants: an experimental approach. *Ecology*, **69**, 801–808.
- Federle, W., Maschwitz, U. & Fiala, B. (1998) The two-partner ant–plant system of *Camponotus (Colobopsis)* sp.1 and *Macaranga puncticulata* (Euphorbiaceae): natural history of the exceptional ant partner. *Insectes Sociaux*, **45**, 1–16.
- Federle, W., Maschwitz, U. & Hölldobler, B. (2002a) Pruning of host plant neighbors as defence against enemy ant invasions: *Crematogaster* ant partners of *Macaranga* protected by ‘wax barriers’ prune less than their congeners. *Oecologia*, **132**, 264–270.
- Federle, W., Riehle, M., Curtis, A.S.G. & Full, R.J. (2002b) An integrative study of insect adhesion: mechanics and wet adhesion of pretarsal pads in ants. *Integrative and Comparative Biology*, **42**, 1100–1106.
- Fiala, B., Maschwitz, U., Tho, Y.P. & Helbig, A.J. (1989) Studies of a South East Asian ant–plant association: protection of *Macaranga* trees by *Crematogaster borneensis*. *Oecologia*, **79**, 463–470.
- Fitt, B.D.L., McCartney, H.A. & Walklate, P.J. (1989) The role of rain in dispersal of pathogen inoculum. *Annual Review of Phytopathology*, **27**, 241–270.
- 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.
- Heil, M. & McKey, D. (2003) Protective ant–plant interactions as model systems in ecological and evolutionary research. *Annual Review of Ecology & Systematics*, **34**, 425–453.
- Hölldobler, B. & Wilson, E.O. (1990) *The Ants*. Belknap Press, Cambridge, MA.
- Janzen, D.H. (1972) Protection of *Barteria* (Passifloraceae) by *Pachysima* ants (Pseudomyrmecinae) in a Nigerian rain forest. *Ecology*, **53**, 885–892.
- Letourneau, D.K. (1983) Passive aggression: an alternative hypothesis for the *Piper–Pheidole* association. *Oecologia*, **60**, 122–126.
- Letourneau, D.K. (1998) Ants, stem-borers, and fungal pathogens: experimental tests of a fitness advantage in *Piper* ant–plants. *Ecology*, **79**, 593–603.
- Madden, L.V. (1997) Effects of rain on splash-dispersal of fungal pathogens. *Canadian Journal of Plant Pathology*, **19**, 225–230.
- Maynard Smith, J. (1989) Generating novelty by symbiosis. *Nature*, **341**, 284–285.
- Merbach, M.A., Zizka, G., Fiala, B., Merbach, D., Booth, W.E. & Maschwitz, U. (2007) Why a carnivorous plant cooperates with an ant – selective defense against pitcher-destroying weevils in the myrmecophytic pitcher plant *Nepenthes bicalcarata* Hook. f. *Ecotropica*, **13**, 45–56.
- Moran, J.A., Hawkins, B.J., Gowen, B.E. & Robbins, S.L. (2010) Ion fluxes across the pitcher walls of three Bornean *Nepenthes* pitcher plant species: flux rates and gland distribution patterns reflect nitrogen sequestration strategies. *Journal of Experimental Botany*, **61**, 1365–1374.
- Osunkoya, O.O., Daud, S.D. & Wimmer, F.L. (2008) Longevity, lignin content and construction cost of the assimilatory organs of *Nepenthes* species. *Annals of Botany*, **102**, 845–853.
- Osunkoya, O., Daud, S.D., Di-Giusto, B., Wimmer, F.L. & Holige, T.M. (2007) Construction costs and physico-chemical properties of the assimilatory organs of *Nepenthes* species in Northern Borneo. *Annals of Botany*, **99**, 895–906.
- Risch, S. (1982) How *Pheidole* ants help *Piper* plants. *Brenesia*, **19**, 545–548.
- Schuitemaker, J.P. & Staercke, A. (1933) Contributions à l'étude de la faune Nepenthicole. Art. III. Un nouveau *Camponotus* de Borneo, habitant les tiges creuses de *Nepenthes*. *Naturhistorisch Maandblad*, **22**, 29–31.
- Stout, J. (1979) An association of an ant, a mealy bug, and an understory tree from a Costa Rican rain forest. *Biotropica*, **11**, 309–311.
- Thompson, J.N. (1981) Reversed animal–plant interactions – the evolution of insectivorous and ant-fed plants. *Biological Journal of the Linnean Society*, **16**, 147–155.
- Wojtusiak, J., Godzińska, E.J. & Dejean, A. (1995) Capture and retrieval of very large prey by workers of the African weaver ant, *Oecophylla longinoda* (Latreille 1802). *Tropical Zoology*, **8**, 309–318.

Received 8 July 2011; accepted 17 October 2011

Handling Editor: Alison Brody

## Supporting Information

Additional supporting information may be found in the online version of this article.

**Video S1.** Video recordings of cleaning behaviour by *C. schmitzi* ants on the peristome of *N. bicalcarata*.

**Appendix S1.** Effect of *C. schmitzi* ants on pitcher retention of *Polyrhachis pruinosa* ants.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.