

A novel resource–service mutualism between bats and pitcher plants

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Mutualistic relationships between vertebrates and plants apart from the pollen and seed-dispersal syndromes are rare. At first view, carnivorous pitcher plants of the genus *Nepenthes* seem to be highly unlikely candidates for mutualistic interactions with animals, as they form dimorphic terrestrial and aerial pitchers that trap arthropods and small vertebrates. Surprisingly, however, the aerial pitchers of *Nepenthes rafflesiana* variety *elongata* are poor insect traps, with low amounts of insect-attractive volatile compounds and low amounts of digestive fluid. Here, we show that *N. rafflesiana elongata* gains an estimated 33.8 per cent of the total foliar nitrogen from the faeces of Hardwicke's woolly bats (*Kerivoula hardwickii hardwickii*) that exclusively roost in its aerial pitchers. This is the first case in which the faeces-trapping syndrome has been documented in a pitcher plant that attracts bats and only the second case of a mutualistic association between a carnivorous plant and a mammal to date.

Keywords: *Kerivoula hardwickii*; *Nepenthes rafflesiana* variety *elongata*; mutualism; nitrogen acquisition

1. INTRODUCTION

Carnivorous pitcher plants of the genus *Nepenthes* grow in nutrient-poor soils and rely on trapping arthropods to acquire sufficient nitrogen [1–3]. In many pitcher plant species, pitcher morphology, fluid viscoelastic properties, extent of epicuticular wax crystals and peristome design predict, to a large extent, prey composition (e.g. [4,5]). In Borneo, *Nepenthes rafflesiana* has five distinct growth forms, one of which is extraordinary in several ways. *Nepenthes rafflesiana elongata* (figure 1a) possesses aerial pitchers that, compared with the typical variety *Nepenthes rafflesiana typica*, are up to four times longer, produce fewer human-perceptible fragrances, exhibit a unique UV light absorption spectrum and capture insects at rates up to seven times lower [6,7]. Surprisingly, we regularly found woolly bats (*Kerivoula hardwickii hardwickii* [8]) roosting above the digestive fluid in the aerial

pitchers of *N. r. elongata*, and hypothesized that pitcher plants entice bats to roost in their pitchers in return for nitrogen that they sequester from bat faeces or urine. Other bat species use a variety of daytime roosts, including furled leaves and bamboo culms [9], but none are known to roost in pitcher plants. The aim of this study was to quantify pitcher use by woolly bats and to determine how much nitrogen the plant derives from woolly bat faeces. We hypothesized that a mutualistic relationship exists between Hardwicke's woolly bats and *N. r. elongata*.

2. MATERIAL AND METHODS

From 14 June to 30 July 2009, Hardwicke's woolly bats were caught by daily patrols of 423 pitchers of *N. r. elongata* in a peat swamp forest in Brunei Darussalam (4°35'16.80" N, 114°30'48.80" E). Using skinbond adhesive (Manfred Sauer GmbH), we fixed transmitters (Holohil Systems Ltd; weight: 0.4 g) onto bats' backs, which fall off after 3–12 days. To localize the bats, we used a TRX-1000S Wildlife Materials receiver (Carbondale, IL, USA).

We compared the isotopic signature of leaf blades from pitchers used as roosts with those from control pitchers that were never occupied, which had been monitored daily since they had opened. Occupied ($n = 38$) and control pitchers ($n = 17$) and their associated leaf blades were collected after three to six weeks of daily monitoring, for analysis of total nitrogen and stable isotope ratios of $^{15}\text{N}/^{14}\text{N}$ ($\delta^{15}\text{N}$). The contribution from bats to total foliar nitrogen was estimated using a two-member isotopic mixing model [10] using mean $\delta^{15}\text{N}$ values for leaf blades of bat-occupied aerial pitchers ($n = 38$), aerial leaf blades without faecal input ($n = 17$) and woolly bat faeces ($n = 3$, see details of the model in the electronic supplementary material).

For the analysis of total N and $\delta^{15}\text{N}$, samples were sent to the Cornell isotope laboratory (COIL; Ithaca, NY, USA), which used a Thermo Delta V isotope ratio mass spectrometer (IRMS) interfaced to an NC2500 elemental analyser. The primary reference scale for $\delta^{15}\text{N}$ was atmospheric air. An internal standard (s.d. = 0.09‰ for $\delta^{15}\text{N}$) was analysed after every 10 samples to ensure accuracy. We measured pitcher length and fluid levels in *N. r. elongata* and *N. r. typica* to evaluate the available roosting space. Means and s.d. are given as descriptive statistics.

3. RESULTS

We found 14 male and 18 female Hardwicke's woolly bats roosting in aerial pitchers of *N. r. elongata* in 2008 and 2009 (figure 1b). Of these, we radio-tracked 17 males and females over an average of 6.1 ± 3.3 days (range 1–12 days) and found that each tagged individual exclusively used pitchers of *N. r. elongata* as daytime roosts, despite the widespread abundance of other potential roosting sites (furled leaves, hollow trees and other *Nepenthes* (*N. r. typica*, *N. bicalcarata*, *N. ampullaria*)). During our 6.5 week study period in 2009, 87 (20.8%) of 418 monitored *N. r. elongata* pitchers were occupied by a total of 25 bats. In total, 64 plants (out of 223) harboured at least one bat in one of its pitchers. Thus, the incidence of plant occupancy was 28.7 per cent over the total study period of 6.5 weeks, compared with a 20.8 per cent pitcher occupancy rate. On average, $1.30 \pm 0.70\%$ of the 423 monitored pitchers was occupied on a single day. On two separate occasions, a juvenile bat (one male, one female) shared the same pitcher with their mother.

The elongated pitcher in *N. r. elongata* provided bats ample room to roost. The distance between the lower rim of the peristome and the pitcher fluid was 151.4 ± 37.8 mm ($n = 58$; range: 49.7–228.5 mm). With the bats' body length averaging 36.6 ± 0.8 mm ($n = 4$), most *N. r. elongata* aerial pitchers provided enough roosting space for two bats stacked above

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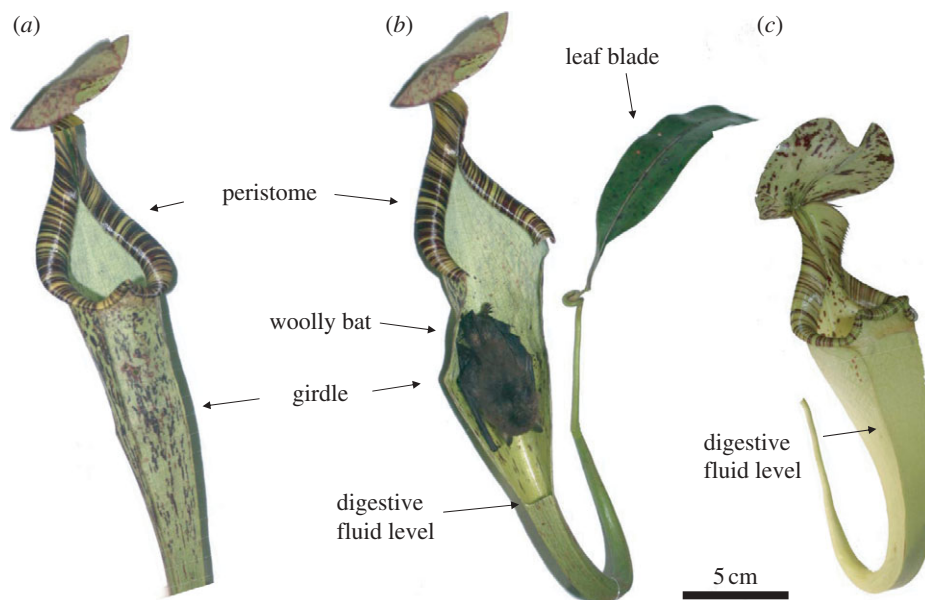


Figure 1. Service benefit provided by *N. r. elongata* to *K. h. hardwickii*. (a) Aerial pitcher of *N. rafflesiana* var. *elongata*. (b) The same pitcher with the front tissue removed to reveal a roosting Hardwick's woolly bat. (c) The shorter aerial pitcher of *N. rafflesiana* variety *typica*.

each other. In contrast, *N. r. typica* aerial pitchers, which were not occupied by bats, provided significantly less roosting space (*t*-test, $t = -912$, d.f. = 68, $p < 0.001$) with average distances of only 50.5 ± 12.3 mm ($n = 12$, range: 31.6–67.7 mm) between peristome and fluid (figure 1c). Fluid levels were significantly lower in *N. r. elongata* (25.0 ± 23.7 mm) than in *N. r. typica* (54.0 ± 12.0 mm; *t*-test, $t = 4.12$, d.f. = 68, $p < 0.001$).

To test the hypothesis that pitcher plants sequester nitrogen from bat faeces or urine, we compared the total nitrogen and $^{15}\text{N}/^{14}\text{N}$ ($\delta^{15}\text{N}$) stable isotopic signature of leaf blades from pitchers used as roosts with those of leaf blades from pitchers never occupied by bats. We found that total foliar nitrogen was significantly higher in leaf blades of pitchers in which bats had been found roosting ($1.48 \pm 0.31\%$) than in control leaf blades ($1.31 \pm 0.19\%$; Mann–Whitney *U*-test, $U = 208$, $n_1 = 38$, $n_2 = 17$, $p < 0.05$; figure 2a). Moreover, foliar $\delta^{15}\text{N}$, used to infer the nitrogen source (i.e. its trophic level; [11]), was significantly higher when pitchers harboured bats ($1.30 \pm 1.53\text{‰}$) than when they did not ($0.51 \pm 1.09\text{‰}$; Mann–Whitney *U*-test, $U = 181.5$, $n_1 = 38$, $n_2 = 17$, $p < 0.01$; figure 2b). Using a two-member mixing model [10], we estimate that between 11 and 56 per cent (95% CI; $n = 38$) or on average 33.8 per cent of foliar nitrogen in *N. r. elongata* is derived from woolly bat faeces.

4. DISCUSSION

The results of the radio-telemetry and the stable isotope analyses suggest a resource–service relationship between a bat and a carnivorous pitcher plant, with clear benefits to both mutualistic partners. The woolly bats (*K. h. hardwickii*) that we observed solely used aerial pitchers of *N. r. elongata* as daytime roosts, providing the plant with nitrogen in an nitrogen-deprived environment. This is an unusual

case of an animal–plant mutualism in which nutrients are supplied by the animal and not vice versa [12].

Both morphological and physiological characters of *N. r. elongata* aerial pitchers facilitate bat roosting. Bats depend on shelter for survival, reproduction and protection from predators [13]. The morphology of the *N. r. elongata* pitchers offers protection for *K. hardwickii* (figure 1b). Bats are hardly visible through the pitcher wall and they are safe from rain or direct solar radiation. Moreover, the elongated form of the pitchers, which distinguishes them from the typical form [1,7,6], ensures that not only single bats but also mother–juvenile pairs comfortably fit into the pitchers. This might be a critical feature, as mothers and juveniles in many bat species remain associated for several weeks to months [14]. Another conspicuous feature of *N. r. elongata* aerial pitchers is that they taper distinctly after a lignified ‘girdle’ in the lower half of the pitcher. Woolly bats were usually wedged head first in the pitcher with their heads below the girdle, suggesting that this girdle in combination with the slender pitcher form obviates the need for bats to cling to the slippery pitcher wall or peristome. Moreover, fluid levels are much reduced in aerial pitchers of *N. r. elongata* compared with its terrestrial pitchers or those of the typical form (own observations), suggesting that physiological modifications regulating fluid production are in place to accommodate bats.

Our findings show that the enigma of *N. r. elongata*'s lower arthropod capture rate compared with the typical form [7] is solved when the role of bats is considered. Attracting bats appears to be facilitated by both pitcher morphology and physiology: narrow and cylindrical aerial pitchers in combination with reduced liquid levels. Bat faecal pellets can provide high rewards, with an estimated 33.8 per cent of the total foliar nitrogen provided suggesting that trapping faeces in *N. r. elongata* has been successful and might even be superior to trapping insects in areas in which bats are abundant.

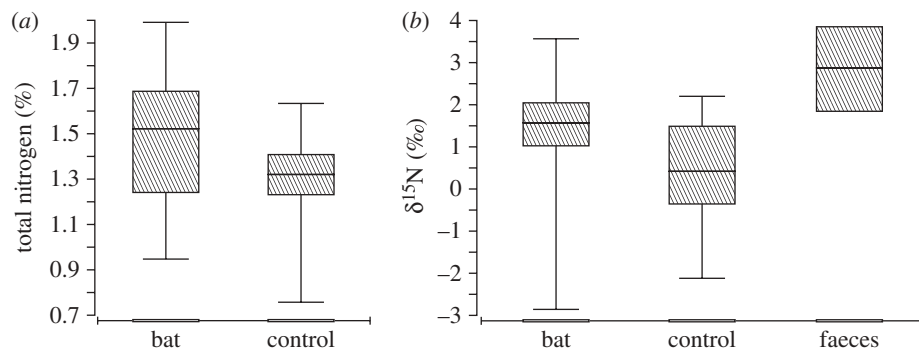


Figure 2. Resource benefit provided by roosting *K. h. hardwickii* to *N. r. elongata*. (a) Box-plot of total foliar nitrogen of pitchers used as roosts ($n = 38$) and of control pitchers ($n = 17$). (b) Box-plot of foliar $\delta^{15}\text{N}$ of pitchers used as roosts ($n = 38$), control pitchers ($n = 17$) and bat faeces ($n = 3$). The scarce stable isotope ^{15}N accumulates from one trophic level to the next and thus stable isotope ratios of $^{15}\text{N}/^{14}\text{N}$ ($\delta^{15}\text{N}$) can be used to indicate the nitrogen source. Faeces from insectivorous bats have a higher $\delta^{15}\text{N}$ signature than the insects they feed on or the insects that are trapped by the pitcher plant.

Recently, it has been suggested that several large montane *Nepenthes* also have reduced abilities to catch arthropod prey and obtain most of their nitrogen critical for growth from treeshrew (*Tupaia montana*) faeces [15,16]. Our study shows that the faeces-trapping syndrome occurs not only in montane *Nepenthes* but also in pitcher plants growing in lowland forests, suggesting that low arthropod densities at mountain sites cannot be the sole factor responsible for the origin and maintenance of this nitrogen acquisition strategy. Our study supports recent evidence that simple modifications of pitcher morphology and levels of digestive fluid can open up novel nitrogen acquisition strategies [15]. However, giving up the insect-capturing strategy completely may be risky from an evolutionary perspective, since the probability of attracting a bat to a particular individual aerial pitcher was only 22.8 per cent in our study area. Instead, it should be beneficial for pitcher plants to pursue a dual strategy by retaining the ability to trap insects, especially if bats are absent from some habitat patches. Such conditional asymmetries in interspecific interactions are common.

Our study shows that the interaction is mutualistic, with the bat possibly more dependent on the pitcher plant than vice versa. This mutualistic relationship seems to be restricted to Borneo, involving one particular subspecies of *K. hardwickii* and one variety of *N. rafflesiana*. Our study shows that woolly bats consistently use pitchers as daytime roosts. However, since woolly bats in other areas of Borneo make occasional use of other pitcher plant species (*N. bicalcarata* and *N. ampullaria*) that are clearly less well suited as roosts (own observations and A.-M. Seibert 2010, personal communication), *Nepenthes*–woolly bat associations are prime candidate models to study the evolution of mutualistic relationships with opportunities to investigate varying degrees of exploitation.

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