

Costs and benefits in a bat-pitcher plant mutualism

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Look deep into nature,
and then you will understand everything better.

Albert Einstein

For Reinhold Steinl,
an inspirational teacher and biologist.

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ABSTRACT

Abstract

Mutualisms are ubiquitous in nature and shape whole ecosystems. Although species benefit by interacting with each other, they permanently act selfishly. As a consequence, the involved partners must balance gaining the maximal benefit while accepting a certain amount of costs. Changes in the environment, however, may alter selection pressures and lead to a shift in the relative costs and benefits for both involved species. Due to this complexity, many mutualisms and their underlying processes, such as the dependence of the involved species on each other, are only poorly understood. Moreover, in several so-called mutualistic interactions it is unclear if they are in fact beneficial for all partners because detailed cost-benefit analyses are missing.

The aim of my thesis was to contribute to a better understanding of the basic principles of mammal-plant mutualisms with special emphasis on the interdependence of the involved species. Using the interaction between an insectivorous bat species (*Kerivoula hardwickii*) and carnivorous pitcher plants (genus *Nepenthes*) as a model system, I conducted a detailed cost-benefit analysis to test if the partners interact mutualistically and are strongly dependent on one another. I hypothesised that pitchers of these plants serve as high quality roosts for the bats while the bats in turn fertilise the plants via their nutritious faeces. For the involved species the costs of the interaction should be lower than the gained benefits, but general costs should increase in the absence of the partner.

Over the course of my field research, I found the bats roosting in three *Nepenthes* species, but the bats occupied intact pitchers of only one species, *Nepenthes hemsleyana*. In *Nepenthes bicalcarata* and *Nepenthes ampullaria*, the bats used senescing or damaged pitchers whose high amount of digestive fluid had drained off. Thus, only *N. hemsleyana* was potentially able to digest bat faecal matter, and thereby benefit from the bats. My cost-benefit analysis showed that *N. hemsleyana* plants strongly benefited from their bat interaction partner: depending on the degree of nutrient deprivation, the plants gained between 34% and 95% of their nitrogen from bat faeces, which significantly improved their growth, photosynthesis and survival. In feeding experiments, *N. hemsleyana* plants could not fully compensate an induced lack of nutrients by using arthropod prey when bat faeces were not available. Field observations revealed no obvious costs for the pitcher plant. *N. hemsleyana* pitchers occupied by bats did not differ in their lifespan from unoccupied ones as bats did not injure the plants' tissue. The interaction was also advantageous for *K. hardwickii* because *N. hemsleyana* offered high

quality roosts with a favourable microclimate and low parasite infestation risk. Consequently, bats roosting in *N. hemsleyana* pitchers were in better condition than those roosting in dead *N. bicalcarata* pitchers. Although *N. hemsleyana* pitchers are rare in the natural habitat, bats did not have to invest much time and energy to find them. Most *N. hemsleyana* plants continuously provided at least one intact pitcher meaning bats could return to the same plants over a period of several months or even years. Additionally, the pitcher's back wall acted as an echo reflector, reducing costs for pitcher detection and identification.

The interaction between *K. hardwickii* and *N. hemsleyana* can be classified as an asymmetric facultative mutualism with stronger dependence of the plant partner. *N. hemsleyana* has outsourced arthropod capture and digestion to its mutualistic bat partner while arthropod attraction is strongly reduced. Contrastingly, several populations of *K. hardwickii* frequently use alternative roosts. Strong selective pressure on the plants could be the consequence to attract bats with a potential stabilising effect on the interaction: *N. hemsleyana* has to outcompete the involuntarily offered roosts of the other *Nepenthes* species in terms of quality and accessibility.

My thesis revealed complex interdependencies in an animal-plant mutualism. This study exemplifies that rigorous cost-benefit analyses are crucial for the classification of interspecific interactions and the characterisation of how the involved species affect and depend on each other.

1 INTRODUCTION

1 INTRODUCTION

1.1 The Importance of Mutualisms

Interspecific mutualistic relationships where the partners exchange beneficial commodities (i.e. resources and/or services) are ubiquitous in nature, shape whole ecosystems, and are drivers of biodiversity (Bronstein 2001b; Bascompte & Jordano 2007). They allow the interacting species to utilise resources and/or to colonise habitats where neither of them can persist alone (Boucher *et al.* 1982; Bronstein 1994a, 2001b; de Mazancourt *et al.* 2005). Without mutualisms some key stages for life on earth, such as the development of the eukaryotic cell (Margulis & Chapman 1998), would not have been possible (Bronstein 2001b). Other influential examples include the close relationships between terrestrial plants and mycorrhizal fungi that accompanied the initial settlement of plants on land (Remy *et al.* 1994) and the interactions between plants and their pollinators or seed dispersers that influenced the radiation of angiosperms (van der Niet & Johnson 2012).

Mutualisms are highly diverse: the interacting species may associate for their whole life, or the interaction can be limited to short periods of resource/service exchange (Leigh Jr 2010). Moreover, mutualisms can range from facultative dependency where the interaction is not essential to the interacting species, to obligatory dependency where the interacting species cannot survive or reproduce without their partners (Bronstein 1994a). In many mutualistic interactions, partner species do not depend equally on each other (Jordano 1987), which can lead to asymmetric mutualisms. This can be seen in mutualistic interactions between mobile and sedentary species. While mobile partners often are not highly specialised, sedentary partners commonly have evolved special traits that meet the behaviour of their particular interaction partner (Bronstein 2009). How strongly the interacting species depend on each other not only varies on an evolutionary timescale, but is also influenced by contemporary biotic and abiotic factors (Bronstein 1994a; Hom & Murray 2014) such as the abundance of predators (Del-Claro & Oliveira 2000) or access to light (Kersch & Fonseca 2005).

Despite their importance and omnipresence, “the evolution and maintenance of mutualisms remains a largely unsolved puzzle“ (Jander & Herre 2010). To maintain their mutualistic interaction and to avoid parasitism, the involved species are in a constant evolutionary race. They must persistently adapt to their partners, keeping their own costs lower than the resulting beneficial outcome, to avoid exploitation by or the loss of the partner species

(Doebeli & Knowlton 1998; Bronstein 2001b; Sachs *et al.* 2006; Leigh Jr 2010). Species may abandon facultative mutualistic interactions and live autonomously when benefits can also be gained from the environment. Instead, they may switch to alternative partners if that is more beneficial for them (Denison & Kiers 2004; Moeller & Gebre 2005; Stadler & Dixon 2005; Sachs *et al.* 2006; Thompson & Fernandez 2006).

Investigating mutualistic interactions requires that involved costs and benefits have to be revealed first. This can be challenging as in some cases it is difficult to determine if, for example, “services” of the associated partners are beneficial at all, or if they are just commensalistic or even parasitic actions. This can be seen in the relationship between fungi and algae in lichens (Ahmadjian & Jacobs 1981; Gargas *et al.* 1995). Currently, a detailed cost-benefit analysis is missing in many presumed mutualisms. Approaches are often unilateral where only one of the involved species is investigated (Bronstein 1994b, Currie 2001), e.g., due to challenges in manipulating the other species. Moreover, there is still a profound lack of knowledge concerning context dependency in mutualisms (Chamberlain & Holland 2009). Thus, for detailed analyses regarding the costs and benefits of the interacting species different abiotic and biotic environmental factors have to be taken into consideration (Bronstein 1994b). Understanding the costs and benefits for the involved species and hence the degree of evolved dependence, the geographic variation and stability over time, as well as the evolution of mutualistic relationships, are central topics in evolutionary biology and ecology (de Mazancourt *et al.* 2005; Ferrière *et al.* 2009).

Overall I aimed to contribute to a better understanding of mutualisms, especially those between animals and plants, the incurred costs and benefits, and the interdependence of the partner species. To do so, I focused on the interactions between a tropical bat species and several carnivorous plants of the genus *Nepenthes*. In order to classify whether these interactions are mutualistic or not, I intended to investigate the costs and benefits of all involved species and to determine how strongly they depend on each other.

1.2 Animal-Plant Mutualisms

The exchange of services and/or resources is fundamental in animal-plant mutualisms. Plants, as sedentary partners, often rely on mobile animal partners that transfer gametes to the other sex or disperse their seeds. In return, animals receive food for their services (Rosas-Guerrero *et al.* 2014). Other plants offer shelter to their animal partners, which defend them as in the case of *Acacia* trees that harbour ants (Janzen 1966). Several plant species, including maize and tobacco, chemically attract parasitoid wasps that rid the plants of their herbivorous pests (De Moraes *et al.* 1998). The majority of such mutualisms comprise plants and insects but there are also prominent examples for mutualistic interactions between plants and mammals: A minimum of 528 angiosperm species from 67 families rely on pollinating bats (Fleming *et al.* 2009). Many of those bats and plants strongly depend on their respective partner species, which is indicated by highly adapted morphological traits. Nectarivorous bats, for example, have extremely long tongues, an elongated rostrum and reduced dental structures, which allow them to reach the nectar of flowers (Winter & von Helversen 2003; Gregorin & Ditchfield 2005; Muchhala 2006a). Morphological and physiological adaptations can also be found in the plants as their flowers have, for example, wide apertures and open (only) at night. Such adaptations not only facilitate pollination by the preferred pollinator, but also exclude those pollinators that are not effective (Muchhala 2006b). Some of these plants additionally have characteristic traits for the attraction of their bat pollinators. It has been shown that many bat-pollinated flowers produce a sulphuric odour that attracts bats (von Helversen *et al.* 2000). However, the main orientation sense of bats is echolocation, a multifunctional system which meets various demands of the bats (Jones & Holderied 2007). Some plant species in the Neotropics have thus developed flowers or leaves that are highly echo-reflective and attractive for their bat pollinators (von Helversen & von Helversen 1999; Simon *et al.* 2011).

For many other bat-plant interactions it remains unclear whether the plants gain benefits. Several bat species are known to roost in foliage such as developing tubular leaves of various plant families, e.g., Musaceae, Zingiberaceae, or Strelitziaceae. These leaves are occupied by single individuals or by aggregations of bats (Happold & Happold 1996; Ralisata *et al.* 2010; McArthur 2012). Moreover, other bat species modify leaves, producing tent-like roost structures (Kunz *et al.* 1994; Kunz *et al.* 1996). Even though the bats often damage these leaves, it has been suggested that the plants could also benefit from the bats as the damage is

often limited (Cholewa *et al.* 2001) and the bats' nutrient-rich faeces could serve as highly effective fertiliser for the plants (Foster & Timm 1976).

The use of animal faeces as fertilisers is a successful strategy in several (carnivorous) plant species to compensate for low nutrient content in soils (Anderson 2005; Romero *et al.* 2006). For example, the mountainous pitcher plant *Nepenthes lowii* occurs at high elevations with low abundance of potential arthropod prey and produces aerial pitcher-shaped trapping organs that lack typical traits for attracting arthropods.



Fig. 1. *Tupaia montana* approaching the peristome of the mountainous pitcher plant *Nepenthes rajah*. (Photo courtesy of Christian Ziegler).

These pitchers are visited by tree shrews (*Tupaia montana*), which feed on exudates from the pitchers' lid. In turn, they provide the pitchers with nutrients via their faeces (Clarke *et al.* 2009). Recent studies showed that several mountainous *Nepenthes* species (*Nepenthes macrophylla*, *Nepenthes rajah*; Fig. 1) mutualistically interact with animals as they are visited by *T. montana* during daytime and additionally by nocturnal rats (*Rattus baluensis*) (Chin *et al.* 2010; Wells *et al.* 2011).

Nepenthes pitchers are hosts for a huge variety of organisms such as larvae of mosquitos or flies, spiders, crabs, and frogs, e.g., *Microhyla nepenthicola* that can be found in *Nepenthes ampullaria* pitchers (Beaver 1985; Ratsirarson & Silander Jr 1996; Das & Haas 2010; Rembold *et al.* 2013). Associated organisms can be classified as nepenthebionts if they cannot live outside *Nepenthes* pitchers, nepenthephiles if they do not totally rely on them in every life stage, and nepenthexenes if they only sometimes can be found in the pitchers. For many of these organisms the mode of interaction with the pitcher plants is unclear (Clarke 2006). If these species make use of the pitchers' captured prey, they are usually considered as parasites. However, it is likely that many of these presumed parasites mutualistically interact with the plants by receiving domicile, protection and food in exchange for support in breaking down the pitchers' prey and thus accelerated digestion processes. One example is an ant, *Camponotus schmitzi*, which feeds on the prey of the pitcher plant *Nepenthes bicalcarata*. These ants had first been considered as parasites (Clarke & Kitching 1995) but further investigations showed the contrary: They not only keep the pitchers' peristomes slippery (Thornham *et al.* 2012), but these ants also reduce the pitchers' nutrient loss by preying upon kleptoparasites in the pitcher fluid (Scharmman *et al.* 2013).

A further puzzle is represented by different *Nepenthes* species that are occupied by bats of the species *Kerivoula hardwickii* (Payne *et al.* 1985; Clarke 2006; Arthur & Lemaire 2009). Before the start of this work nothing was known about the relationships between the involved species and whether they could be classified as mutualistic, commensalistic or even parasitic. I hypothesised that bats of the species *K. hardwickii* interact mutualistically with one or several *Nepenthes* species, which the bats use as roosts. To test this hypothesis, I conducted cost-benefit analyses with those pitcher plants that potentially benefit from the bats as well as with the bats themselves and classified the interaction as mutualism if for both partners the benefits exceeded the costs.

1.3 Study Organisms

Carnivorous Plants in the Genus *Nepenthes*

Nepenthes pitcher plants grow on nutrient-poor soils in diverse habitats such as peat swamp, heath, and mountain forests (Bohn 2004; Clarke 2006) but gain nutrients from capturing arthropods (Box 1). For this purpose, these plants have specialised leaves that form pitchers (Fig. 2) (Clarke 2006). Each pitcher is characterised by four different zones: lid, peristome, the inner upper waxy zone, and the glandular zone at the bottom of the pitcher (Clarke 2006). The lid protects pitchers from entering rainwater that would dilute the digestive fluid contained in the pitchers (Clarke 2006; Bauer *et al.* 2012). Moreover, the lid plays an important role in prey attraction and prey capture (Bauer *et al.* 2012). The second pitcher zone, the peristome, is very slippery and lures arthropods, with nectar, to approach the inner edge where they easily fall into the pitchers. In the interior of the pitchers the upper zone is coated with epicuticular wax crystals or lunate cells that point downwards (Bohn 2004; Gaume *et al.* 2002) to prevent captured arthropods from escaping the pitchers. The lower zone comprises the digestive fluid and the wall of the pitcher is full of glands that produce and secrete digestive enzymes and acids (Thornhill *et al.* 2008; Bazile *et al.* 2015). The acidity of the digestive fluids inside the pitchers varies from neutral to highly acidic, reaching pH values below 2 in species such as *Nepenthes rafflesiana* and *Nepenthes gracilis* (Clarke 2006). The digestive fluid contains various enzymes such as proteases, lipases, and chitinases (Tökés *et al.* 1974; Eilenberg *et al.* 2006; Hatano & Hamada 2008), which allow the plant to break down prey. In many species the digestive fluid is viscoelastic, a trait that significantly

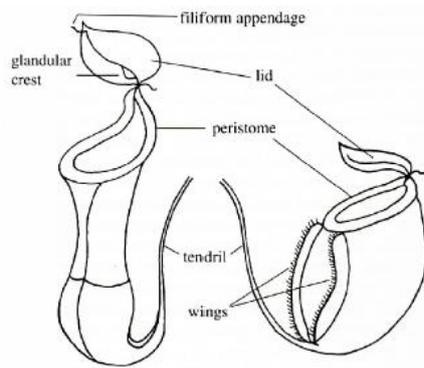


Fig. 2. Pitcher morphology of terrestrial and aerial *Nepenthes* pitchers (Clarke 2006).

contributes to the plants' prey capture success as arthropods are drawn into the fluid (Gaume & Forterre 2007; Bonhomme *et al.* 2011). Most *Nepenthes* species produce dimorphic pitcher types: 1) terrestrial pitchers that touch the ground and have an ovoid shape, and 2) aerial pitchers that are located above the ground (Fig. 2). The latter pitchers are produced by the plants when stems start climbing and the plant reaches maturity (Clarke 2006).

Box 1: Carnivory in Plants

Carnivory has convergently evolved in different lineages of angiosperms (Albert *et al.* 1992). Pitcher plants occur in seven genera in the Americas, Asia, and Australia. Five of these genera are restricted to the New World, with the majority of plants belonging to the family Sarraceniaceae (genera *Heliamphora*, *Sarracenia*, and *Darlingtonia*). The two other New World genera are bromeliads (*Catopsis* and *Brocchinia*). In the Old World the genus *Cephalotus* is monotypic and solely occurs in south-western Australia (Clarke 2006). Pitcher plants of the largest genus *Nepenthes* are common in northern Australia, in South-East Asia, and southern China. Some species occur in Sri Lanka, India, the Seychelles, and Madagascar as well as in New Caledonia. However, the largest diversity of *Nepenthes* species has been described on Borneo (Clarke 2006; Meimberg & Heubl 2006). Carnivory is a strategy of plants that occur in wet, sunny, and oligotrophic habitats to compensate the low nutrient content in soils (Givnish *et al.* 1984). Until recently it was unclear to which degree carnivory is important for carnivorous plants. Only few studies have focused on the benefits of prey capture in terms of growth and/or photosynthesis gain, and results were partly controversial but with a general agreement that carnivory is at least in some aspects beneficial for the plants (Moran & Moran 1998; Mendez & Karlsson 1999; Wakefield *et al.* 2005; Farnsworth & Ellison 2007; Pavlovič *et al.* 2009; Pavlovič *et al.* 2013). In some species, such as *Nepenthes talangensis*, prey capture seems to additionally stimulate reproductive activity (Pavlovič *et al.* 2009).

Nepenthes hemsleyana and *Nepenthes rafflesiana*

N. hemsleyana was previously characterised as the elongate form of *N. rafflesiana* Jack (*elongata* *nom. nud.*; Cheek & Jebb 2001; Phillipps *et al.* 2008). In northwest Borneo, the ‘elongate’ and ‘typical’ varieties often occur sympatrically. The elongate form was recently elevated to species status by Clarke *et al.* (2011) who named it *Nepenthes baramensis*. However, this name is a junior synonym of *N. hemsleyana* (Macfarlane 1908; Scharmann & Grafe 2013). *N. hemsleyana* can attain a maximal height of 6 m. The pitchers are soft, due to the low amount of incorporated lignin (Osunkoya *et al.* 2008), and can be green, white, or speckled with purple patches (Fig. 3a) (Clarke 2006). The length of the terrestrial pitchers is up to 20 cm with a width of up to 5 cm. They are thin-chartaceous and arise directly from the tendril. The lower third is ovoid and a clear hip can be observed. The aerial pitchers are 18 to 25 cm long with a width of 3 to 5 cm. They are narrowly infundibular in the lowest third of the pitcher and broaden towards the hip, which can be found between the middle and upper third of the pitcher. The pitcher section above the hip is cylindrical (Clarke *et al.* 2011). Volatile emission and UV light patterns, as typically observed in the closely related *N. rafflesiana* (Scharmann, personal communication; Fig. 3b), cannot be found in the aerial pitchers of *N. hemsleyana* (Moran 1996). The plants occur in the periphery of peat swamp and heath forests of Western Borneo (Brunei Darussalam, Sarawak) usually below 200 meters above sea level (Clarke 2006; Clarke *et al.* 2011).



Fig. 3. a) Aerial pitcher of *Nepenthes hemsleyana*. b) A mature (left) and a developing aerial pitcher of *Nepenthes rafflesiana*.

Nepenthes bicalcarata

N. bicalcarata (Hooker, 1873) plants are able to climb 20 m upwards to reach the forest canopy. Pitchers of *N. bicalcarata* have a bulbous or urceolate shape (Clarke 2006) (Fig. 4) and wax crystals are missing from the surface of these plants (Bohn 2004). There are two characteristic thorns in the upper part of the pitchers that are derived from the apical ribs of the peristome (Fig. 5) (Clarke 2006). As nectar glands can be found in these thorns, it is probable that they serve for prey attraction (Merbach *et al.* 1999). Aerial pitchers vary from the lower ones because of their smaller size and lower liquid quantity (Clarke 1997). The pitchers are often yellow, orange, or sometimes green. The species predominantly occurs in the periphery of peat swamp and heath forests (Clarke 2006).



Fig. 4. An aerial pitcher of *Nepenthes bicalcarata*.

Nepenthes ampullaria

Similar to *N. bicalcarata*, *N. ampullaria* (Jack, 1835) can climb to a height of up to 15 m (Fig. 5). Pitchers grow in rosettes, have an urceolate shape, and are smaller than 10 cm. Each pitcher has two fringed wings that reach from the tip to the bottom of the pitcher (Clarke 2006). The lid is small and narrow and in contrast to other species it is not located above the pitchers' orifice (Moran *et al.* 2003). These traits enable the plant to gain a considerable amount of nutrients from digesting leaf litter (Moran *et al.* 2003; Pavlovič *et al.* 2011). The plants only rarely produce upper pitchers. *N. ampullaria* grows sympatrically with *N. hemsleyana* and *N. bicalcarata* (Clarke 2006).



Fig. 5. Rosette pitchers of *Nepenthes ampullaria* (Photo courtesy of Christian Ziegler).

Bats (Chiroptera) and the Genus *Kerivoula*

The order Chiroptera (approximately 1,230 species) comprises more than 20% of the currently described mammal species and is the second largest mammalian order (Wilson & Reeder 2005; Kunz *et al.* 2011). As in many other taxa there is a global biodiversity gradient in bats, with the highest bat diversity in the subtropics and tropics. Some bat families, e.g., the Old World fruit bats (Pteropodidae), are completely restricted to these areas (Marshall 1985). Bats are the only mammals capable of active flight, which was probably key to their enormous radiation and their wide distribution as they occur on every continent except Antarctica (Thomas & Suthers 1972; Wilson & Reeder 2005). Another key factor that has allowed bats to occupy diverse ecological niches is their acoustic orientation system, which is adapted to their primarily nocturnal lifestyle (Neuweiler 2000). Most bats produce ultrasound calls in the larynx and emit them through the mouth or nose (Schnitzler & Grinnell 1977). Some Pteropodidae produce clicks with their tongues (i.e. bats of the genus *Rousettus*) (Jones & Teeling 2006) or wings (Boonman *et al.* 2014). It is highly likely that echolocation developed several times independently (Boonman *et al.* 2014).

The genus *Kerivoula* comprises 22 species, a number that has increased during the last few years as new species especially from South-East Asia, such as *Kerivoula kachinensis* (Bates *et al.* 2004), *Kerivoula krauensis* (Francis *et al.* 2007), and *Kerivoula titania* (Bates *et al.* 2007), have recently been described. More cryptic species are likely to be discovered as genetic and morphological analyses are on-going (Douangboubpha *et al.* 2015). Bats in the genus *Kerivoula* are characterised by a dense, woolly fur, a non-specialised dentition, and funnel-shaped ears with a long tragus (Payne *et al.* 1985). *Kerivoula* usually roost solitarily or in little groups of about six individuals (Nowak 1994) (for a general overview see Box 2). Their echolocation calls are downward-frequency-modulated with low intensity and are characterised by short call durations, extremely large bandwidths, and high starting frequencies (152.0 – 180.0 kHz) (Kingston *et al.* 1999).

Box 2: Roosting Ecology of Bats

Small vertebrates frequently suffer high predation risk (Lagos *et al.* 1995) or adverse climatic conditions (Roper *et al.* 2001; Shimmin *et al.* 2002). Thus, they often strongly depend on shelters for survival and reproduction. As a consequence, low shelter availability can lead to population decline (Grillet *et al.* 2010; Lagarde *et al.* 2012). Similarly, the availability of roosts seems to influence the number of occurring bats in a certain area probably due to competition between bats and other vertebrate or invertebrate species (Humphrey 1975; Kunz & Lumsden 2005).

Most bats are highly social with group sizes ranging from only few individuals to aggregations of several million bats in some locations (McCracken & Wilkinson 2000; Kunz & Lumsden 2005; Kerth 2008). While females roost solitarily in only a few species, such as *Lasiurus borealis* (Mager & Nelson 2001), solitary lifestyle is more common in males (McCracken & Wilkinson 2000). Besides commonly used roosts, such as caves and tree cavities (Kunz & Lumsden 2005), some bats also use exceptional roost types such as ant and termite nests (Hodgkison *et al.* 2003; Dechmann *et al.* 2004) or abandoned bird nests (Schulz 2000). Roost selection is not only variable between but also within species as it often depends on the developmental stage and reproductive status of the bats (Lewis 1995; Kerth *et al.* 2001; Chruszcz & Barclay 2002). Here, abiotic factors such as microclimatic conditions inside the roosts play an important role. Reproductive female bats of several species are known to select warm roosts to accelerate the development of the juveniles (Hamilton & Barclay 1994; Kerth *et al.* 2001). Similarly, in the bat *Lophostoma silvicolum*, males excavate active termite nests to create roosts that provide the associated females with higher inner temperatures than the ambient one. By roosting in active termite nests, these bats can reduce their daily energy needs by up to 5% (Dechmann *et al.* 2004; Dechmann *et al.* 2005). A further important microclimatic factor is the humidity inside roosts, which is especially relevant in bats whose water loss is high due to their large and exposed wing membranes (Chew & White 1960). Thus, bat roosts are commonly characterised by high air humidity (Baudunette *et al.* 1994; van der Merwe & Stirnemann 2009).

Furthermore, roost selection in bats is often influenced by parasite infestation risk. Bats suffer from various highly specialised ectoparasites such as mites (Spinturnicidae, Macronyssidae), and bat flies (Nycteribiidae). While some of those parasites (e.g., Spinturnicidae) spend their whole lifecycle on the bats, others (e.g., Nycteribiidae) need the roosts of their hosts for

certain developmental stages (Rudnick 1960; Zahn & Rupp 1999). Parasites of the latter group deposit their larvae in the bats' roosts, where they pupate and subsequently wait for new hosts to arrive (Dick & Patterson 2006; Reckardt & Kerth 2006, 2007). It is likely that such ectoparasites negatively influence the body condition of animals, although several studies investigating the cost of parasitism have produced contrasting results (Brown & Brown 1986; Lewis 1996; Zahn & Rupp 1999; Giorgi *et al.* 2001; Lourenço & Palmeirim 2007). Roost switching in many bat species seems thus to be a behavioural adaptation that minimises parasite load (Lewis 1996; Reckardt & Kerth 2007). Pallid bats, *Antrozous pallidus*, for example, show very low roost fidelity and switch roosts highly frequently to disrupt the reproductive cycle of their parasites (Lewis 1996). However, bats are faced with a trade-off as not only the parasites themselves but also roost switching incurs costs, for example, it may be more difficult to keep a social group intact when roost switching occurs frequently (Lewis 1995, 1996).



Fig. 6. The insectivorous bat *Kerivoula hardwickii*.

Kerivoula hardwickii

K. hardwickii (Horsfield, 1824) is a small bat with a forearm length of 32.0 – 34.0 mm and a weight of 3.5 – 4.2 g. The fur colour on the dorsal surface is grey-brown with dark grey at the base, while the ventral surface is lighter grey (Fig. 6). It can be found in the undergrowth of mature forests (Payne *et al.* 1985) and especially in primary forests (Heaney 1998). The range of *K. hardwickii* includes Sri Lanka, India, southern China, Malay Peninsula, Sumatra, Java, Kangean Islands, Bali, Borneo, Sulawesi, Philippines (Payne *et al.* 1985; Corbet & Hill 1992; Nowak 1994; Esselstyn *et al.* 2004), and Singapore (Leong & Lim 2009). Several subspecies of *K. hardwickii* have been described including *K. h. hardwickii* that has been reported to occur in Sabah, Sarawak as well as in East and West Kalimantan (Payne *et al.* 1985; Hill & Rozendaal 1989). However, the classification of subspecies based on morphological and multiple genetic datasets has produced contradictory results (for an overview see Douangboubpha *et al.* 2015).

2 RESULTS AND DISCUSSION

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2.1 General Findings

We found bats of the species *K. hardwickii* roosting in pitchers of three *Nepenthes* species: in aerial pitchers of *N. hemsleyana* and *N. bicalcarata*, as well as in terrestrial pitchers of *N. ampullaria* (Grafe *et al.* 2011; Schöner *et al.* 2013; Schöner C. *et al.* 2015), which is in accordance with previous publications (Payne *et al.* 1985; Clarke 2006; Arthur & Lemaire 2009). The bats were present in all study sites in North-western Borneo (Brunei Darussalam and Sarawak) where one or several of these pitcher plant species occurred except for one area where the pitcher plants grew outside the forest (Fig. 7). As bats are highly sensitive to the microclimatic conditions inside their roosts (Kerth *et al.* 2001; Dechmann *et al.* 2004), it seems that the relationship between *K. hardwickii* and the pitcher plants is restricted to areas with dense canopy cover that buffers the microclimatic conditions (Schöner *et al.* 2013).

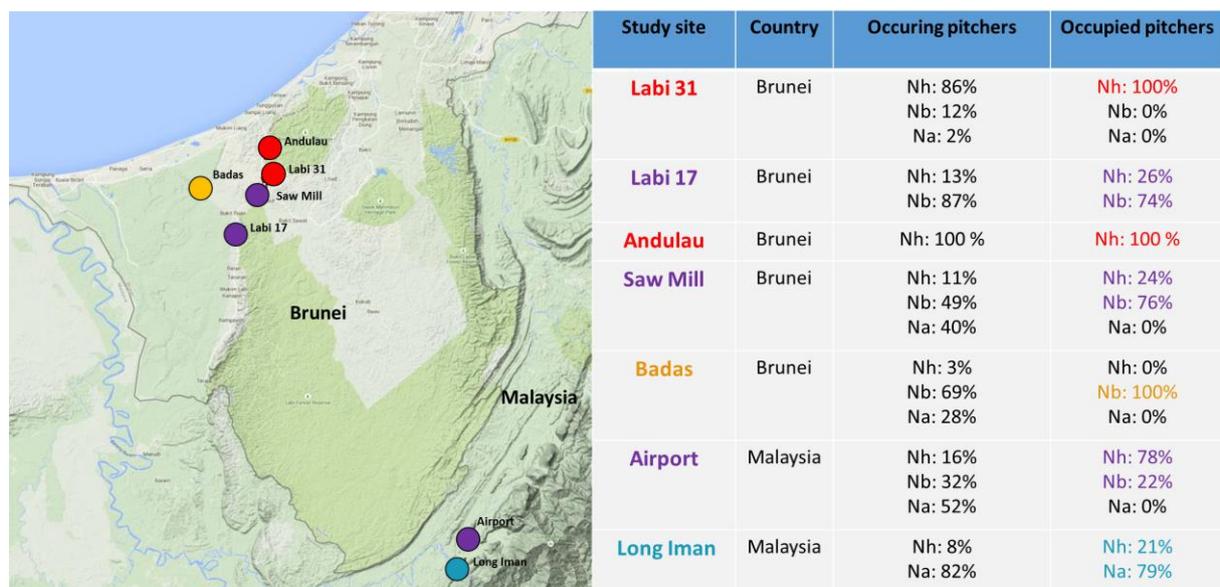


Fig. 7. Study sites with populations of *Kerivoula hardwickii* using *Nepenthes* pitchers as roosts. The percentages of occurring pitcher types and the ratios of occupied roosts are shown (data from 2009 till 2014 included). Nh = *Nepenthes hemsleyana*; Nb = *Nepenthes bicalcarata*; Na = *Nepenthes ampullaria*. Colours indicate which pitcher type(s) were used by *K. hardwickii*: red = Nh, orange = Nb, violet = Nh and Nb, turquoise = Nh and Na.

Intact pitchers of *N. bicalcarata* and *N. ampullaria* are characterised by high levels of digestive fluid and an urceolate shape (Clarke 2006) so that bats cannot use them. Instead, we found bats roosting in senescing and damaged pitchers of those species where the digestive fluid had drained off (Schöner *et al.* 2013). Therefore, the interaction with *K. hardwickii* is not beneficial for *N. bicalcarata* and *N. ampullaria* as they cannot gain nutrients from the bats' faeces (Schöner *et al.* 2013). In contrast to *N. bicalcarata* and *N. ampullaria* pitchers,

only intact pitchers of *N. hemsleyana* can be used because these pitchers lose their shape as soon as they start senescing (Schöner C. *et al.* 2015). This makes them an optimal candidate for a mutualistic interaction with the bats. In the following cost–benefit analyses I will thus focus on this interaction between the bats and *N. hemsleyana*.

2.2 Benefits for the Interacting Species

Benefits for *K. hardwickii*



Fig. 8 *Kerivoula hardwickii* roosting in a *Nepenthes hemsleyana* pitcher. The shape of the pitcher and the low fluid level perfectly match the bats' body size.

Interspecific roost competition has been discussed as a potential cause for roost partitioning in bats (Kunz & Lumsden 2005). By choosing a particular type of roost, such as pitchers of carnivorous plants, bats could benefit by avoiding interspecific competition. In fact, we never found another bat species other than *K. hardwickii* roosting in pitcher plants (Grafe *et al.* 2011; Schöner *et al.* 2013). One possible reason why only *K. hardwickii* use *N. hemsleyana* pitchers is that the pitchers' dimensions are well matched to *K. hardwickii*'s body lengths and widths as we showed in Grafe *et al.* (2011) and Lim *et al.* (2015). Compared to the closely related *N. rafflesiana*, *N. hemsleyana*'s pitchers are characterised by a reduced fluid level (Fig. 8) (Lim *et al.* 2015). Thus, the inner volume of *N. hemsleyana* pitchers is large enough to host a maximum of two *K. hardwickii* individuals (normally a mother with her offspring). In contrast, many other sympatric bat species would either be too big and would not fit into the pitchers or they would be too small and thus at risk of falling into the digestive fluid (Payne *et al.* 1985).

K. hardwickii particularly benefit more from roosting in pitchers of *N. hemsleyana* than in those of the non-mutualistic *N. bicalcarata*. During the hottest hours of the day the temperature inside *N. hemsleyana* pitchers is continuously lower than in wilted *N. bicalcarata* pitchers. In contrast to *N. bicalcarata* pitchers, the humidity inside *N. hemsleyana* pitchers is higher than the ambient humidity and highly stable during the course of the day. The presence of evaporating digestive fluid in combination with the elongated shape, which retains the high air humidity, supports beneficial microclimatic conditions inside *N. hemsleyana* pitchers (Schöner *et al.* 2013). This helps bats to avoid heat stress and dehydration (Herreid &

Schmidt-Nielson 1966; Vonhof & Barclay 1997). Additionally, *K. hardwickii* roosting in pitchers of *N. hemsleyana* are free from parasite species such as fleas and bat flies that strongly depend on the roosts of their host for reproduction (Reckardt & Kerth 2007). The inner surface of *N. hemsleyana* pitchers is covered with waxy crystals (Gaume & Di Giusto 2009; Bauer *et al.* 2011), which prevent the attachment of eggs or larvae on the roosts' walls. Such a waxy layer is missing in *N. bicalcarata* pitchers (Bohn 2004).

The higher quality of *N. hemsleyana* pitchers also affects the bats' physical condition and behaviour: Bats that exclusively roost in *N. hemsleyana* pitchers are in better body condition compared to those individuals that at least partially use *N. bicalcarata* pitchers (Schöner *et al.* 2013). As a consequence, the bats use *N. hemsleyana* pitchers more frequently (Schöner *et al.* 2013). Such behaviour can also be seen in other bat species that use those roosts more intensively which correspond to their current requirements (Kerth *et al.* 2001; Lausen & Barclay 2002). Could the roost preference of *K. hardwickii* also be beneficial to *N. hemsleyana*?

Benefits for *N. hemsleyana*

Despite the fact that the production of trapping organs is costly (Osunkoya *et al.* 2007; Pavlovič *et al.* 2009), carnivory is beneficial for *Nepenthes* and other carnivorous plants (Aldenius *et al.* 1983; Chapin & Pastor 1995; Pavlovič *et al.* 2013). In this context, *N. hemsleyana* seems to be a paradox as its large pitchers capture a low amount of arthropods (Moran 1996). To determine whether *N. hemsleyana* profits from harbouring bats, we compared the isotopic signature of leaf blades from pitchers used as roosts with those from control pitchers that had never been occupied and which had been monitored on a daily basis since they had opened (Grafe *et al.* 2011). Our results showed that *N. hemsleyana* gains more than one third of its foliar nitrogen from bat faeces (Fig. 2 in Grafe *et al.* 2011). Yet, the value of this nitrogen source and its fitness relevance were still unclear. To compare the beneficial effect of bat faeces on *N. hemsleyana* with that of arthropod prey, we conducted feeding experiments both in the wild and in the greenhouse (Schöner *et al.* submitted). We determined growth, photosynthesis and survival of all experimental *N. hemsleyana* plants. Plants fed with bat faeces performed better in all measured parameters than plants fed with arthropods only. After long-term nutrient deprivation plants that we fed with both faeces and arthropods even covered 95.4% of their nitrogen demand from bat faeces (Schöner *et al.* submitted) probably

because faecal nitrogen is typically bound in urea molecules that can be more readily assimilated by plants than protein-bound nitrogen (McFarlane *et al.* 1995). Arthropod prey alone was not sufficient for the plants as 21.4% of the arthropod-fed plants died during the experiment while all of the faeces-fed plants survived. *N. hemsleyana* has thus outsourced prey capture and digestion to its animal-interaction partner (Schöner *et al.* submitted), similar to other mammal-*Nepenthes* interactions (Clarke *et al.* 2009; Chin *et al.* 2010). This results in multiple benefits for the plants: The associated mammals have large foraging areas and access to a variety of food items that otherwise would be inaccessible for the plants (Payne *et al.* 1985). Finally, all of these mammals predigest their food, and deliver it to the plants via their faeces (Clarke *et al.* 2009; Chin *et al.* 2010; Grafe *et al.* 2011) in a readily absorbable format (Schöner *et al.* submitted).

2.3 Costs for the Interacting Species

Costs for *K. hardwickii*

Finding new roosts is a critical and challenging task for bats (Ruczyński *et al.* 2007) especially when roosts are highly ephemeral (Chaverri *et al.* 2010). *N. hemsleyana* pitchers are relatively rare, grow hidden in the dense peat swamp forests of Borneo, and are short-lived (Osunkoya *et al.* 2008; Schöner C. *et al.* 2015). Other roosts used by *K. hardwickii* (*N. bicalcarata* and *N. ampullaria*) are more abundant and available for a long time as they keep their shape when they are dead (Schöner C. *et al.* 2015). However, most *N. hemsleyana* plants continuously provide at least one suitable pitcher (Schöner C. *et al.* 2015) so that the bats are able to remain associated with the same plant for months or years (Schöner *et al.* 2013).

Costs to find new pitchers could additionally be reduced if *N. hemsleyana* pitchers have characteristics that help the bats to find them. We detected a concave structure in the elongated back wall of *N. hemsleyana*, which serves as an effective echo reflector with a species-specific spectral signature that makes the pitchers acoustically stand out in cluttered environments. Behavioural experiments showed that this echo reflector helps the bats to find and identify their pitcher roosts (Schöner C. *et al.* 2015). Convergent structures have previously been found in Neotropical plants which however are pollinated and not fertilised by bats (von Helversen & von Helversen 1999; Simon *et al.* 2011). Taken together, it seems that the costs for *K. hardwickii* to find new pitchers are relatively low. It is highly likely that the bats find new pitchers by inspecting plants that they already know and even if they have to

find new plants, the pitchers' echo-reflective structure will facilitate the search. Other potential costs, which we investigated, appeared to be marginal (Schöner M. *et al.* 2015).

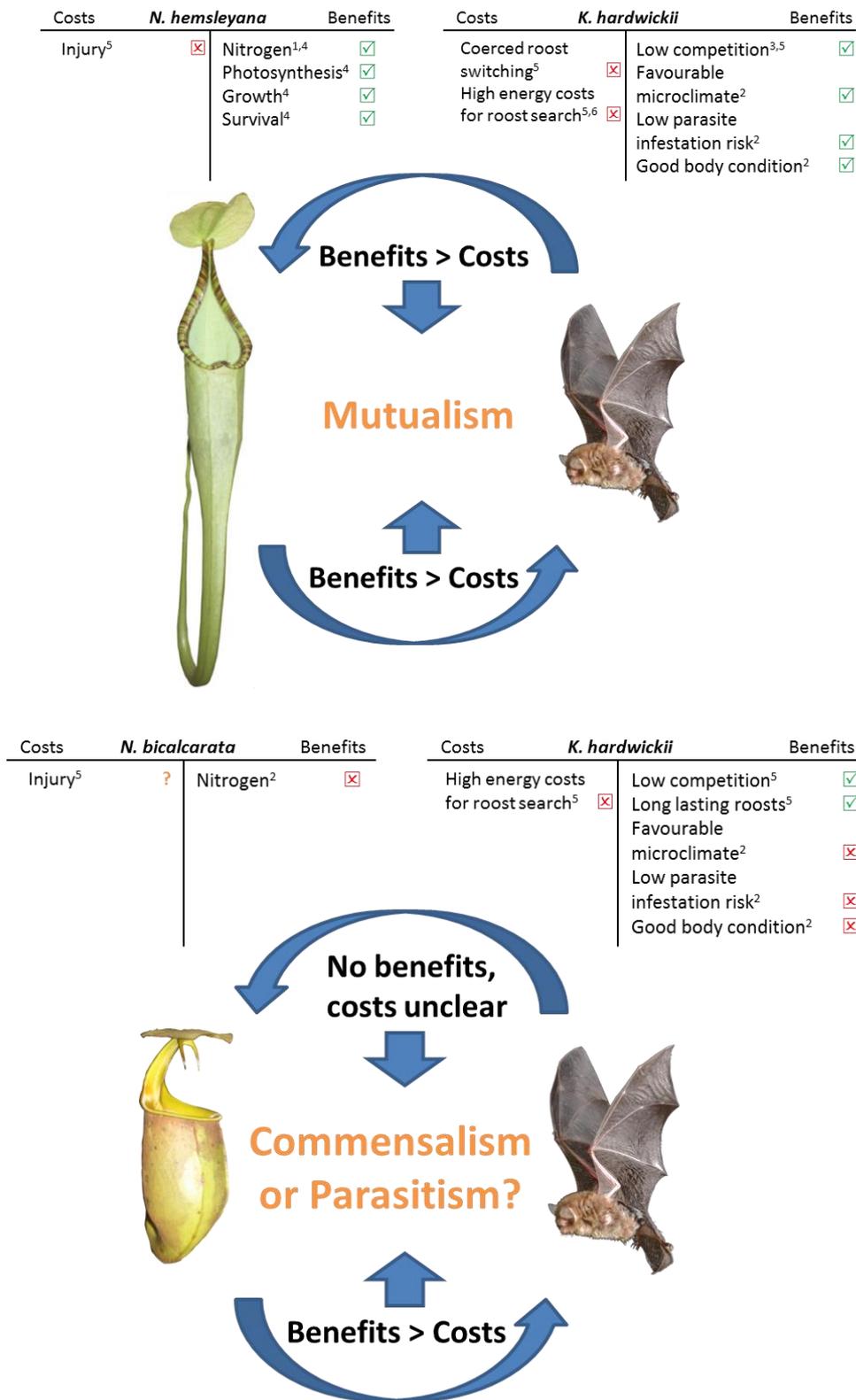
Costs for *N. hemsleyana*

Voluntarily or involuntarily, some bat species are known to damage plant tissue while or in order to roost in it (Kunz *et al.* 1996). *N. hemsleyana* pitchers are highly delicate due to their low lignin content and thin walls (Osunkoya *et al.* 2008). By monitoring the lifespan of pitchers and whether and how often they were occupied by bats, we could neither find visible damage in pitchers used by the bats nor a reduction in their lifespan compared to pitchers that had never been used by *K. hardwickii* (Schöner C. *et al.* 2015). As the rare pitchers of *N. hemsleyana* are a valuable resource for the bats (Schöner C. *et al.* 2015), it is likely that they avoid damage to their foliage roosts in order to use them for as long as possible (Cholewa *et al.* 2001).

2.4 Classification of the Interaction between Bats and Pitcher Plants

Mutualisms are defined as interactions where the benefits of each involved species outweigh the costs (Bronstein 1994a) – a situation that seems to be the case in the interaction between *K. hardwickii* and *N. hemsleyana* (Fig. 9). Despite a net-benefit resulting from the interaction, some mutualisms cause high costs for the involved species. Some ant species, for example, limit growth and reproduction of their host trees that they defend against herbivores (Stanton & Palmer 2011). Several obligate pollinators feed on the seeds of associated plants and thereby impair the reproduction of the mutualism partner (Pellmyr 1989; Herre & West 1997). To quantify the outcome for *K. hardwickii* and *N. hemsleyana*, I examined the most prominent factors that were already known to be beneficial (e.g. the use of faeces as fertilisers (Clarke *et al.* 2009)) or costly (e.g., the ephemerality of plant roosts (Chaverri *et al.* 2010)) in other mammal and (pitcher) plant mutualisms. Even though the list of possible benefits and costs could still be extended (Schöner C. *et al.* 2015), the benefits are so significant that the consideration of further costs is unlikely to reverse this ratio. The effect of the mutualism between *K. hardwickii* and *N. hemsleyana* on the reproduction of both species is still unknown but we found that the survival of *N. hemsleyana* plants and the body condition of *K. hardwickii* improved when both species interacted (Schöner *et al.* 2013; Schöner *et al.* submitted). Thus, our studies provide indirect evidence that this mutualism has a beneficial effect on the fitness of both species.

As common in other facultative mutualisms (Bshary & Bronstein 2004; Moeller & Gebre 2005; Stadler & Dixon 2005; Thompson & Fernandez 2006) *K. hardwickii* and *N. hemsleyana* still use alternative sources outside the mutualism to meet their requirements (Grafe *et al.* 2011; Schöner *et al.* 2013; Schöner *et al.* submitted). *N. hemsleyana* has retained some traits that are effective in arthropod capture such as the viscoelasticity of the digestive fluid (Bazile *et al.* 2015). These traits may be important during early stages of the plants' development when they do not produce pitchers that are suitable bat roosts or during times when bats are not available. Similarly, the bats use alternative roost sites. By additionally using resources and services outside the mutualism the involved species retain flexibility and are not strictly bound to the occurrence and distribution of the partner as is the case in obligatory mutualisms such as those between figs and fig wasps (Harrison 2000). However, when one of the species utilises resources outside the mutualism, selective pressure may act on the respective partner to prevent the former escaping the mutualism.



¹Grafe et al. 2011; ²Schöner et al. 2013; ³Lim et al. 2015; ⁴Schöner et al. submitted; ⁵Schöner C. et al. 2015; ⁶Schöner M. et al. 2015

Fig. 9. Classification of the interaction between *Kerivoula hardwickii* and *Nepenthes hemsleyana* (upper panel), and between *Kerivoula hardwickii* and *Nepenthes bicalcarata* (which also represents *Nepenthes ampullaria*; lower panel).

There are several ways for species to become more attractive for the partner and thus to strengthen the mutualism: first, species can increase the probability to be more frequently encountered by their partner than alternative resources. Neotropical flowers, for example, catch the attention of phyllostomid bats that pollinate a broad range of different plants with olfactory or acoustic signals (Raguso 2004). Second, species can provide high quality resources and services to be more competitive. Many organisms are able to distinguish partners that offer high quality resources from those offering low quality resources. Bees, for example, avoid unrewarding flowers (Smithson & Gigord 2003) and in cleaner-fish mutualisms high quality cleaners are preferred partners (Bshary & Grutter 2002). The pitcher plant *N. hemsleyana* uses both mechanisms: the conspicuous echo reflector, which is missing in *N. bicalcarata* and *N. ampullaria*, helps the plants to be located and identified quickly by the bats (Schöner M. *et al.* 2015). Additionally, the roost quality of *N. hemsleyana* pitchers exceeds that of the other *Nepenthes* species in respect to microclimate and parasite infestation risk (Schöner *et al.* 2013), which should have a beneficial effect on the bats. Often, partners that offer greater benefits also gain more benefits themselves, which improves their cost-benefit ratio and enforces the mutualism (Ferrière *et al.* 2007). *N. hemsleyana* also benefits from offering conspicuous and high quality roosts as it is used more intensively than other *Nepenthes* pitchers (Schöner *et al.* 2013; Schöner M. *et al.* 2015).

A consequence, which can often be seen when mutualistic partners strongly depend on each other, is trait loss (Ellers *et al.* 2012). Leaf cutter ants, for example, have lost arginine biosynthesis pathway due to fungus cultivation (Suen *et al.* 2011). Although the mutualism between *N. hemsleyana* and *K. hardwickii* is not an obligate one, *N. hemsleyana* seems to strongly depend on its bat partner to whom it outsourced prey capture and digestion. The plant has largely reduced its arthropod attracting traits (Moran 1996) and therefore partially abandoned one of the key characteristics of carnivorous plants: carnivory itself (Schöner *et al.* submitted). Notably, captured arthropods cannot fully compensate for the beneficial effects of bat faeces and *N. hemsleyana*'s mortality increases when bats are excluded (Schöner *et al.* submitted). The loss of its bat mutualism partner would thus imply high costs for the plants. The bats seem to have retained more flexibility than the plants. Although alternative roosts of *K. hardwickii*, such as pitchers of *N. bicalcarata* and *N. ampullaria*, are of low quality with a direct or indirect negative influence on the bats' body condition, they are consistently used by the bats in several populations (Schöner *et al.* 2013) (Fig. 7). Therefore, the mutualism between *K. hardwickii* and *N. hemsleyana* is not completely symmetrical, a characteristic

observed in many mutualistic interactions (Guimaraes *et al.* 2006). This could partially be explained by abundance effects of the involved species (Vazquez *et al.* 2009). Bascompte *et al.* (2006) suggested that such evolved asymmetries could have a stabilising effect on mutualisms. Accordingly, in the interaction between *N. hemsleyana* and *K. hardwickii* the plant seems to have acquired highly attractive traits that meet the requirements of their mammal partner (Grafe *et al.* 2011; Lim *et al.* 2015; Schöner, M. *et al.* 2015).

My thesis emphasises the necessity of rigorous cost-benefit analyses to determine how the involved species affect and depend on each other. Such analyses have regularly been conducted incompletely, often neglecting the determination and quantification of benefits and especially costs for one or several of the interacting partners (Bronstein 2001a). This may lead to preliminary classifications not revealing the true nature of these complex interspecific interactions especially as in most cases not only two organisms but whole networks of different organisms interact with each other (Knowlton & Rohwer 2003). Examples can be seen in *Acacia* trees and their symbiotic ant species (Palmer *et al.* 2010). The complex interplay between multiple species often opens up advantages for species but also poses problems such as increased competition and opportunities for cheating (Addicott 1978; Palmer *et al.* 2010). Cost-benefit analyses of all involved partners would be fundamental for the improved understanding of such complex systems.

Moreover, this study can serve as basis for future projects that will investigate the evolution of this and similar mutualisms. Although mutualisms are omnipresent and shape whole ecosystems (Bronstein 2001a; Bronstein 2009; Vazquez *et al.* 2009), our understanding of how mutualisms originate is incomplete. Current knowledge is mainly limited to theoretical approaches (Connor 1995; Yamamura *et al.* 2004), and a few exemplary systems that have been extensively examined (Anstett *et al.* 1997; Yoder *et al.* 2010).

Finally, the interaction between *K. hardwickii* and *N. hemsleyana* is likely to be in an early stage of evolution as molecular analyses suggest that speciation processes in *Nepenthes* only took place after the last glacial period (± 10000 ya) (Meimberg & Heubl 2006; Chin *et al.* 2010). This comparatively young interaction therefore gives us the rare opportunity to experimentally reconstruct and identify which components have been important for its development and which traits of the partners facilitate the maintenance of the mutualistic relationship.

3 REFERENCES

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4 PUBLICATION LIST

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4.1 Benefits in a Bat-Pitcher Plant Mutualism



Manuscript 1

Grafe, T.U., Schöner, C.R., Kerth, G., Junaidi, A. & Schöner, M.G. (2011). A novel resource-service mutualism between bats and pitcher plants. *Biol. Lett.*, 7, 436–439.

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

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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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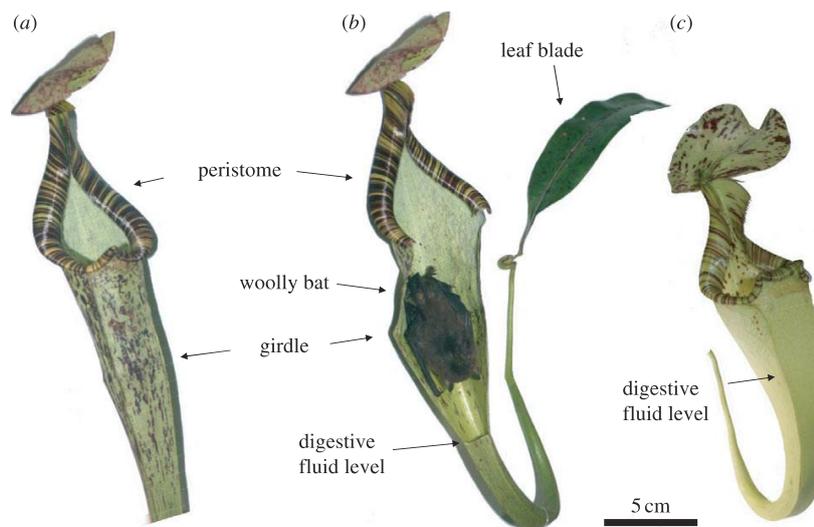


Figure 1. Service benefit provided by *N. r. elongata* to *K. h. hardwickii*. (a) Aerial pitcher of *N. rafflesiana* var. *elongata*. (b) The same pitcher with the front tissue removed to reveal a roosting Hardwick's woolly bat. (c) The shorter aerial pitcher of *N. rafflesiana* variety *typica*.

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 ($1.30 \pm 1.53\%$) than when they did not ($0.51 \pm 1.09\%$; 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.

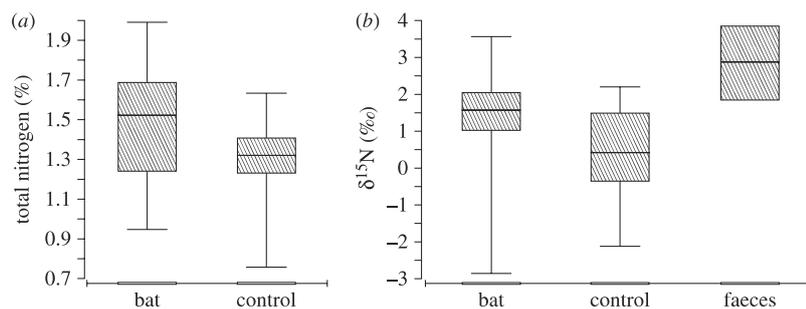


Figure 2. Resource benefit provided by roosting *K. h. hardwickii* to *N. r. elongata*. (a) Box-plot of total foliar nitrogen of pitchers used as roosts ($n = 38$) and of control pitchers ($n = 17$). (b) Box-plot of foliar $\delta^{15}\text{N}$ of pitchers used as roosts ($n = 38$), control pitchers ($n = 17$) 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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Supplementary material

Electronic supplementary material for **A novel resource-service mutualism between bats and pitcher plants** -- This is additional material to clarify and augment the Methods, Results and Discussion sections of the paper

2. MATERIAL AND METHODS

Study species

Nepenthes rafflesiana variety *elongata*

Pitcher plants of the genus *Nepenthes* grow on nutrient-poor soils, found e.g. in the threatened heath and peat swamp forests of Borneo. As a result, pitcher plants strongly depend on additional nitrogen supply that they gain by catching arthropods and small vertebrates in their pitchers. Stoichiometric analyses have shown that *Nepenthes* species are nitrogen limited (Osunkoya *et al.* 2007). Most *Nepenthes* species show an ontogenetic dimorphism: they produce terrestrial and aerial pitchers (Clarke 2006, Gaume & Di Giusto 2009). With at least 31 described species (Jebb & Cheek 1997) of about 120 species worldwide (McPherson 2009), Borneo harbours the greatest regional diversity of *Nepenthes* species (Moran 1996). In addition, many species comprise several subspecies further increasing the genetic and ecological diversity of pitcher plants.

Our study species, *N. rafflesiana* comprises at least nine subspecies, five of which occur in Borneo and can be discriminated from each other by the form of their aerial pitchers (e.g. *typica*-form vs. *elongata*-form that commonly co-occur in lowland forests in northern Borneo; Clarke 2006). *N. r. typica* occurs in Borneo, Sumatra, Singapore and Peninsular Malaysia. The less widespread *N. r. elongata* is restricted to the heath and peat swamp forests of Borneo (Clarke 2006; Phillipps *et al.* 2008). The aerial pitchers of the conspicuous subspecies *elongata* are characterized by their elongated, narrow and cylindrical form, a lack of the typical volatiles (as detected by smell; DiGusto *et al.* 2010),

and reduced UV-absorption of the peristome (Moran 1996; Phillipps *et al.* 2008). The two latter features are important mechanisms of the *typica*-form that help it to attract insects (Bauer *et al.* 2009). As a result, the insect catching rate of the *typica*-form is about seven times higher than that of the *elongata*-form (Moran 1996, Gaume & Di Giusto 2009). Aerial pitchers of *N. r. typica* have a lifespan of about two months (Di Giusto *et al.* 2008), whereas aerial pitchers of *N. r. elongata* are functional for 2-4 months (own observations). Active aerial pitchers of *N. r. elongata* can be found all year round (own observations).

We monitored a total of 223 *N. r. elongata* plants of which 64 (28.7%) harboured a total of 25 bats during our 6.5 week study period in 2009. Individual plants had a maximum of five intact pitchers of which bats used up to three pitchers per plant. Only a single leaf blade was sampled from a plant for subsequent nitrogen analysis.

Kerivoula hardwickii

Hardwicke's woolly bat can be found in the undergrowth of tall forests (Payne & Francis, 2005) particularly in primary forests (Heaney *et al.* 1998). *K. harwickii* is an insectivorous bat that forages in narrow-spaces or clutter (Struebig *et al.* 2010). The range of Hardwicke's woolly bats includes Sri Lanka, India, southern China, Malay Peninsula, Sumatra, Java, Kangean Islands, Bali, Borneo, Sulawesi, Philippines (Corbet & Hill, 1992; Esselstyn *et al.* 2004; Nowak, 1994; Payne & Francis, 2005) and Singapore (Leong & Lim, 2009). However, the subspecies *K. h. hardwickii*, which we found to roost in *Nepenthes* pitchers, occurs only in Borneo (Brunei, Sabah, Sarawak as well as in East and West Kalimantan; Payne & Francis, 2005). Thus, the mutualistic relationship between pitcher plants and bats seems to be restricted to Borneo involving one particular subspecies of *Kerivoula hardwickii* and one variety of *Nepenthes rafflesiana*. It should be noted, however, that additional cryptic species of *Kerivoula* are currently being described from Borneo (M.J. Struebig pers. communication) and it appears likely that the *hardwickii* subspecies found on Borneo may be a separate species (Barcode of Life Data Systems, <http://www.barcodinglife.org/>).

Details of sampling and stable isotope analysis

Observations of faeces in aerial pitchers of *N. r. elongata* confirmed that bats were defecating into the pitchers (own observations). Bats presumably defecate on entering or exiting the pitcher and we have seen them turn around in the pitcher, suggesting that they can easily avoid soiling themselves if they choose not to. However, defecation within the pitcher could be uncommon and the droppings themselves do not show that the pitcher plants sequester any of the nitrogen from the faeces. To test the hypothesis that pitcher plants obtain nitrogen from bat faeces, we determined the source of foliar nitrogen in *N. r. elongata* using stable isotope analyses. Nitrogen obtained from pitchers is stored within the associated leaf blades and is likely to be transported to the youngest, uppermost leaf of the plant to facilitate pitcher construction there, as has been shown in *N. mirabilis* (Schulze *et al.* 1997). We opted not to sample the youngest leaf to avoid picking up nitrogen signal from other pitchers on the plant, as each plant usually has multiple pitchers. Since some of the sequestered nitrogen from a pitcher that has harboured bats is lost to younger, developing leaves, our estimate of the contribution of bats to nitrogen must be regarded as conservative. Likewise, we could not exclude the possibility that pitchers known not to have had bats roosting in them might have been on plants that had bats roosting in other pitchers. Thus, sampling the youngest leaves on a plant might have introduced considerable error. Nitrogen is not known to be transported to already established pitchers and their associated leaf blades. After harvesting leaf blades, they were freeze dried in a specimen freeze dryer (Virtis model 24DX24) and stored in a dessicator over silica gel for one to two weeks before they were ground to a fine powder using liquid nitrogen.

We used a two-member isotopic mixing model (Shearer & Kohl 1989; Treseder *et al.* 1995) to determine the contribution by bats to the nitrogen intake by pitcher plants. Our

model included mean $\delta^{15}\text{N}$ values for aerial leaf blades with potential faecal input ($n = 38$), aerial leaf blades without faecal input ($n = 17$) and woolly bat faeces ($n = 3$). The bat faeces was collected from bats defecating upon handling and immediately dried over silica gel. We estimated the contribution of bats as:

$$\%N_{\text{faeces}} = ([\delta^{15}\text{N}_{\text{leaf blade with bat}} - \delta^{15}\text{N}_{\text{leaf blade without bat}}] / [\delta^{15}\text{N}_{\text{faeces}} - \delta^{15}\text{N}_{\text{leaf blade without bat}}]) * 100$$

where $\delta^{15}\text{N}_{\text{leaf blade with bat}}$ and $\delta^{15}\text{N}_{\text{leaf blade without bat}}$ represent the nitrogen isotope ratios of the leaf blades associated with aerial pitchers of *N. r. elongata* known to have harboured bats and leaf blades associated with aerial pitchers known not to have harboured bats, whereas $\delta^{15}\text{N}_{\text{faeces}}$ represents the nitrogen isotope ratio of bat faeces.

Ideally the model would have included the nitrogen isotope ratio of *N. r. elongata* pitcher plants that acquire their nitrogen only from bat faeces to exclude influences of isotopic discrimination by the pitcher plant (Shearer & Kohl 1989, Treseder *et al.* 1995; Clarke *et al.* 2009). For practical reasons, however, it is very difficult to exclusively “feed” pitcher plants with animal faeces while excluding nitrogen input from other sources (Clarke *et al.* 2009). We believe that substituting $\delta^{15}\text{N}$ values for *N. r. elongata* aerial leaf blades with 100% faecal input for $\delta^{15}\text{N}$ values of bat faeces is justified, since studies on several non-carnivorous plant species have shown that lower N availability decreases discrimination (e.g. Pritchard & Guy 2005) a situation very likely to apply to *Nepenthes*.

3. RESULTS

Some woolly bat individuals showed high site fidelity, using the same pitcher without interruption for up to 11 days. Other woolly bats switched pitchers daily. Pitchers used by bats had an

average length of 19.8 ± 3.1 cm, with a diameter of 4.6 ± 0.8 cm and a height above ground of 123.4 ± 60.6 cm ($n=87$). In total, 75 (86.2%) of the 87 occupied *N. r. elongata* pitchers showed single occupancy, 10 pitchers (11.5%) had double occupancy and two (2.3%) pitchers were visited by three different 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 % over the total study period of 6.5 weeks, compared to a 20.8% pitcher occupancy rate. On average, $1.30 \pm 0.70\%$ of the 423 monitored pitchers were occupied on a single day.

4. DISCUSSION

Bats use a variety of daytime roosts (Kunz & Lumsden 2003), including furled leaves (Chaverri *et al.* 2010) and bamboo culms (Feng *et al.* 2008), but none are known to roost in pitcher plants. None of the other woolly bats are known to use pitchers as roosts although some (*K. minuta* and *K. intermedia*) are smaller than *K. hardwickii* and thus would find ample space within pitchers. Bats are known to be highly selective in their choice of roosting sites. For example, the neotropical bat *Lophostoma silvicolum* roosts in active termite nests that the bats modify (Dechmann *et al.* 2004). Likewise, the Malaysian bat *Balionycteris maculata* excavates arboreal ant nests for use as day roosts (Hodgkison *et al.* 2003) and *Kerivoula papuensis* roosts predominantly in abandoned birds' nests in Australia (Schulz 2000). These examples show that the highly specialized nature of daytime roost use in *K. hardwickii* is not unusual.

Additional advantages of roosting inside pitchers can be identified. A significant advantage could be the absence of blood-sucking ectoparasites, which are frequently found in other bat roosts (Zahn & Rupp 2004; Reckardt & Kerth 2007). Roost longevity may be another factor that has selected for woolly bat roosting behaviour. Although the pitchers of *N.r. elongata* are ephemeral in nature, remaining intact for only two to four months, they are much more reliable as roosts than short-lived alternatives such as furled leaves. For example, the neotropical bat *Thyroptera tricolor* is only able to

use furled leaves as roosts for one day (Vonhof & Fenton 2004). Finally, the narrow and cylindrical form and the presence of a prominent girdle in the aerial pitchers of *N.r. elongata* might suit woolly bats by allowing them to wedge themselves into the pitcher. We have often found it difficult to extract bats from their pitchers because they were so tightly lodged.

We suggest that the low fluid levels, the prominent girdle as well as the narrow, elongate, and cylindrical pitcher form may be adaptive traits, derived or ancestral, that facilitate bat roosting behavior. This study has identified a suit of characters that facilitate bat roosting, some may be derived, others (such as the elongate form) are likely to be ancestral in which case selection is likely to maintain them in the presence of bats.

This study has also documented a significant contribution by bats to the nitrogen needs of pitcher plants. It should be noted that our estimate of a 33.8% contribution to the total foliar nitrogen is conservative because some of the nitrogen sequestered from the pitchers by leaf blades is likely to be transported to younger leaves. For comparison, ants, the most common arthropod trapped in aerial pitchers of *N. r. typica*, provide 68% of total foliar nitrogen (Moran *et al.* 2001) whereas the tropical epiphyte *Dischidia major* derives 29% of its nitrogen from debris deposited by ants (Treseder *et al.* 1995).

Pitcher plants could also benefit from this relationship if bats glean off herbivores from pitchers and associated leaves or reduce herbivore population levels in the surrounding habitat. Such tritrophic cascades have been documented for vertebrate insectivores (Kalka *et al.* 2008; Mooney *et al.* 2010). Further research is needed to assess if pitcher plants benefit in this way from attracting bats.

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PLANT-ANIMAL INTERACTIONS - ORIGINAL RESEARCH

Supply determines demand: influence of partner quality and quantity on the interactions between bats and pitcher plants

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Abstract Interspecific relationships such as mutualism and parasitism are major drivers of biodiversity. Because such interactions often comprise more than two species, ecological studies increasingly focus on complex multi-species systems. However, the spatial heterogeneity of multi-species interactions is often poorly understood. Here, we investigate the unusual interaction of a bat (*Kerivoula hardwickii hardwickii*) and two pitcher plant species (*Nepenthes hemsleyana* and *N. bicalcarata*) whose pitchers serve as roost for bats. *Nepenthes hemsleyana* offers roosts of higher quality, indicated by a more stable microclimate compared to *N. bicalcarata* but occurs at lower abundance and is less common than the latter. Whereas *N. hemsleyana* benefits from the roosting bats by gaining nitrogen from their feces, the bats' interaction with *N. bicalcarata* seems to be commensal or even parasitic. Bats stayed longer in roosts of higher quality provided by *N. hemsleyana* and preferred them to pitchers of *N. bicalcarata* in a disturbance experiment. Moreover, bats roosting only in pitchers of *N. hemsleyana* had a higher body condition and were less infested with parasites compared to bats roosting in pitchers of *N. bicalcarata*. Our study shows how the local

supply of roosts with different qualities affects the behavior and status of their inhabitants and—as a consequence—how the demand of the inhabitants can influence evolutionary adaptations of the roost providing species.

Keywords *Kerivoula hardwickii* · *Nepenthes* · Mutualism · Roost selection · Roost quality

Introduction

Mutualisms are common in nature and represent a key evolutionary adaptation. They may be facultative or obligate and enable certain interacting species to utilize resources or colonize habitats where they could not persist alone (Boucher et al. 1982; Bronstein 1994, 2001; de Mazancourt et al. 2005). However, mutualisms may change to parasitic relationships that result in risks to one or both of the interacting partners (Bronstein 2001; Sachs and Simms 2006). The same species can act as a mutualist, commensalist, or parasite, depending on the partner species (Redman et al. 2001) or on the quality of the resource provided by one partner (Heil et al. 2009). Finally, if the costs increase or the benefits decrease (e.g., because the offered commodity is already available in the environment), species can become autonomous again or switch to another partner that secures a higher benefit (Sachs and Simms 2006).

The situation is even more complex, as species often interact with several partners that differ in quality (Fraser et al. 2001) depending on the abiotic and biotic environment (Gomulkiewicz et al. 2003). In such a situation, mutualistic interactions may follow the biological market model (Noë and Hammerstein 1994). While simultaneous interactions with several high- and low-quality partners can

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lead to higher benefits (Palmer et al. 2010), they also may result in higher costs (Miller 2007). Even though specialization on one high-quality partner appears advantageous in many situations (Frederickson 2005), it is prevented when the availability of high-quality partners is spatially or temporarily limited (Howe 1984; Waser et al. 1996).

As a consequence of the evolutionary and ecological flexibility of interspecific interactions, detailed data on the costs and benefits for the interacting species as well as on the alternatives of each partner are required for a full understanding of mutualistic relationships. Furthermore, spatial variations that occur in multispecies interactions (Moeller 2005) should also be considered. Although many mutualisms involve animals and plants (Janzen 1974; Fiala et al. 1994; Bronstein 2001; Thornham et al. 2012), relationships between vertebrates and plants are exceptional outside the pollen- and seed-dispersal syndromes (Boucher et al. 1982).

Several mutualisms between plants and animals involve plants that provide shelter for animals in exchange for services such as protection from herbivores or in exchange for resources such as energy or nutrients (Janzen 1974; Beattie 1985; Fiala et al. 1994). Optimal shelters are of enormous importance for many animals as they protect them from predators (Manser and Bell 2004) and adverse climatic conditions (Schwarzkopf and Alford 1996). The Bornean woolly bat *Kerivoula hardwickii hardwickii* roosts in aerial pitchers of the carnivorous plants *Nepenthes hemsleyana* and *N. bicalcarata* (Payne et al. 1985; Grafe et al. 2011). *Nepenthes hemsleyana* was previously reported as *N. rafflesiana* var. *elongata* and was recently elevated to species status (*N. baramensis*) by Clarke et al. (2011; this paper also provides details on the nomenclature). However, this name is a junior synonym of *N. hemsleyana* Macfarlane (Macfarlane 1908; M. Scharmann, personal communication). *Nepenthes* spp. grow on nutrient-poor soils and are characterized by a high diversity of nitrogen and phosphate acquisition strategies, which are often reflected in the morphology of their catching organs (Moran et al. 1999; Clarke et al. 2009; Grafe et al. 2011). The pitchers of *N. albomarginata*, for example, specifically trap termites (Merbach et al. 2002) and some *Nepenthes* species capture leaves (Moran et al. 2003) or animal feces (Clarke et al. 2009; Chin et al. 2010; Bauer et al. 2012). On average, *N. hemsleyana* gains an estimated 33.8 % of total foliar nitrogen from the feces of *K. h. hardwickii* (Grafe et al. 2011). However, the costs and benefits to *N. bicalcarata* of bat roosting have not so far been examined.

In this paper, we further evaluate the costs and benefits of *Nepenthes*–*K. h. hardwickii* interactions. Although *K. h. hardwickii* have been radio-tracked previously and observed to roost in pitcher plants (Grafe et al. 2011), these earlier data were collected over a limited spatial scale from

bats previously found roosting in pitchers. By radio-tracking bats captured in harp traps during the night, we aimed to determine whether only some *K. h. hardwickii* individuals specialize on roosting in pitchers while others may choose other roosts, e.g., tree holes. In this study, we investigated the extent to which *K. h. hardwickii* specializes on *Nepenthes* species and evaluated whether this is an obligate or facultative relationship. Moreover, we tested whether there is variation in roost choice as a function of habitat type or population.

We further assessed whether roost quality differs between pitchers of *N. hemsleyana* and *N. bicalcarata*. First, we assumed that the bats used high-quality roosts more intensively whenever they are able to choose between different pitcher plant species, as is the case in other bats using tree cavities and bat boxes as day roosts (Kerth et al. 2001). We used the stability of microclimate inside the roosts as a second criterion for roost quality (cf. Dechmann et al. 2004). As a third criterion, we used the absence of parasites that develop in roosts, e.g., bat flies (Reckardt and Kerth 2007). In contrast, bats roosting in high- and low-quality roosts should not differ in the infestation of parasites that spend their whole life cycle directly on the bat, e.g., wing mites (Reckardt and Kerth 2009). As a fourth criterion, we used body condition which is often indicated by ectoparasite infestation (e.g., Lewis 1996; Lourenço and Palmeirim 2007). We assumed that bats with better body condition are those that roost in high-quality roosts in which fewer parasites develop. However, parasite infestation can also depend on other variables than the roost, e.g., animal abundance (Brown and Brown 1986).

It is known that under time pressure some animals alter their shelter choice, accepting shelters of inferior quality (Franks et al. 2003). Therefore, we conducted a disturbance experiment to test the stability of the roosting behavior of *K. h. hardwickii*. Our goal was to determine whether time constraints during roost selection alter preferences for certain *Nepenthes* species compared to undisturbed roost choices.

Materials and methods

Site descriptions

We chose eight study areas within different forest types in the Belait district of Brunei Darussalam, Borneo (we called those study sites Andulau, Badas, Bukit Teraja, Labi 17 front, Labi 17 back, Labi 31, Saw Mill, and Wasai Teraja). Andulau, Bukit Teraja and Wasai Teraja were within lowland mixed-dipterocarp rainforest that contained few or no pitcher plants. The other study sites were within peat swamps and heath forests (so-called *kerangas*) with up to

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six species of pitcher plants. Six of the eight study sites were relatively undisturbed by human impact (Ashton et al. 2003; Anonymous 2008). Only Saw Mill experienced moderate logging, while Labi 17 front was a degraded heath forest with sparse vegetation as it had burned repeatedly over the past several years (Davies and Becker 1996; Ashton et al. 2003).

Procedural methods

During two field seasons (14 June to 30 July 2009; see Grafe et al. 2011 and 14 August 2011 to 14 January 2012) we set up one to five four-bank harp traps per night in each study site to catch *K. h. hardwickii*. The harp traps were set up one to four times per week. The distance between the single traps was 30–50 m and we distributed them uniformly over each study site. About 2 h before sunset, the harp traps were placed across on established forest trails and they remained there until the following morning. We placed traps at strategic locations perpendicular to the trail to ensure that bats could not pass on either side. At least every second day, all traps were moved to a new location. Furthermore, during the day, we also searched for bats roosting inside *N. hemsleyana* or *N. bicalcarata* pitchers. To monitor and count these *Nepenthes* plants, we used transects ranging from 10 to 50 m in 20-m intervals along trails through the study sites. All pitchers at a height below 2.5 m were monitored daily. Due to reports of unspecified small bats roosting in the pitcher plant species *N. ampullaria* (Arthur and Lemaire DL 2009; A.-M. Seibert, University of Tübingen, personal communication), we also monitored this pitcher plant species wherever it occurred in our study sites (Badas, Labi 17 back, Labi 31, Saw Mill). If we found a bat in a given site, we continued to stay in the same site, if not, we switched to a different study site after 5–14 days (Electronic Supplementary Material: ESM1).

We determined the sex, age (adult or juvenile), mass (using a portable balance; Voltcraft PS-250 \times 0.05 g, Germany), and forearm length of all captured *K. h. hardwickii*. Moreover, we measured the bats' circumference by wrapping a flexible measuring tape around their bodies. We collected all visible ectoparasites and determined them to their taxonomic order or family using the key of Whitaker et al. (2009). For individual recognition, we marked all adult individuals with transponders (ISO 11784/11785; Paddy-Mark, UK; see Kerth et al. 2006). Transmitters (mass: 0.3 g; battery life: 21 days; frequency: 150.069–150.854 MHz; pulse frequency: 0.6–0.7 p/s; Holohil Systems, Canada) were fixed with skin bond adhesive (Manfred Sauer, Germany) on the dorsal fur of 29 bats. By using radio-telemetry (TRX-1000S Wildlife Materials receiver, fitted with a Yagi three-element antenna; Carbondale, IL, USA), we monitored the bats and

compared the roosting behavior of individuals caught in harp traps (14 bats) to that of bats captured in *Nepenthes* pitchers (15 bats). Data of ten of the latter ones have already been partly published in Grafe et al. (2011). In all study sites, we radio-tracked both bats caught in harp traps and those found in pitcher plants (except in Andulau where bats were only caught in harp traps).

We radio-tracked 1–4 (mean \pm SD: 2.60 ± 1.02) bats caught in harp traps in each study area. For each accessible (<2.5 m height above ground) roost pitcher, we measured morphological features (ESM2). We monitored temperature and humidity in each study area except in Labi 31. To monitor temperature and humidity, we placed 2–8 (5.50 ± 2.32) iButtons (DS1921G Thermochron iButton and DS1923 temperature/humidity logger iButton 712; Maxim, USA) within the *N. hemsleyana* and *N. bicalcarata* pitchers and on the stem of the plant (within a distance of 30 cm to the focal pitcher). Paired sets of iButtons were installed: one logger measured the temperature (T_P) and humidity (H_P) inside the pitcher and the other the ambient temperature (T_A) and humidity (H_A) near the pitcher. The iButtons inside the pitchers were wrapped in a fine net that was fixed on the pitchers' peristome and hung in that part of the pitcher that would have been used by the bats. The iButtons outside the pitchers were also fixed on the *Nepenthes* plant. In total, 22 iButton sets (8 in *N. hemsleyana*, 14 in *N. bicalcarata*) recorded the temperature and 8 sets (4 in *N. hemsleyana*, 4 in *N. bicalcarata*) measured the humidity every 10 min for 7 days (iButton Viewer 3.22). In two pitchers, T_P and H_P as well as T_A and H_A were measured for 6 days. Measurements started at 1900 hours on day 1 and ended at the same time on day 7. In areas where we could not find *K. h. hardwickii*, only T_P was measured.

Experimental design

By radio-tracking the 29 bats for 2–23 (9.38 ± 4.85) successive days we examined whether individuals specialize on a particular *Nepenthes* species or if some bats roost in both species. To analyze the bats' preference for the two *Nepenthes* species, we observed their pitcher choice both under natural conditions and after forcing them to switch their roost. For this purpose we disturbed the bats by touching them inside the pitchers with one finger. We only disturbed bats without juveniles that were roosting in pitchers with a height <1.8 m and no dense vegetation around them. This disturbance experiment was conducted in all study sites in which the bats occurred (except in Andulau where only one pitcher plant species suitable for bat roosting occurred; ESM3). We then followed the bats to their new roost by tracking them. Our aim was to determine if the disturbed bats still chose pitchers as roosts and if they

preferred one of the pitcher plant species in a situation of induced roost switching.

Data analysis

If data were normally distributed, Welch two-sample t tests were applied, if not we used Mann–Whitney U tests (two data groups) and Kruskal–Wallis tests (>two data groups). We tested if the number of days that bats use a pitcher in succession depends on the pitcher plant species (Mann–Whitney U test) or on the study site (Kruskal–Wallis test). For these tests, only pitchers were used in which bats were not disturbed by us. With a Chi-squared test, we compared the number of available pitchers (*N. hemsleyana* and *N. bicalcarata*) with those that had been used as roosts. For the analysis of the bats' preference for a pitcher plant species, we determined how often each bat used *N. hemsleyana* pitchers (%) and compared these values to the percentage of *N. hemsleyana* pitchers in the study area using Wilcoxon matched-pairs signed-ranks tests. Since the bats switched to roosts that were up to 759 m from their last roost (77.23 ± 89.49 m), we assumed that all *N. hemsleyana* and *N. bicalcarata* pitchers in the study site were potential roosts for *K. h. hardwickii*. We also tested if the bats' preference changed when bats were forced to switch a roost. We counted how often bats use *N. hemsleyana* after roosting in *N. hemsleyana* or *N. bicalcarata* pitchers and vice versa, and applied a Fisher's exact test. Since in all the study sites the numbers of pitchers of the two species were not equal, we conducted a further Fisher's exact test taking into account the relative abundance of each pitcher type. Each bat's pitcher choice was divided by the relative abundance of the chosen pitcher type using the following formula:

$$\text{corrected pitcher choice} = \frac{1}{\frac{n(\text{pitchers of chosen species in study site } x)}{n(\text{pitchers of both species in study site } x)}}$$

The corrected pitcher choice of each bat was assigned to one of four categories (stay in *N. hemsleyana*, change from *N. hemsleyana* to *N. bicalcarata*, stay in *N. bicalcarata*, change from *N. bicalcarata* to *N. hemsleyana*).

For the temperature and humidity analysis, we followed the approach of Dechmann et al. (2004). We calculated mean temperature and mean humidity of each full hour inside and outside the pitchers. Moreover, we averaged temperatures and air humidities for each 7-day period. We used Wilcoxon matched-pairs signed-ranks tests to examine whether the median of the mean T_P differs from the median of the mean T_A of the same iButton sets and whether hourly T_P and T_A differences differ between *N. bicalcarata* and *N. hemsleyana* pitchers. Furthermore, we subtracted the lowest hourly mean temperature and

humidity from the highest one of each iButton to receive the average daily fluctuations. Temperature fluctuations measured inside and outside were compared with Wilcoxon matched-pairs signed-ranks tests. To compare maximum T_P of pitchers in Labi 17 front with those of *N. hemsleyana* and *N. bicalcarata* pitchers in the other study sites, Welch two-sample t tests were applied. Humidity differences and fluctuation were not tested statistically because of the low number of replicates.

As an index for body condition, we used the residuals of a regression between forearm length and body mass. Welch two-sample t tests were applied to detect differences between the residuals of bats that roosted exclusively in *N. hemsleyana* pitchers and those that used *N. bicalcarata* pitchers at least once. To determine the preferred pitcher plant species, we only used data collected on bats that had been observed for 5 days or more. We assumed that after a 5-day period a robust preference of the bats' pitcher choice can be estimated. A Mann–Whitney U test was used to determine if there are differences in the parasite infestation of bats that use different pitcher plant species. All tests were conducted with R (v.2.13.1; R Foundation for Statistical Computing, Vienna, Austria).

Results

Roosting behavior of *K. h. hardwickii*

We caught a total of 69 *K. h. hardwickii* (18 adult males, 40 adult females, 5 male juveniles, 6 female juveniles). Twenty-four bats were captured in harp traps and 45 were first located and captured in pitchers. Of these 69 bats, 60 were re-located later in their day roosts, 29 by using radio-tracking, and 31 during the pitcher controls. All were exclusively roosting in pitchers of *N. hemsleyana* (including the hybrid with *N. rafflesiana*) or *N. bicalcarata*, regardless whether the bats had been originally captured in harp traps or pitchers. We never found them roosting in any of the 396 monitored *N. ampullaria* pitchers (116 plants).

Except for the lowland rainforest sites, Bukit Teraja and Wasai Teraja, we found pitchers of *N. hemsleyana* (including the hybrid with *N. rafflesiana*) and/or *N. bicalcarata* in all our study areas. However, the study sites differed in the presence of these *Nepenthes* species. In Labi 17 front, we only found *N. hemsleyana*. In Andulau, only hybrids of *N. hemsleyana* \times *N. rafflesiana* occurred, whereas in the remaining sites *N. hemsleyana* and *N. bicalcarata* co-occurred. We could not observe *K. h. hardwickii* in the two areas where neither of the two pitcher plants were found (Bukit Teraja and Wasai Teraja), but did so in all sites where the pitchers were common, except in Labi 17 front (ESM3). Bats in Labi 31 only used *N. hemsleyana*

pitchers. In Badas, pitchers of *N. bicalcarata* were exclusively occupied.

In sum, pitcher plant usage in all sites throughout the study period, bats roosted in 8.40 % of the 1,178 monitored *N. bicalcarata* pitchers whereas 20.83 % of the 600 monitored *N. hemsleyana* pitchers were used (Chi-squared test, $\chi^2 = 40.95$, $df = 1$, $P < 0.0001$). The pitcher choice varied considerably between sites depending on the availability of the two pitcher plant species. For example, in the heath forest site Labi 17 back, *K. h. hardwickii* roosted in 43.75 % of the 32 observed *N. hemsleyana* pitchers and 19.34 % of the 212 *N. bicalcarata* pitchers. In contrast, the bats in Labi 31, a peat swamp forest, used 22.48 % of the 418 monitored *N. hemsleyana* pitchers but none of the 60 *N. bicalcarata* pitchers. While occupied *N. hemsleyana* pitchers were always alive and intact, the bats mostly used dead *N. bicalcarata* pitchers that had lost all their digestive fluid. Only 5 of the 99 occupied *N. bicalcarata* pitchers were alive, but all 5 pitchers had small holes at the pitchers' base through which digestive fluid drained. Among different study sites, daily rates of occupancy of available pitchers by *K. h. hardwickii* were 0.51 ± 0.38 % for *N. hemsleyana* and 0.23 ± 0.17 % for *N. bicalcarata* pitchers. In Andulau, 0.77 % of the *N. hemsleyana* hybrid roosts were used as day roosts.

Undisturbed bats used *N. hemsleyana* pitchers ($n = 48$) on a mean of 1.77 ± 1.72 successive days, *N. bicalcarata* pitchers ($n = 91$) on 1.17 ± 0.52 successive days, and hybrid pitchers ($n = 5$) on 1.50 ± 0.77 successive days. They stayed significantly longer in *N. hemsleyana* pitchers (hybrids included) than in pitchers of *N. bicalcarata* (Mann–Whitney *U* test, $W = 3004.00$, $P < 0.0001$). The duration the pitchers were used by the bats was not dependent on the study area (Kruskal–Wallis test, $\chi^2 = 4.84$, $df = 4$, $P = 0.30$).

Choice of new pitchers

In total, we observed 161 movements from one pitcher to another in the 28 radio-tracked bats (5.75 ± 3.89 movements per bat on average). These observations did not involve movements of the bats caused by the disturbance experiment. In 89 cases, the bats chose one of the monitored 1,178 *N. bicalcarata* pitchers and in 72 cases one of 566 *N. hemsleyana* pitchers (ESM3). Although the average proportion of *N. hemsleyana* pitchers available for all individuals ($n = 28$) across all study sites with both pitcher plant species was 39.22 ± 35.83 %, bats preferred to roost in *N. hemsleyana* pitchers (in 49.33 ± 44.19 % of all cases). For each of the 28 bats, we determined whether they used a higher percentage of *N. hemsleyana* pitchers than expected given the local availability of these pitchers (Wilcoxon matched-pairs signed-ranks tests, $V = 282.00$,

$P = 0.02$). In 136 of 161 natural roost switches (84.47 %), the bats chose a pitcher of the same species they had roosted in before. They moved only 25 (15.53 %) times to a pitcher of the other pitcher plant species. In the disturbance experiment, all bats ($n = 27$) left the pitcher in which they had been disturbed and moved to another pitcher plant. In 23 (85.19 %) cases, the same pitcher plant species was chosen, whereas in 4 (14.81 %) cases, the bats moved to the other species. The undisturbed bats did not differ in their roost-switching behavior from the bats that were disturbed (Fisher's exact test, $P = 1.00$).

Even after disturbance, the bats could not be found in roosts other than pitcher plants. Bats were faithful to either *N. hemsleyana* or *N. bicalcarata* pitchers and chose the same pitcher plant species in the majority of cases (Fisher's exact test, $P = 1.00$; Table 1). However, after correcting each bat's movement for the relative abundance of pitchers, we found that the bats showed higher fidelity to pitchers of *N. hemsleyana* than to those of *N. bicalcarata* in each study area (Fisher's exact test, $P = 0.003$; Table 1).

Environmental conditions in pitchers of different *Nepenthes* species

In total, *N. hemsleyana* pitchers had a mean T_P of 24.91 ± 0.44 °C while the mean T_A was 25.86 ± 1.09 °C. In *N. bicalcarata* pitchers, the mean T_P was 25.50 ± 0.29 °C while the corresponding mean T_A was 26.15 ± 1.14 °C. Comparing the median of the mean T_P of each iButton set with that of the mean T_A , only the T_P in *N. hemsleyana* pitchers were significantly lower (Wilcoxon matched-pairs signed-ranks tests, $V = 0$, $P = 0.008$ for *N. hemsleyana*; $V = 39.50$, $P = 0.43$ for *N. bicalcarata*). With regard to the hourly temperature regime (Fig. 1), the mean T_P/T_A difference was 0.94 ± 0.11 °C in *N. hemsleyana* and 0.95 ± 0.04 °C in *N. bicalcarata* (Wilcoxon matched-pairs signed-ranks tests, $V = 129.00$, $P = 0.56$). However, analyzing the hottest hours of the day (1100–1900) separately, T_P/T_A differences of *N. hemsleyana* (1.07 ± 0.05 °C) exceeded those of *N. bicalcarata* (0.98 ± 0.03 °C) (Wilcoxon matched-pairs signed-ranks tests, $V = 36.00$, $P = 0.01$). T_P/T_A differences in the remaining hours were higher in *N. bicalcarata* (0.92 ± 0.03 °C) than in *N. hemsleyana* (0.86 ± 0.05 °C) (Wilcoxon matched-pairs signed-ranks tests, $V = 3.00$, $P = 0.0006$). This indicates that, compared to *N. bicalcarata*, pitchers of *N. hemsleyana* are cooler during midday and warmer when T_A decreases. Daily temperature fluctuations were slightly lower inside *N. hemsleyana* pitchers than outside (4.48 ± 0.63 °C and 4.79 ± 0.71 °C, respectively). This was not the case in *N. bicalcarata* pitchers (inside: 4.14 ± 0.97 °C; outside: 4.20 ± 1.17 °C) (Wilcoxon matched-pairs signed-ranks tests, $V = 4.00$,

Table 1 *Kerivoula hardwickii hardwickii*'s choice of new roosting pitchers with reference to the preceding roost

Preceding roost	New roost: absolute frequencies		New roost: standardized frequencies	
	<i>N. hemsleyana</i>	<i>N. bicalcarata</i>	<i>N. hemsleyana</i>	<i>N. bicalcarata</i>
<i>N. hemsleyana</i>	17	3	32.40	3.69
<i>N. bicalcarata</i>	1	6	7.63	6.82

Numbers indicate the frequencies the bats chose the same pitcher plant species or changed to the other one. Each bat's pitcher choice was divided by the relative abundance of the chosen species' pitchers at each study site to obtain standardized frequencies

$P = 0.05$ for *N. hemsleyana*; $V = 45.00$, $P = 0.67$ for *N. bicalcarata*). The mean T_P in four *N. hemsleyana* pitchers in Labi 17 front (26.04 ± 0.22 °C) were higher than the mean T_P inside *N. hemsleyana* (Mann–Whitney U test, $W = 32$, $P = 0.004$) and *N. bicalcarata* (Mann–Whitney U test, $W = 53.50$, $P = 0.008$) pitchers in the other study areas. The mean maximum T_P of *N. hemsleyana* pitchers in Labi 17 front were 36.38 ± 0.89 °C whereas those in both *N. hemsleyana* (30.42 ± 1.35 °C) and *N. bicalcarata* pitchers (29.60 ± 1.30 °C) in the other study sites were lower (Welch two-sample t test, $t = 4.00$, $P < 0.0001$ for *N. hemsleyana*; $t = 45.00$, $P < 0.0001$ for *N. bicalcarata*). A qualitative analyses based on only 4 individual pitchers measured for each pitcher type suggests that the average H_P of *N. hemsleyana* was 96.79 ± 0.80 RH %, while the respective H_A was 94.03 ± 2.87 RH %. H_P of *N. bicalcarata* was 94.02 ± 2.50 RH % but H_A was 95.19 ± 1.91 RH % on average. Comparing the hourly differences during the course of the day, H_P was 2.76 ± 2.06 RH % higher in *N. hemsleyana* pitchers and 1.17 ± 0.32 RH % lower in *N. bicalcarata* pitchers compared to the corresponding H_A (Fig. 1). While daily H_P fluctuations inside *N. bicalcarata* pitchers (4.65 ± 4.13 RH %) follow those of H_A (4.83 ± 2.49 RH %), H_P is more stable inside *N. hemsleyana* pitchers (1.38 ± 0.46 RH %) than H_A (7.60 ± 6.54 RH %).

Parasite load of bats using one or both of the *Nepenthes* species

The 38 bats that had been observed more than 5 days were infested with 2 Nycteribiidae, 35 Macronyssidae and 18 Spinturnicidae individuals. The 14 individual bats that had used only pitchers of *N. hemsleyana* during the observation period had significantly fewer parasites compared to the 24 bats that had roosted for at least 1 day in *N. bicalcarata* during the observation period (Mann–Whitney U test, $W = 111.00$, $P = 0.05$). In particular, the bats that only used *N. hemsleyana* pitchers had no parasites that depend on roosts for reproduction (Macronyssidae and Nycteribiidae), whereas such parasites were found on bats also using *N. bicalcarata* pitchers (Mann–Whitney U test, $W = 119.00$, $P = 0.03$). As expected, we found no

significant difference in the number of Spinturnicidae, which spend their whole lives on bats, between the two classes of bats (Mann–Whitney U test, $W = 161.00$, $P = 0.79$; Fig. 2).

Body size and mass of bats using one or both of the *Nepenthes* species

Thirty-one of the 38 bats that had been observed for more than 5 days were adults (22 females, 9 males). Eight of these females and 5 of these males only used *N. hemsleyana* pitchers as roosts. The mean of the residuals of a regression between forearm length and body mass was positive in bats that exclusively roosted in *N. hemsleyana* pitchers (0.18 ± 0.13 for males; 0.29 ± 0.49 for females) while that of those bats that used *N. bicalcarata* as roost (-0.23 ± 0.18 for males; -0.17 ± 0.18 for females) was negative (Welch two-sample t test, $t = 3.35$, $P = 0.02$ for males; $t = 2.40$, $P = 0.04$ for females; Fig. 3). The bats' mass (Kruskal–Wallis test, $\chi^2 = 2.74$, $df = 3$, $P = 0.43$) and forearm length (Kruskal–Wallis test, $\chi^2 = 1.54$, $df = 3$, $P = 0.67$) did not differ between the study sites.

Discussion

Our study provides several lines of evidence that *K. h. hardwickii* strongly depend on *N. hemsleyana* and *N. bicalcarata* pitchers as day roosts with a preference for pitchers of *N. hemsleyana*. Bats were only found in areas with *N. bicalcarata* and *N. hemsleyana* pitchers and the bats captured in harp traps and pitchers always roosted in these pitcher types even after being disturbed. Thus, it is likely that the absence of *K. h. hardwickii* in the lowland mixed-dipterocarp rainforest sites, Bukit Teraja and Wasai Teraja, is related to the absence of pitcher plants. Indeed, monitoring bat diversity in Brunei, Struebig et al. (2012) found only a single *K. h. hardwickii* in both the lowland rainforest of the Ulu Temburong National Park and in Wasai Teraja, while the bats were more common in the sites Andulau and Tasek Merimbun, where pitcher plants are abundant. It has been shown for other bat species that absence of roosts can limit the geographical range of a

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Fig. 1 Inside (T_P) and outside (T_A) hourly mean temperatures in **a** *N. hemsleyana* pitchers ($n = 8$) and **b** *N. bicalcarata* pitchers ($n = 14$) as well as inside (H_P) and outside (H_A) hourly mean air humidities in **c** *N. hemsleyana* pitchers ($n = 4$) and **d** *N. bicalcarata* pitchers ($n = 4$). Temperature and humidity was measured over 7 days. Error bars \pm SD

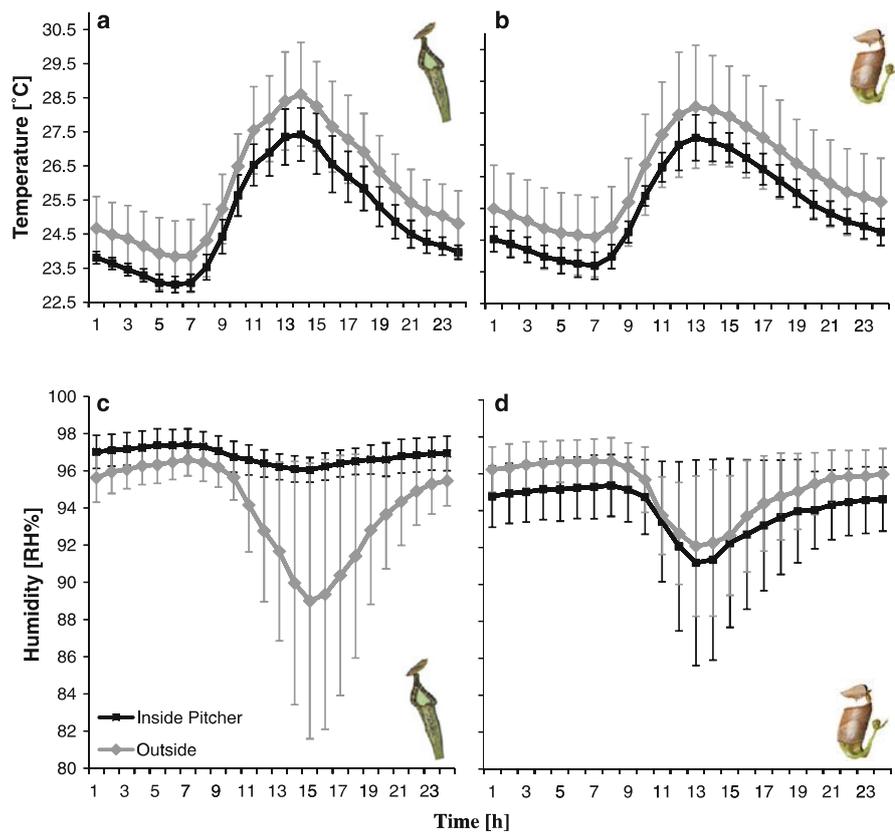


Fig. 2 Number of ectoparasites on *K. h. hardwickii* that only used pitchers of *N. hemsleyana* (left) and those that at least partly roosted in pitchers of *N. bicalcarata* (right)

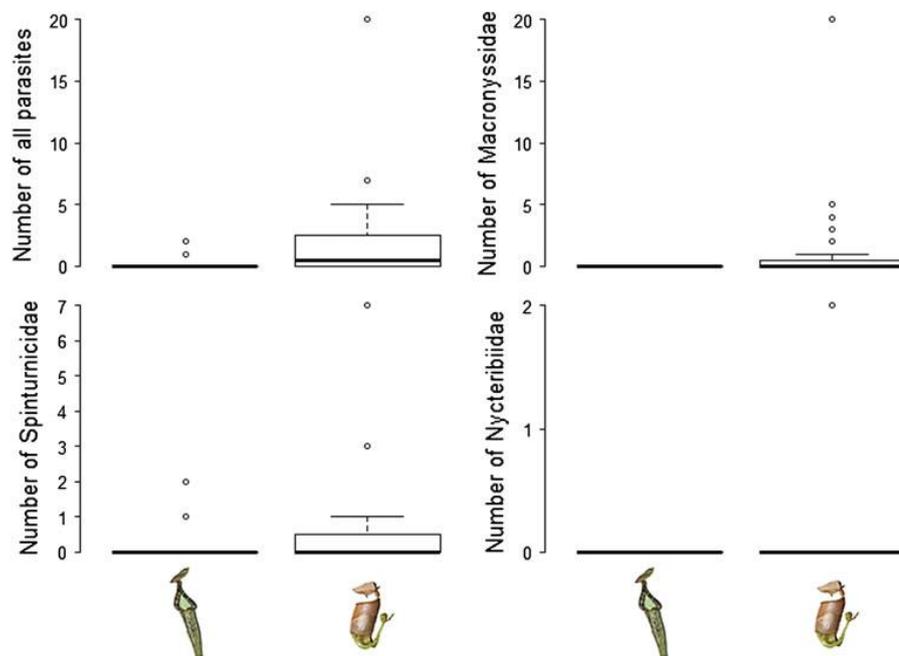
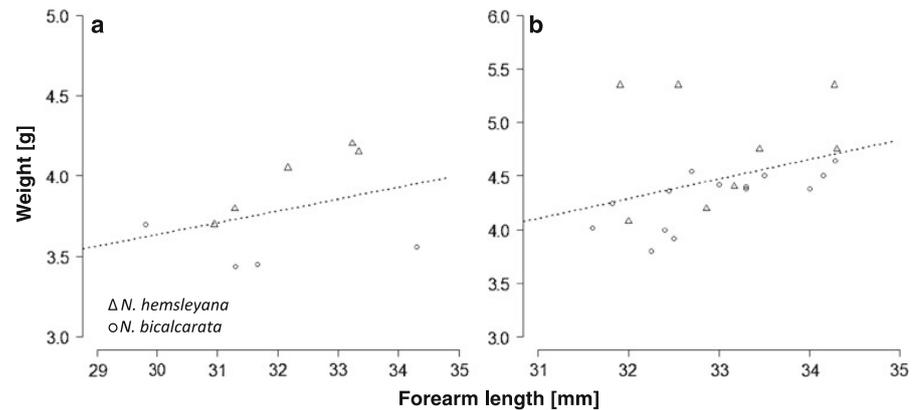


Fig. 3 Regression between forearm length and body mass of **a** *K. h. hardwickii* males and **b** females that only used pitchers of *N. hemsleyana* and those that at least partly roosted in pitchers of *N. bicalcarata*



species (Jenkins et al. 1998; Sedgeley and O'Donnell 1999).

While the relationship between *N. hemsleyana* and the bats is most likely mutualistic, with pitchers providing safe roosts in return for nitrogen from bat feces (Grafe et al. 2011), our study shows that this is not the case for the interactions with *N. bicalcarata*. The vast majority (94.94 %) of the occupied *N. bicalcarata* pitchers were dead and the remaining ones had holes and lacked the digestive fluid with which the pitcher plants retain (Gaume et al. 2007) and digest their prey (Schulze et al. 1999). Thus, *N. bicalcarata* is very unlikely to sequester any nitrogen from bat feces or urine. Further studies will have to clarify whether the relationship is commensal (the bats use already dead or damaged pitchers) or parasitic (the bats actively damage the pitchers by nibbling holes in the pitcher base). Roost modification can occasionally be found in bats and has already been demonstrated in a variety of species (Kunz and MacCracken 1996; Dechmann et al. 2004), including the genus *Kerivoula* (Schulz 2000).

In one of our study sites (Labi 17 front), we found *N. hemsleyana* but *K. h. hardwickii* was not present. Even though reduced fitness is often the consequence when mutualistic partners are not available (Fiala et al. 1994; Stachowicz and Hay 1996), the lack of a mutualistic partner can sometimes be compensated for when abiotic factors can be gained autonomously (Sachs and Simms 2006). Labi 17 front differed from the other study sites as it had been burned several times in previous years and therefore the soil may have contained increased levels of nitrogen and phosphate available for the plants (Giardina et al. 2000). This, in addition to the arthropod-caching strategy as a nitrogen source, could be the reason why *N. hemsleyana* remained common in the area despite the absence of the bats. The significantly higher maximum T_p inside pitchers at this site, that may exceed the thermal neutral zone of the bats, as well as the sparse forest vegetation that may interfere with foraging, may be the reason

why *K. h. hardwickii* was not found in this area. For example, in glossophagine and vespertilionid bats, water loss increases with temperatures above the thermal neutral zone (Carpenter and Graham 1967; Studier 1970).

In comparison to *N. bicalcarata*, *N. hemsleyana* seems to provide roosts of higher quality for the bats. Other studies demonstrate that bat species are highly selective of the microclimate within their roosts (Kerth et al. 2001; Sedgeley 2001; Chruszcz and Barclay 2002). T_p inside *N. hemsleyana* pitchers were significantly lower than T_A and daily temperature fluctuations inside these pitchers were also lower than outside. Bats typically choose roosts with stable temperatures, especially when they are lactating (Sedgeley 2001; Chruszcz and Barclay 2002; Lausen and Barclay 2003). Although the T_p of both *Nepenthes* species fluctuated during the course of the day, *N. hemsleyana* functioned as a more effective buffer for high temperatures around noon than *N. bicalcarata*. Higher temperatures inside their roosts during the day may provide benefits for some species (Dechmann et al. 2004). However, cooler temperatures inside the roosts can help to avoid heat stress (Vonhof and Barclay 1997). Moreover, moderately lower T_A could constrain energy loss if the bats make use of torpor during the daytime as can be seen in several tropical bat species (Geiser and Stawski 2011). In contrast to bats caught in *N. bicalcarata* pitchers, several individuals that we removed from *N. hemsleyana* pitchers seemed to be in shallow torpor as they did not react immediately when we pulled them out of the pitchers.

Bat roosts are often characterized by high humidity (Baudunette et al. 1994). Our humidity data are based on a small sample size, nevertheless they strongly suggest that, compared to the unstable and lower H_p of *N. bicalcarata* pitchers, H_p in *N. hemsleyana* pitchers was higher and more stable than H_A . The high humidity inside *N. hemsleyana* pitchers seems to be the result of the digestive fluid's evaporation and the wind-protected long and narrow interior pitcher volume. In all *N. bicalcarata* pitchers used

by *K. h. hardwickii*, the fluid was missing and the pitcher walls were always dry. Thus, one important advantage of *N. hemsleyana* pitchers may be that H_P is largely independent of daily fluctuations of H_A . During the daytime, active water uptake is almost impossible for bats. Therefore, high humidity inside the roosts is likely to be important to avoid dehydration, although it minimizes evaporative heat loss (Herreid and Schmidt-Nielson 1966; Genoud et al. 1990). In addition, a high metabolic rate, common in small mammals, in combination with a high H_A , contributes to a high body temperature (Genoud et al. 1990). Thus, the observed low T_P as provided by *N. hemsleyana* may be of high importance for maintaining body temperatures in the thermo-neutral range.

Bats that only used *N. hemsleyana* pitchers were free of parasites that partly develop in bat roosts. While parasites belonging to the family Macronyssidae lay their eggs in the bats' roost (Radovsky 1967), Spinturnicidae spend their whole life cycle on the wing and tail membranes of their host (Reckardt and Kerth 2009). The inner surface of some *Nepenthes* pitchers is covered with wax crystals that are slippery for insects (Riedel et al. 2003). This waxy zone is particularly distinctive and well developed in *N. hemsleyana* (Gaume and Di Giusto 2009; Bauer et al. 2011). We assume that eggs of Macronyssidae cannot stick to these walls and so the propagation of these parasites is interrupted. However, this waxy zone is missing in *N. bicalcarata* (Bohn 2004). We found only two Nycteribiidae on bats, but interestingly both bats were known to roost at least partially in *N. bicalcarata* pitchers. Females of these parasites place their larvae in the bats' roost where they immediately develop into pupae (Reckardt and Kerth 2007).

A consequence of roosting in high quality roosts could be the better body condition of bats that had roosted exclusively in *N. hemsleyana* compared to those bats we found roosting at least partially in *N. bicalcarata*. A causal link between the lower mass of the latter individuals and their higher parasite load seems possible. However, while some studies have shown such a negative correlation under certain conditions (Lewis 1996; Lourenço and Palmeirim 2007), others indicate that parasite load and body condition can be independent or show a positive correlation (e.g., Zahn and Rupp 1999; Reckardt and Kerth 2009).

Finally, the higher quality of *N. hemsleyana* pitchers becomes apparent in the roost switching behavior of the bats. Generally, *K. h. hardwickii* are more faithful to *N. hemsleyana* than to *N. bicalcarata* pitchers. Observations on Bechstein's bats (*Myotis bechsteinii*) also confirm that roosts with high quality tend to be used more intensively (Kerth et al. 2001). Our disturbance experiment showed that individual *K. h. hardwickii* preferred one *Nepenthes* species, which they chose more often with or without disturbance. Some studies show that habitat use

can be influenced by natal experience (Klopfer 1963; Davis 2008). This may also be true for bats whose juveniles remain with their mothers for extended periods to learn certain forms of behavior even when they are full-grown (Brigham and Brigham 1989). An alternative explanation for pitcher species fidelity, especially for disturbed bats, was that they searched for new pitchers in the vicinity of their previous roost (own observation). As pitchers of the same species are often aggregated (own observation), the probability of choosing pitchers of the same species is enhanced. However, despite the varying relative abundance of *N. hemsleyana* and *N. bicalcarata* pitchers between sites, the bats preferred *N. hemsleyana* pitchers in most sites.

Our results suggest that *N. hemsleyana* pitchers are higher quality roosts than pitchers of *N. bicalcarata*, but why do *K. h. hardwickii* also use the latter ones? One advantage of using *N. bicalcarata* pitchers is that these plants seem to be more common throughout north-western Borneo than the more sparsely distributed *N. hemsleyana* (Moran 1996; Clarke 2006). By additionally using pitchers of *N. bicalcarata*, *K. h. hardwickii* is able to expand its range. Furthermore, *N. bicalcarata* produces more pitchers per plant than *N. hemsleyana* (ESM3). In addition, *N. hemsleyana* pitchers have shorter life spans and thus are available as roosts for a more limited time period than the pitchers of *N. bicalcarata* (Osunkoya et al. 2008). Therefore, *N. hemsleyana* pitchers should be much harder to locate than the readily available and long-lived *N. bicalcarata* pitchers. By using *N. bicalcarata*, the bats may save energy by reducing the time needed in search of roosts and increasing time for other activities. This could be the case in Badas, where bats never used *N. hemsleyana* pitchers presumably because they only constituted 5.84 % of all pitchers.

There are hints that bats generally compete for limited roosts (Perkins 1996; Schöner et al. 2010). Although *K. h. hardwickii* used only 20.83 % of all *N. hemsleyana* pitchers, competition for optimal roosts could still be occurring, as most of the unoccupied pitchers were probably not optimal roosts due to other costs for the bats (e.g., through predation or dehydration). These pitchers were either too close to the ground (19.83 % of unused pitchers were at a height <1.2 m), too exposed to rain and sunlight (23.67 % of unused pitchers were located at positions where the canopy cover was <50.00 %), or unsuitable in size or shape (personal observation). Moreover, in heath forests where the bats predominantly used the more abundant *N. bicalcarata* pitchers, the *N. hemsleyana* occupation rate was even higher (e.g., Labi 17 back: 43.75 %) than in the peat swamp forest where they only used *N. hemsleyana* pitchers (Labi 31: 22.48 %). In areas in which *N. hemsleyana* pitchers are less abundant they should be occupied and defended by highly competitive individuals of *K. h. hardwickii* as these are the roosts of higher quality. The

less competitive bats might switch to less preferred roosts—the pitchers of *N. bicalcarata*. It is not clear whether the better body condition of bats roosting in *N. hemsleyana* is a result of the higher roost quality or if bats with better body condition and therefore higher competitiveness occupy roosts with higher quality.

Our study shows how supply determines demand on the biological market (Noë and Hammerstein 1994): *N. hemsleyana* offers roosts for bats as a commodity. As *N. hemsleyana* gains a valuable resource—nitrogen from bat feces—from providing accommodation to bats, these plants have to compete against roosts that are involuntarily offered by *N. bicalcarata*. Our results suggest that pitchers of *N. bicalcarata* are of lower quality for the bats, but due to their higher quantity should be interesting for them in areas where *N. hemsleyana* is absent or in very limited supply. From an evolutionary perspective, competition with *N. bicalcarata* pitchers could result in selection pressure for *N. hemsleyana* pitchers to adapt according to the bats' requirements—a similar effect which has been documented in plants that specialize for the exclusive attraction of effective pollinators (Schemske and Horvitz 1984). However, there are also cases in which *N. hemsleyana* only provides a low quality service (if it grows at the forest edge or in degraded habitat without forest cover as in Labi 17 front). Thus, high quality partners can become low quality partners and vice versa, so that it is difficult to order interaction partners in multispecies systems hierarchically (Frederickson 2005). In conclusion, we show that biotic factors such as the interactions between the partners or competing species (Federle et al. 1997) and the abiotic environment (Fraser et al. 2001; Gomulkiewicz et al. 2003; Stanton 2003) can influence the quality of mutualistic partners.

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Conflict of interest The authors declare that they have no conflict of interest.

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Electronic Supplemental Material 1

Supply determines demand: Multispecies interactions between bats and pitcher plants

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Study period and harp trap setup per study site.

Study site	Andulau	Badas	Bukit Teraja	Labi 17 front	Labi 17 back	Labi 31	Saw Mill	Wasai Teraja
Duration [d]	19	28	5	14	41	50	29	5
No. of harp traps per study site	44	48	14	8	32	17	22	16
Trapping nights	14	13	4	4	15	6	6	4

Electronic Supplemental Material 2

Supply determines demand: Multispecies interactions between bats and pitcher plants

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Overview of the numbers of captured *K. h. hardwickii*, monitored *Nepenthes* plants and pitchers and numbers of *Nepenthes* used by the bats.

	Andulau	Badas	Bukit Teraja	Labi 17 front	Labi 17 back	Labi 31	Saw Mill	Wasai Teraja
Total no. of bats	3	11	0	0	14	32	9	0
Bats caught in harp traps	3	10	0	0	5	3	3	0
No. of monitored <i>N. hemsleyana</i> plants	11*	30	0	218	14	223	36	0
No. of monitored <i>N. bicalcarata</i> plants	0	70	0	0	38	8	60	0
No. of monitored <i>N. hemsleyana</i> pitchers	34*	34	0	489	32	418	82	0
No. of monitored <i>N. bicalcarata</i> pitchers	0	548	0	0	212	60	358	0
No. of used <i>N. hemsleyana</i> plants	5*	0	0	0	9	91	8	0
No. of used <i>N. bicalcarata</i> plants	0	28	0	0	39	0	25	0
No. of used <i>N. hemsleyana</i> pitchers	5*	0	0	0	14	94	12	0
No. of used <i>N. bicalcarata</i> pitchers	0	32	0	0	41	0	26	0

*Note that the observed *Nepenthes* in Andulau seemed to be hybrids (*N. hemsleyana* × *N. rafflesiana*).

Electronic Supplemental Material 3

Supply determines demand: Multispecies interactions between bats and pitcher plants

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Morphology of the *Nepenthes* species' pitchers

For each accessible (< 2.5 m height above ground) roost pitcher, we measured length, peristome diameter, and inner volume available for the bats. In *N. hemsleyana* and *N. bicalcarata* pitchers, the upper part is relatively cylindrical while the lower part has the shape of a cone. We measured the height and the radius of both, cylinder and cone to calculate the corresponding volumes and added them. In *N. hemsleyana* pitchers the lower part of the pitcher's cone is too narrow for the bats. As the bats' perimeter was 49.63 ± 4.25 mm we subtracted the pitchers' inner volume that is not available for the bats (pitcher perimeter < 49.63 mm) from the cone.



	<i>N. hemsleyana</i>	<i>N. bicalcarata</i>	Mann-Whitney-U test
Number of measured pitchers	98	47	
Length of the pitcher [cm]	19.92 ± 2.96	8.42 ± 1.24	$W = 4605.00, P < 0.0001$
Diameter of the peristome [cm]	4.53 ± 0.85	3.96 ± 0.54	$W = 3215.00, P = 0.0001$
Pitcher volume available for the bats [cm ³]	73.22 ± 38.80	64.52 ± 21.82	$W = 286.00, P = 0.78$

Manuscript 3

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How a pitcher plant facilitates roosting of mutualistic woolly bats

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ABSTRACT

Question: How does the pitcher plant *Nepenthes hemsleyana* facilitate roosting of mutualistic bats?

Hypothesis: Pitchers have adaptations that match the shape and body size of small woolly bats.

Organisms: The pitcher plant *Nepenthes hemsleyana*, its close relative *N. rafflesiana*, and the woolly bat *Kerivoula hardwickii*.

Field sites: Peat swamps and heath forests in western Brunei Darussalam on the island of Borneo.

Methods: We measured various morphological traits of *N. hemsleyana* that might facilitate bat roosting. We compared these traits with those of *N. rafflesiana*, which is not visited by bats. We compared the sizes and characteristics of the pitchers with the body sizes of roosting bats.

Conclusions: As predicted, aerial pitchers matched the body size of bats and had lower digestive fluid levels than pitchers of a close relative. Thus, small morphological differences between closely related species have caused rapid dietary niche divergence.

Keywords: Borneo, carnivorous plants, *Kerivoula hardwickii*, mutualism, *Nepenthes*, roosting behaviour.

INTRODUCTION

Carnivorous plants trap arthropod prey using a variety of independently evolved trapping mechanisms (Darwin, 1875; Juniper *et al.*, 1989; Ellison *et al.*, 2003; Phillipps *et al.*, 2008; Bauer *et al.*, 2011). The pitcher plants of the genus *Nepenthes* (Nepenthaceae) capture and digest arthropod prey in their fluid-filled pitchers. Pitcher shapes and sizes, growth forms, and habitat preferences are

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highly variable within this genus. Over 120 species occur across their palaeotropical range, with the centre of diversity in Borneo (Meimberg and Heubl, 2006; McPherson *et al.*, 2009).

Pitcher-associated phenotypes are highly polymorphic even at the species level. As such, they are assumed to be under diversifying selection and play a crucial role in the genesis of plant diversity (Clarke, 1997; Phillipps *et al.*, 2008). Studies of the ecology of *Nepenthes* have typically focused on pitcher-related characteristics, particularly the structures and mechanisms related to attracting and trapping prey (e.g. Bohn and Federle, 2004; Bauer and Federle, 2009; Gaume and Di Giusto, 2009). These include the nectar glands (Merbach *et al.*, 2001; Bauer *et al.*, 2008), olfactory and visual cues (Moran, 1996; Moran *et al.*, 1999, 2012; Bauer *et al.*, 2011), the slippery peristome surfaces (Bauer *et al.*, 2009, 2015), viscoelastic digestive fluids (Gaume and Forterre, 2007), and waxy inner surfaces (Gaume *et al.*, 2004).

These studies have also revealed that there is more to *Nepenthes*' plant–animal interactions than carnivory. For example, *N. bicalcarata* Hook. f. has developed a mutualistic relationship with the swimming ant *Camponotus schmitzi* Stärke, which enhances trapping efficiency by regularly cleaning the pitcher rim (peristome) (Thornham *et al.*, 2012) and prevents infaunal larvae from leaving the pitchers as adults (Scharmann *et al.*, 2013) in exchange for extrafloral nectar and refuge in the pitcher's tendril (Clarke and Kitching, 1995; Bonhomme *et al.*, 2011a; Bazile *et al.*, 2012). Furthermore, associations between *Nepenthes* and small mammals have been documented. Tree shrews [*Tupaia montana* Thomas (Clarke *et al.*, 2009; Chin *et al.*, 2010; Greenwood *et al.*, 2011)] and nocturnal rats [*Rattus baluensis* Thomas (Wells *et al.*, 2011)] feed on pitcher lid exudates of montane *Nepenthes* species [*N. rajah* Hook. f., *N. lowii* Hook. f., and *N. macrophylla* (Marabini) Jebb and Cheek], whose large pitchers are modified to 'capture' the feces of these small mammals. The focus of this study is another mammal–pitcher plant relationship: *N. hemsleyana* Macfarlane obtains nitrogen from the feces of *Kerivoula hardwickii* Horsfield (Hardwicke's woolly bat) that roosts in its aerial pitchers (Grafe *et al.*, 2011).

Study species

Nepenthes hemsleyana was previously reported as the elongate form of *N. rafflesiana* Jack [*elongata* *nom. nud.* (Cheek and Jebb, 2001; Phillipps *et al.*, 2008)]. In northwest Borneo, the 'elongate' and 'typical' varieties can often be found in close sympatry. The elongate variety was recently elevated to species status by Clarke *et al.* (2011), who gave it the name *N. baramensis*. However, this name is a junior synonym of *N. hemsleyana* Macfarlane (Macfarlane, 1908; Scharmann and Grafe, 2013).

Nepenthes hemsleyana appears to have a relatively narrow distribution in northwestern Borneo, with high densities found in the interior peat swamps and heath forests of Brunei Darussalam (Clarke *et al.*, 2011). In contrast, its close relative *N. rafflesiana* Jack has a broad biogeographical distribution and is relatively common in heath forests with acidic soils. Like most *Nepenthes* species, *N. hemsleyana* and *N. rafflesiana* plants produce lower and upper pitchers over their lifetime (Cheek and Jebb, 2001). The upper or 'aerial' pitchers are conical in shape. In *N. hemsleyana*, the aerial pitchers are divided into an upper waxy zone and a lower secretory zone, whereas *N. rafflesiana* aerial pitchers only have a secretory zone (Gaume and Di Giusto, 2009; Bauer *et al.*, 2011).

Nepenthes rafflesiana catches more prey of higher diversity (Moran, 1996; Bauer *et al.*, 2008, 2009, 2011) and has a higher pitcher fluid viscosity and more human-perceptible fragrances than *N. hemsleyana* (Moran, 1996; Clarke *et al.*, 2011). *Nepenthes hemsleyana* produces longer and

more tapered pitchers that are much less effective as an insect trap. The aerial pitchers of *N. hemsleyana* obtain an average of 33.8% (and up to 56%) of the plant's foliar nitrogen from bat feces and urine, whereas bats avoid the fluid-filled ground pitchers, which have a distinctly different morphology (Grafe *et al.*, 2011). Thus, different pitcher structures seem to facilitate alternative prey-trapping strategies in these *Nepenthes* species (Gaume and Di Giusto, 2009; Bauer *et al.*, 2011). Preliminary genetic data indicate that *N. hemsleyana* and *N. rafflesiana* are direct sister taxa (M. Scharmann, unpublished). Due to their close relatedness, *N. hemsleyana* and *N. rafflesiana* can be used as model taxa to investigate the evolution of different nutrient acquisition strategies.

The woolly bat *K. hardwickii* (Vespertilionidae) is a small gleaning bat that lives and forages in forest interiors in large parts of tropical Asia (Payne *et al.*, 1985). Multiple lines of evidence strongly suggest that numerous genetic lineages, if not species, exist under this name (Douangboubpha *et al.*, 2015). In Brunei, *K. hardwickii* is abundant in forests that contain pitcher plants (Struebig *et al.*, 2012; Schöner *et al.*, 2013), suggesting a link in the geographic distribution of this subspecies (or cryptic species) and *N. hemsleyana*, although the bats do occasionally use alternative roosts [e.g. *N. bicalcarata* (Schöner *et al.*, 2013)]. Previous studies that measured pitcher length and diameter have highlighted obvious allometric differences between *N. hemsleyana* and *N. rafflesiana* (Moran, 1996; Gaume and Di Giusto, 2009; Grafe *et al.*, 2011). However, these measures provide only a partial answer to the suitability of pitchers as roosting sites for woolly bats because there are no data on bat body size and how well they fit into the pitchers that they choose.

This study aims to compare key morphological traits of *N. hemsleyana* and *N. rafflesiana* relevant to the roosting behaviour of *K. hardwickii*. We hypothesized that the aerial pitchers of *N. hemsleyana* are matched in size and shape to the body size of woolly bats and that they have lower digestive fluid levels and thus offer more space for roosting bats than the sympatric, closely related *N. rafflesiana*. In particular, we hypothesized that pitcher orifice diameter, the degree of pitcher tapering, fluid level, and space availability between the two species of pitcher plants would differ significantly. If so, relatively minor morphological modifications of trap characters in *N. hemsleyana*'s aerial pitchers could have a profound effect on its function and allow it to occupy a hitherto unexploited niche.

MATERIALS AND METHODS

Over a period of eight weeks in May and June 2011, we measured 51 *N. hemsleyana* aerial pitchers within a lightly disturbed peat swamp and heath forest mosaic in western Brunei Darussalam at elevations between 20 and 50 m asl: at Badas, on the northern edge of the Badas Forest Reserve (4°4'N, 114°24'E), and Lumut, east of the Lumut pipeline road (4°38'N, 114°25'E). During the same period, we measured 42 aerial pitchers of *N. rafflesiana* at White Sands, a degraded heath forest with white, acidic sands (4°44'N, 114°35'E). Furthermore, between August 2011 and January 2012, we measured *N. hemsleyana* pitchers that had been occupied by bats in three additional sites within the same forest mosaic in western Brunei: Saw Mill (4°33'N, 114°29'E), Labi 31 (4°35'N, 114°30'E), and Labi 17 (4°30'N, 114°27'E). Some of these data have been published previously in Schöner *et al.* (2013). Following Schöner *et al.* (2013), we also captured *K. hardwickii* while pitchers were being monitored using harp traps.

We measured the length and orifice diameter of aerial pitchers of both *Nepenthes* species. Orifice diameter was taken as the average between the broadest point of the pitcher opening

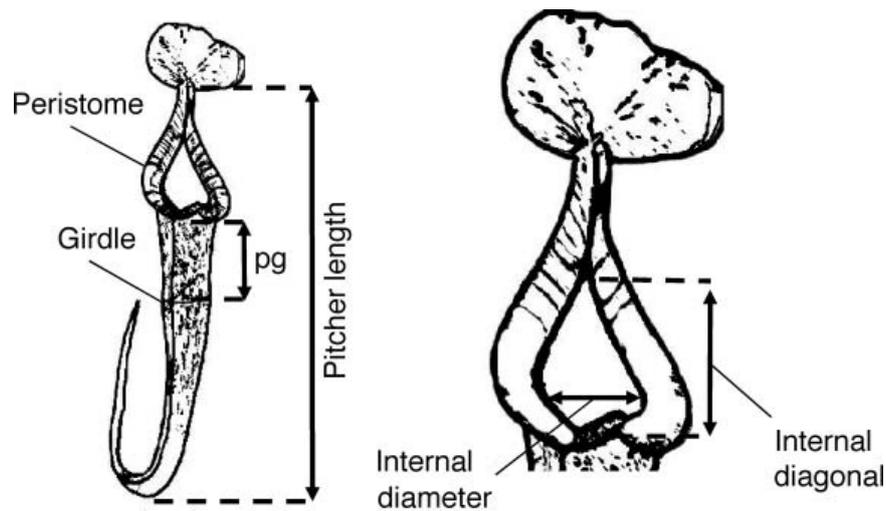


Fig. 1. Measurements undertaken of the aerial pitchers of *N. hemsleyana* and *N. rafflesiana* (pg = distance between peristome and girdle of *N. hemsleyana*; see text for details).

(internal diagonal) and the narrowest point of the pitcher opening (internal diameter) (Fig. 1).

In contrast to *N. rafflesiana*, the pitchers of *N. hemsleyana* have a girdle-like structure or hip that separates the pitcher into an upper, cylindrical section and a lower, more conical and tapered section (Fig. 1) (see Grafe *et al.*, 2011). Thus, to determine space available to bats, we measured the diameter of the pitcher at the girdle (girdle diameter), circumference of the pitcher at the girdle, and peristome–girdle length only for *N. hemsleyana*.

As a cone-shaped or tapered pitcher is likely to contribute to the bats' ability to wedge or stem themselves between the pitcher walls, we measured pitcher diameter at the orifice (DO) and pitcher diameter at the fluid level (DF). We calculated the ratio DF/DO as an index of the amount of taper in the part of the pitcher that is habitable to bats. The lower the value of this index, the higher the degree of tapering (a ratio of 1 = no tapering).

To estimate pitcher volume available to bats, we measured internal diameter, girdle diameter, and the lengths of the tapered and non-tapered zones of the pitchers. We calculated the total habitable space available to bats by assuming that pitchers could be reduced to cylinders and cones.

We measured the bats' body length and shoulder width to evaluate the fit between *K. hardwickii* and their roost. Although females were approximately 8% larger than males, females and males were pooled for the purposes of this study. Body length was measured as the distance between forehead and base of the tail wing membrane using a hand-held calliper ($n = 22$). We measured body width at shoulder height because this is the broadest and least compressible body part. Bats do not cling or hold on to the peristome but wedge themselves head first into the pitcher (Grafe *et al.*, 2011). To determine if bats fit comfortably into the pitcher without slipping into the digestive fluid, we measured the distance between peristome and fluid as well as the pitcher diameter at fluid height. We measured these variables in *N. hemsleyana* pitchers used by bats and in pitchers not known to be used by bats. The same measures were also taken for *N. rafflesiana* pitchers. All analyses were conducted with SPSS v.13 and Bias (v.8.2; epsilon-Verlag GbR 1989–2015). Descriptive statistics are given as means \pm standard deviations.

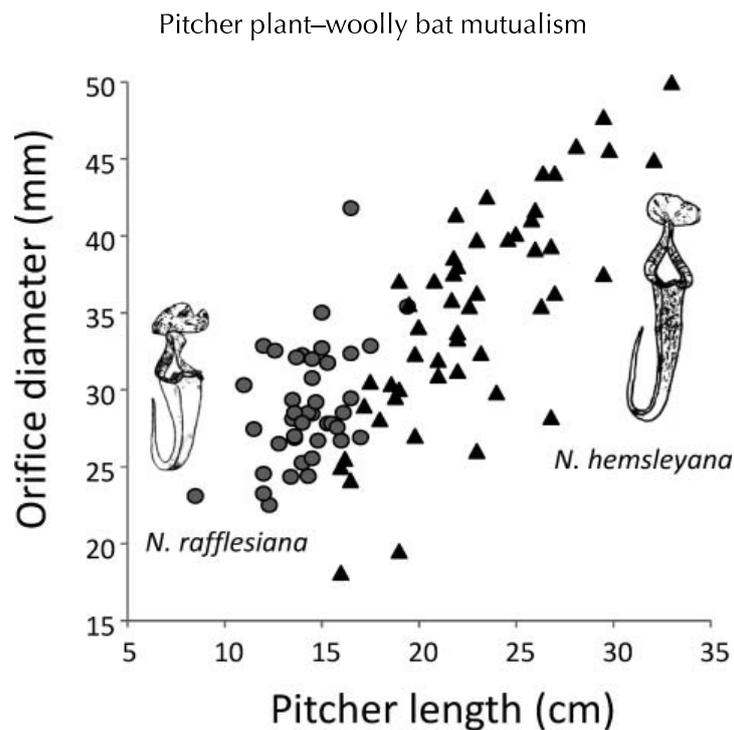


Fig. 2. Relationship between orifice diameter and pitcher length in *N. hemsleyana* (▲) and *N. rafflesiana* (●).

RESULTS

Pitcher length and orifice diameter

The aerial pitchers of *N. hemsleyana* were significantly longer than those of *N. rafflesiana* (Welch *t*-test: $t_{73} = 12.89$, $P < 0.001$; Fig. 2). Likewise, pitcher orifice diameter was larger in *N. hemsleyana* than in *N. rafflesiana* (Welch *t*-test, $t_{75} = 5.35$, $P < 0.001$) with higher variance (*F*-test: $F_{50,41} = 3.42$, $P < 0.001$; Fig. 2).

Tapering

Nepenthes hemsleyana pitchers are highly tapered between the peristome and the pitcher at fluid level (index = 0.37 ± 0.13 , range = 0.06–0.74, $n = 51$). Tapering was significantly lower in *N. rafflesiana* (index = 0.56 ± 0.06 , range = 0.47–0.66, $n = 42$) (Welch *t*-test, $t_{75} = 9.84$, $P < 0.001$).

Fluid volume and space availability

Median fluid volumes were significantly lower in *N. hemsleyana* (4.3 mL, range = 0–20.0 mL) than in *N. rafflesiana* (7.0 mL, range = 4.3–21.8 mL; Mann-Whitney *U*-test: $U = 313.5$, $n_1 = 37$, $n_2 = 37$, $P < 0.001$; Fig. 3). The conical space below the girdle (but above the fluid) in *N. hemsleyana* pitchers contributed considerably to the total habitable space. All *N. hemsleyana* aerial pitchers had girdle diameters above the average width of the bats at shoulder height ($15.8 \text{ mm} \pm 1.4 \text{ mm}$; $n = 22$), suggesting that bats could manoeuvre into a part of the space below the girdle. Together with their respective calculated cylindrical

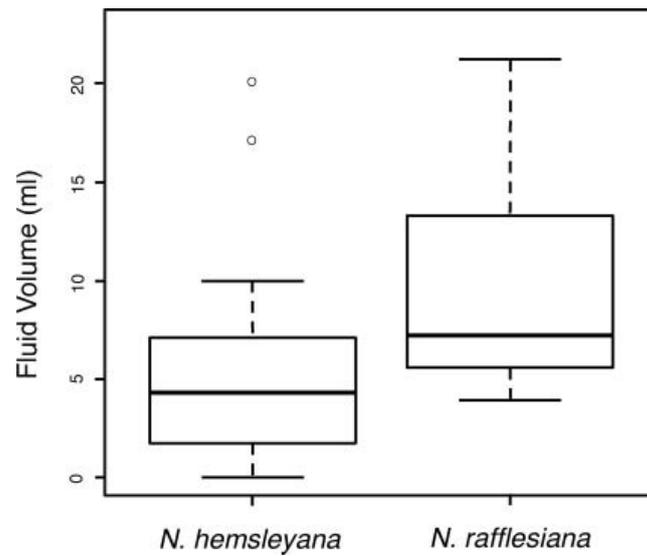


Fig. 3. Fluid volume in *N. hemsleyana* and *N. rafflesiana*. Boxes indicate the 25th and 75th percentiles, the line in the box represents the median, the whiskers are the 10th and 90th percentiles, and the dots show outliers.

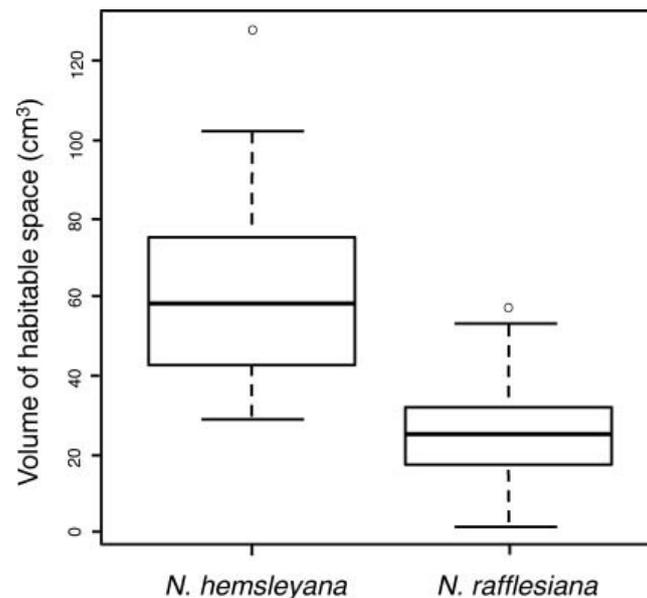


Fig. 4. Volume of habitable space in *N. hemsleyana* ($n = 23$) and *N. rafflesiana* ($n = 37$). See caption to Fig. 3 for further details.

volumes, the total habitable volume of $59.19 \pm 25 \text{ cm}^3$ for *N. hemsleyana* pitchers averaged more than twice the habitable volume in *N. rafflesiana* pitchers ($27.5 \pm 13.25 \text{ cm}^3$; Welch *t*-test, $t_{75} = 6.43$, $P < 0.001$; Fig. 4). The distances between peristome and fluid were also significantly different between the two pitcher-plant species (Mann-Whitney *U*-test: $U = 86.5$, $n_1 = 42$, $n_2 = 72$, $P < 0.001$) with 97.2% of the *N. hemsleyana* pitchers having sufficient space to accommodate a single bat of average body length, compared with only 78.6% of *N. rafflesiana* pitchers (Fig. 5).

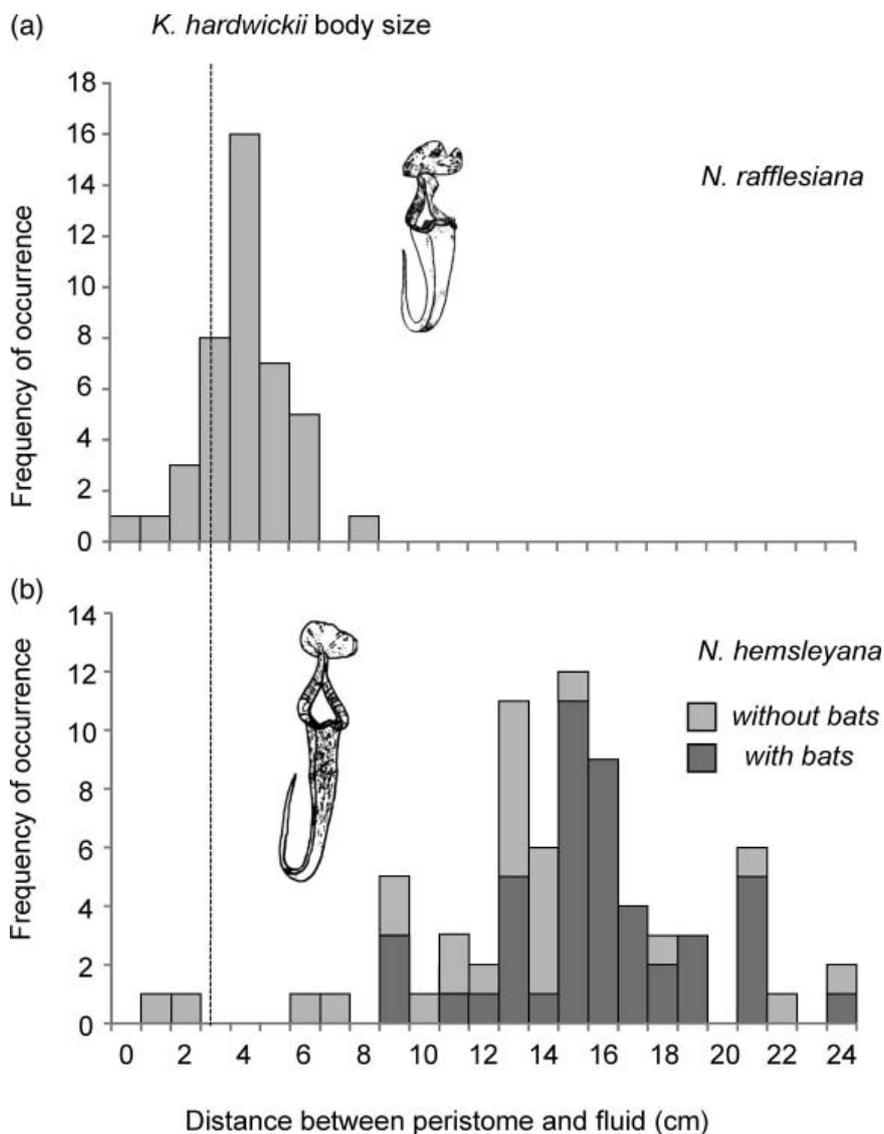


Fig. 5. Size distribution of aerial pitchers of (a) *N. rafflesiana* ($n = 42$) and (b) *N. hemsleyana* ($n = 46$) used as roosts by *K. hardwickii* (dark grey) and not seen to be used by bats ($n = 26$; light grey). Dashed line indicates the average body size of *K. hardwickii*.

Pitcher size relative to body size of *Kerivoula hardwickii*

The average body length of *K. hardwickii* was 32.6 ± 2.3 mm (range = 28.5–36.2 mm; $n = 18$; 14 females, 4 males; Fig. 5). Since *N. hemsleyana* aerial pitchers were strongly tapered below the girdle, the pitcher diameter at fluid level was significantly smaller than the shoulder diameter of *K. hardwickii* (Mann-Whitney U -test: $U = 100$, $n_1 = 22$, $n_2 = 17$, $P = 0.013$) (Fig. 6), allowing bats to wedge themselves between the pitcher walls well above the fluid. None of the pitchers with pitcher diameter above 22 mm at fluid level were used by *K. hardwickii* (Fig. 6). Pitchers known to have been used by bats had significantly smaller pitcher diameters at fluid level than pitchers not known to have been used as roosts (Mann-Whitney U -test: $U = 261$, $n_1 = 17$, $n_2 = 52$, $P = 0.011$). Pitcher diameter at fluid level was significantly larger in *N. rafflesiana* (Mann-Whitney U -test: $U = 523$, $n_1 = 52$, $n_2 = 42$,

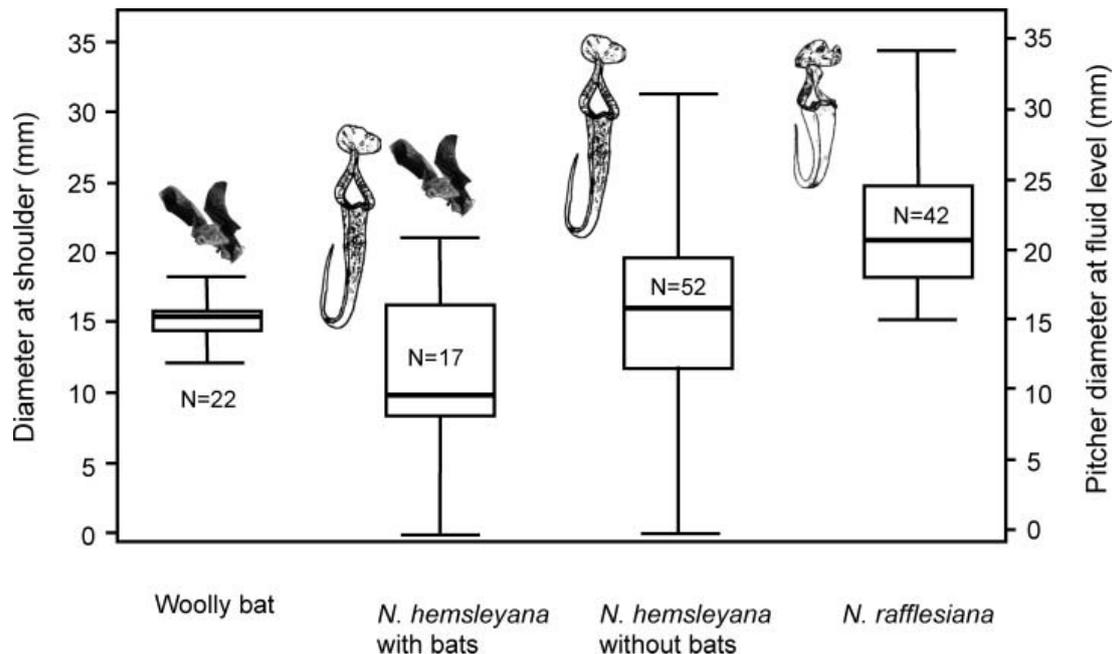


Fig. 6. Diameter of *K. hardwickii* at shoulder height versus pitcher diameter at fluid level in aerial pitchers of *N. hemsleyana* used by bats, aerial pitchers of *N. hemsleyana* not known to be used by bats, and in aerial pitchers of *N. rafflesiana* (photo of bat by C.C. Lee). See caption to Fig. 3 for further details.

$P < 0.001$) (Fig. 6), and, hypothetically, bats would slip into the fluid unless they were able to hold onto the edge of the peristome, thus exposing themselves to direct sunlight and potential predators.

DISCUSSION

Suitability of *N. hemsleyana* aerial pitchers as roosting sites for bats

This study identified a set of characteristics in *N. hemsleyana* that facilitate bat roosting. Particularly revealing are those traits that appear to be derived in *N. hemsleyana* and thus might have evolved to attract bats: low fluid levels, relatively large orifices, as well as the elongate, cylindrical, and basally strongly tapered pitchers.

The geometry of the *N. hemsleyana* aerial pitcher indicates that these pitchers are excellent roosting sites for *K. hardwickii*. The enlarged orifice in *N. hemsleyana*, created by the elongated rear pitcher wall, allows bats easier access to the pitcher interior. Once inside the pitcher, both the body length and body width of *K. hardwickii* are well matched to pitcher dimensions. The elongated, narrow pitchers provide sufficient space and appropriate morphology to accommodate individual bats well above the digestive fluid. We did not observe bats use as roosts pitchers that were wider than 22 mm at fluid level, suggesting selection on *N. hemsleyana* pitchers to either reduce fluid levels or to produce narrow, strongly tapering pitchers. Similarly, under conditions of few invertebrate species, the pitchers of several montane *Nepenthes* species produce pitchers that are highly adapted to the body size of *Tupaia montana*, whose feces are captured and digested by those pitcher plants (Chin *et al.*, 2010).

The dual strategy of *Nepenthes hemsleyana*

Although *N. hemsleyana* obtains about a third of its total foliar nitrogen from the feces or urine of *K. hardwickii* (Grafe *et al.*, 2011), the ability of its pitchers to trap insects, albeit reduced (Moran, 1996), suggests that *N. hemsleyana* follows a dual strategy of nitrogen acquisition. The orifice diameter of *N. hemsleyana* is significantly larger than that of *N. rafflesiana*, but it retains the ability to trap arthropod prey by aquaplaning when the peristome is wet [whether by rain, humidity or nectar (Bohn and Federle, 2004; Bauer *et al.*, 2009, 2011, 2015)]. The wettable peristome and the long waxy zone between the peristome and the girdle are nearly 100% effective in retaining prey that has fallen into the pitcher (Gaume and Di Giusto, 2009; Bauer *et al.*, 2011). Extra-floral nectaries along the inner rim of the peristome may also attract prey, although the rates of nectar production are much reduced compared with *N. rafflesiana* (Bauer *et al.*, 2011). Thus, the morphological traits of *N. hemsleyana*'s aerial pitchers compromise between attracting bats and capturing insects.

The adoption of a dual strategy is not unique to *N. hemsleyana*. *Nepenthes ampullaria* has also evolved to glean nutrients from trapping invertebrates and leaf litter that falls into its pitchers (Moran *et al.*, 2003; Pavlovič *et al.*, 2011). *Nepenthes rajah* and *N. macrophylla*, two species that attract small mammals, also show a combination of pitcher characteristics that serve dual functions of trapping arthropods and collecting feces (Chin *et al.*, 2010). In *N. lowii*, the dual strategy is divided between lower pitchers that trap insects and aerial pitchers that trap shrew feces (Clarke *et al.*, 2009). The retention of pitcher characters useful to capturing insects in *N. hemsleyana* suggests that the relative importance of the feces-trapping and carnivorous syndrome fluctuates temporally in their contributions to the plant's nitrogen demands. Alternatively, this could be a snapshot of ongoing evolutionary divergence, with some pitcher traits underlying genetic or developmental constraints that prevent it from completely abandoning highly conserved trap structures such as the peristome.

Evidence from morphological and molecular phylogenetic studies indicates that a long, cylindrical waxy zone in upper pitchers is a basal feature (Bauer *et al.*, 2012). As such, the partly cylindrical, funnel-shaped aerial pitcher of *N. hemsleyana* was most likely pre-adapted and later modified to accommodate roosting bats leading to a functional divergence away from the typical arthropod-trapping strategy. Our results thus support the notion that slight allometric differences in pitcher morphologies facilitate divergence to new ecological niches and nitrogen sequestration strategies (Gaume and Di Giusto, 2009; Chin *et al.*, 2010; Bauer *et al.*, 2011; Bonhomme *et al.*, 2011b; Grafe *et al.*, 2011; Greenwood *et al.*, 2011).

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Manuscript 4

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Ecological outsourcing: a pitcher plant benefits from transferring pre-digestion of prey to a bat mutualist

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Title:

2 Ecological outsourcing: a pitcher plant benefits from transferring pre-digestion of prey to a bat
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Running headline:

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27 Outsourcing of carnivory in plants

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2

30 **Summary**

- 31 1. Mutualistic interactions between animals and plants are one of nature's success stories. The
32 exchange of resources and/or services between mutualistic partners often involve tasks that
33 species originally accomplished themselves but which have been taken over by or transferred
34 to the more efficient partner during the evolution of the mutualism. Such "*ecological*
35 *outsourcing*" can be seen, e.g., in several carnivorous plants that have transferred prey
36 attraction and digestion to animal partners. However, the beneficial outcome of this transfer
37 and its fitness relevance has rarely been quantified in general and never for carnivorous plants.
- 38 2. Using a mutualistic interaction between a carnivorous pitcher plant and a bat as a model, we
39 tested the hypothesis that the benefits of ecological outsourcing are significant for the
40 outsourcing partner. In a series of field and greenhouse experiments we measured survival,
41 growth, photosynthesis, and nutrient content of plants fed with faeces and/or arthropods.
- 42 3. We found that plants fed with bat faeces had increased survival, growth and photosynthesis
43 compared to plants fed with arthropods only. On average, plants covered 95 % of their
44 nitrogen demand from bat faeces under strong nutrient-deprivation.
- 45 4. *Synthesis*. Outsourcing prey capture and digestion can explain the evolutionary trend of
46 several carnivorous plants to lose their carnivorous traits while increasing their attractiveness
47 to mutualistic partners. We propose that ecological outsourcing could be one of the major
48 drivers for the evolution and maintenance of mutualisms.

49

50 **Keywords:**

51 animal-plant interaction, fluorescence, *Kerivoula*, *Nepenthes*, nitrogen, photosynthesis, stable
52 isotopes, trait loss

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3

55 **Introduction**

56 Mutualisms are interspecific interactions that provide benefits to each of the species involved.
57 They are ubiquitous in nature and may be facultative or obligatory, e.g., when one of the involved
58 species completely relies on the services provided by the other(s) (Howe 1984; Bronstein 2001).
59 Such services include the dispersal of gametes between the sexes of the partner species (Rosas-
60 Guerrero *et al.* 2014) or the conveying and processing of nutrients for them (Perry 1998;
61 Humphreys *et al.* 2010). The degree of mutual dependence between partner species may vary in
62 response to a variety of ecological and environmental factors (Bronstein 1994; Chamberlain,
63 Bronstein & Rudgers 2014). Species that strongly depend on each other may be subject to strong
64 selective pressures that profoundly affect each other's morphology, physiology and behaviour, as
65 can be seen in the flowers of plants that attract specialized pollinators (Stiles 1978) or in leaf
66 cutter ants that cultivate fungi (North, Jackson & Howse 1997).

67 One prominent characteristic of mutualisms is the evolution of specialized morphological
68 and/or physiological traits in the participating species (Bronstein 2009). However, adaptations to
69 mutualism may also involve the loss of traits, particularly when one species "transfers" one or
70 more of its key biological functions to its partner. Trait loss may occur for cost-saving reasons
71 when a species' relevant traits to achieve certain benefits become redundant because these
72 benefits are now delivered more efficiently by the mutualistic partner species (Ellers *et al.* 2012).
73 In analogy to the outsourcing of performances in economics (Deardorff 2001), we here introduce
74 the term "ecological outsourcing" to describe this process in mutualisms.

75 A principal challenge to understanding the nature of mutualisms is quantification of the
76 costs and benefits that the interaction provides to the species involved (Bronstein 2001; Holland
77 2002). Although an increasing number of studies has succeeded in quantifying the benefits of
78 mutualisms (usually by demonstrating improved rates of nutrient acquisition or reproductive

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79 success) (Holland 2002; Hosokawa 2010), the logistical challenges to designing informative field
80 experiments remain significant. In the case of mutualists that engage in ecological outsourcing,
81 these challenges are amplified, as it is necessary to additionally quantify the compensated trait
82 loss (Ellers *et al.* 2012).

83 In this study, we investigated whether benefits derived from ecological outsourcing could
84 explain the loss of morphological and physiological traits that facilitate arthropod capture in a
85 carnivorous pitcher plant. The majority of carnivorous plants are highly effective in attracting and
86 capturing arthropods to compensate for a lack of nutrients in their substrates. However, several
87 species from Borneo deploy pitchers that aid resource-service mutualisms with vertebrates, at the
88 expense of traits for arthropod capture (Clarke *et al.* 2009; Chin, Moran & Clarke 2010; Grafe *et*
89 *al.* 2011). One of these is *Nepenthes hemsleyana* (Macf., 1908) from NW Borneo, whose pitchers
90 capture seven times less arthropods than the closely related, and sometimes co-occurring
91 *Nepenthes rafflesiana* (Jack, 1835) due to reduced chemical attractants and altered UV-
92 reflectance patterns (Moran 1996). *Nepenthes hemsleyana* is unique among carnivorous plants by
93 providing high quality roosts for the insectivorous woolly bat, *Kerivoula hardwickii* (Horsfield,
94 1824) that in return supplies the plant with nutrients via faeces that fall into the pitchers'
95 digestive fluid (Grafe *et al.* 2011; Schöner *et al.* 2013). The pitchers of *N. hemsleyana* have a
96 unique combination of traits to attract and host the bats such as a very low fluid level that leaves
97 sufficient roosting space for the bats (Grafe *et al.* 2011), elongated, narrow pitchers with large
98 openings (Lim *et al.* 2015) or an echo reflector that facilitates pitcher detection and identification
99 (Schöner, M.G. *et al.* 2015).

100 Grafe *et al.* (2001) recently showed that on average one third of *N. hemsleyana*'s foliar
101 nitrogen derives from bat faeces. Across many plant species nitrogen content is positively
102 correlated with growth and photosynthesis (Ellison & Farnsworth 2005; Pavlovič, Masarovičová

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103 & Hudák 2007). However, a quantification of the benefits for *N. hemsleyana* and other
104 carnivorous plants that gain nitrogen from their mutualism partners is missing so far. This is
105 relevant to determine 1) the value of the mutualism to the plant and 2) the plant's dependence on
106 the mutualism partner.

107 We hypothesized that *N. hemsleyana* has effectively “outsourced” its arthropod prey
108 capture and digestive mechanisms to *K. hardwickii* and has partially or completely lost several of
109 the traits that facilitate direct predation upon arthropods. Potential benefits to the plant include a
110 reduction in the physiological costs of trap production and function, facilitated by inputs of pre-
111 digested arthropod prey caught by *K. hardwickii*. Arthropods that have passed through the
112 digestive tract of *K. hardwickii* before being deposited inside *N. hemsleyana* pitchers have
113 already been chemically and physically broken-down, meaning that the nutrients they contain are
114 likely to be assimilated more efficiently by *N. hemsleyana* than those derived from direct
115 arthropod capture. Increased nutrient uptake of faeces-fed plants should result in increased
116 photosynthesis and growth compared to arthropod-fed plants. Strong benefits from digesting
117 faeces instead of captured arthropods would provide evidence for a high dependence of *N.*
118 *hemsleyana* on their mutualistic bat partner. If this is the case, this system could serve as a
119 candidate model for the investigation and elucidation of other vertebrate-plant mutualisms
120 (Clarke *et al.* 2009; Chin, Moran & Clarke 2010). Moreover, the concept of ecological
121 outsourcing provides broad application possibilities to investigate the loss of morphological,
122 physiological and behavioural traits in all kinds of mutualisms.

123

124 Materials and methods**125 DESCRIPTION OF STUDY SITES AND EXPERIMENTAL DESIGN**

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126 Field experiments took place in the Belait district of Brunei Darussalam/Borneo from 20 June to
127 3 December 2012. All experimental *N. hemsleyana* plants grew on predominantly sandy soils at
128 the forest edge of the study site “Labi 31”. Precise location data are withheld to protect sensitive
129 populations of plants and animals at this site. We chose five plants per set, with sets defined as
130 plots of 20 x 20 m all with similar environmental conditions, e.g., canopy cover and soil
131 composition (*N. hemsleyana*: 10 sets, 50 plants). To ensure that plants were distinct individuals
132 and not connected by rhizomes, we kept a minimum distance of 5 m between the plants. We
133 selected young plants (total lengths < 2m) with a single aerial pitcher and no lower pitchers to
134 ensure comparability of growth parameters. On the day the pitcher first opened its lid the
135 pitcher’s opening was manually closed with a wrapping film and cotton wool moistened in
136 distilled water, to prevent the entry of arthropods or bat faeces. As soon as we had five freshly
137 opened pitchers within one plot and within a maximal time period of three weeks, we started to
138 feed the pitchers that were randomly assigned to one of the following treatments: fed with faeces
139 and arthropods (AF), fed only with bat faeces (F), fed only with arthropods (A), and an unfed
140 control (C). In addition, one plant per set with an open pitcher (O) that allowed a natural capture
141 rate was used as a second control. All pitchers (except the O-pitchers) remained artificially closed
142 for the complete eight weeks of the experiment apart from feeding times. In pre-tests we had
143 determined the natural insect and bat faeces capture rate of *N. hemsleyana* pitchers (Supporting
144 Information). Based on these data, we fed all AF- and F-pitchers with 42 mg bat faeces/week.
145 AF- and A-pitchers received 35 mg arthropods (also see Supporting Information for chosen bat
146 faeces and arthropod prey).

147 To control for biotic and abiotic influences, e.g. soil properties, and to generate a highly
148 nutrient-deprived environment over a long time period, we additionally performed a comparable
149 feeding experiment with cultivated specimens of *N. hemsleyana* in the greenhouse in Greifswald/

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150 Germany from 24 June 2013 to 12 October 2014. We grew 58 plants on a mixed substrate
151 (sphagnum/bark/leaves/moss) with an average temperature of 25°C during daytime and 23°C
152 during night-time and high humidity (80% - 100%). The photoperiod was at least 12 hours of
153 light per day. As the plants were too young to produce aerial pitchers, we used lower pitchers for
154 feeding the plants (Supporting Information). Four months prior to the experiment we had closed
155 all pitchers with a plastic film and cotton wool, preventing any source of nutrition gained through
156 the pitchers. Again, all plants were randomly distributed to different feeding treatments (AF, F,
157 A, and C). The youngest pitcher per plant was chosen and fed once a week with the same amount
158 of arthropods/faeces as in the field experiment for 19 weeks (= feeding period one). When the
159 original feeding pitchers died or became inoperative, a newly developed pitcher was used for
160 feeding. To investigate long-term effects, seven A- and seven F-plants were fed for an additional
161 period of 31 weeks (= feeding period two; one F-plant had to be excluded as it did not develop a
162 new pitcher when the experimental one wilted).

163

164 MEASUREMENTS OF PLANT GROWTH

165 We counted the number of newly developed leaves (as soon as they started to unfurl) and pitchers
166 (> 0.5 cm in length). To determine changes in a given plants' photosynthetic area, we measured
167 the surface of the three youngest leaves at the beginning of the experiment and again at the end of
168 the field experiments, along with all subsequently developed leaves. In the greenhouse
169 experiment we only measured the youngest leaf when the experiment started because the older
170 leaves partly wilted during the long duration of the first and second feeding period (Supporting
171 Information). To determine the dry weight of the newly developed plant parts, new leaves and
172 pitchers were removed at the end of the field experiment, dried on silica gel for three weeks and
173 weighed. For the greenhouse experiment we only cut the newest fully developed leaves (we had

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174 to exclude one A- and two C-plants from the greenhouse experiments due to their bad condition)
175 at the end of feeding period one, dried them in a drying oven at 75°C for 20 h and weighed them.

176

177 ELEMENTAL ANALYSIS

178 We determined the total nitrogen, potassium, and phosphorus content and the stable isotope ratio
179 of nitrogen of the plants' youngest leaves (field and greenhouse experiments). The same was
180 done for the spent prey (arthropods and/or faeces) that was left in five randomly selected pitchers
181 of AF-, F-, and A-plants, respectively, two weeks after the last prey addition of feeding period
182 one to see how efficiently the plants absorb different nutrients (for results and discussion see
183 Supporting Information). We also calculated the plants' stoichiometry of nitrogen, phosphorus
184 and potassium to determine nutrients limiting plant growth (Olde Venterink 2003) (Supporting
185 Information).

186

187 MEASUREMENTS OF CHLOROPHYLL *A* FLUORESCENCE, CHLOROPHYLL AND
188 CAROTENOID CONTENT

189 To determine the different plants' photosynthetic activity, we measured chlorophyll *a*
190 fluorescence (MINI-PAM Fluorometer, Walz GmbH, Effeltrich, Germany) of the greenhouse
191 plants' youngest fully developed leaf after feeding period one and two (Supporting Information).
192 All tested leaves were dark adapted for 12 hours. After measuring minimal fluorescence (F_0) we
193 determined maximum fluorescence (F_m) by applying a saturation pulse and calculated the
194 maximum quantum yield ($F_v/F_m = (F_m - F_0)/F_m$), which is diagnostic for stress in plants (Parkhill,
195 Maillet & Cullen 2001). Then plants were adapted to moderated light ($150 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 12.5
196 min. We switched off the actinic light for 30 s and started a Rapid Light Curve (WINCONTROL
197 software, Walz GmbH, Effeltrich, Germany) that reveals the photosynthetic performance of the

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198 plants (Ralph & Gademann 2005). We increased actinic light from 60 to 1874 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR
199 (photosynthetic active radiation) in eight steps. At the end of each irradiation period (10 s) we
200 applied saturation pulses to compute effective photochemical quantum yield of photosystem II
201 (Φ_{PSII} ; measures the proportion of light used for photochemistry and light absorbed by
202 chlorophylls that are associated with photosystem II) and non-photochemical quenching (NPQ;
203 i.e. the share of photon energy that is converted to heat and not used for photosynthesis,
204 Supporting Information).

205 For the analyses of chlorophyll and carotenoid content we randomly selected nine
206 greenhouse plants from each treatment. We punched a circular area (diameter of 1 cm^2) out of the
207 youngest fully developed leaf and stored it in liquid nitrogen. After grinding the samples
208 photosynthetic pigments were extracted (using methanol, MgCO_3). We determined the
209 concentration of assimilation pigments spectrophotometrically (Evolution 200, Thermo
210 Scientific, Waltham, USA, Supporting Information).

211

212 STATISTICAL ANALYSIS

213 We tested our data for normality (Shapiro test), homogeneity of variance (Bartlett test), and
214 equality of distribution (Kolmogorov-Smirnov test). As some of our data deviated from these
215 assumptions, instead of using parametric tests, we applied permutation tests to investigate if the
216 feeding treatments affected the growth, nutrient content and photosynthesis of the respective
217 plants (Supporting Information). Values are either given as median (range) or mean \pm SD. All
218 tests were conducted with R (v.3.1.3; R Foundation for Statistical Computing, Vienna, Austria).
219 By computing Bayesian stable isotope mixing models (MixSIAR GUI; Parnell *et al.* 2013) we
220 analysed the proportions of nitrogen uptake from faeces and arthropods in AF-plants.

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222 **Results**

223 IMPACT OF BAT FAECES VERSUS ARTHROPOD PREY ON PLANT GROWTH

224 In the field experiment, feeding bat faeces had a positive influence on *N. hemsleyana*'s growth as
225 indicated by higher numbers of newly developed pitchers, increased leaf area and weight in
226 plants fed with faeces and arthropods (AF) or in those fed only with bat faeces (F) compared to
227 random distributions. Plants with an open pitcher (O) that allowed a natural capture rate did not
228 differ from random distributions while plants fed only with arthropods (A) and the unfed control
229 plants (C) grew less than expected (Fig. 1). This effect was even more pronounced in the
230 greenhouse experiment. After feeding period one, all measured parameters provided evidence for
231 a strong growth in AF- and F-plants while in the A- and C-plants growth was lower than expected
232 from random distribution (Fig. 1). During feeding period two, F-plants continued to produce
233 significantly more pitchers than A-plants (F: 2.00 (1.00-2.00), A: 1.00 (0.00-2.00), $P = 0.007$).
234 However, there was no longer a significant difference in the amount of new leaves (F: 2.50 (2.00-
235 3.00)); A: 2.00 (0.00-2.00)); $P = 0.33$), which showed a partial recovery from nutrient stress after
236 an extended feeding period. Sizes of the youngest fully developed leaves increased to a greater
237 extent in F-plants than in A-plants (leaf size differences between the start of the experiment and
238 the end of feeding period one, F: 28.51 (6.04-45.97) cm², A: -3.21 (-9.36-9.73) cm², $P = 0.002$;
239 leaf size difference between the end of feeding period one and the end of feeding period two, F:
240 49.64 (16.83-79.41) cm², A: 9.83 (-0.23-15.08) cm², $P < 0.0001$; Fig. 2). Noteworthy, three of the
241 14 A-plants and nine of the 15 C-plants did not recover at all and died six months after the end of
242 feeding period one while none of the AF- and F-plants died.

243

244 IMPACT OF BAT FAECES VERSUS ARTHROPOD PREY ON NUTRIENT CONTENT IN

245 LEAVES

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246 In the field experiment, the nitrogen, phosphorus, and potassium content in leaves of *N.*
247 *hemsleyana* plants did not differ significantly between treatments (Table 1). However, the
248 Bayesian stable isotope mixing model (MixSIAR GUI) revealed that $55.9 \pm 28.7\%$ of the foliar
249 nitrogen in the AF-plants originated from bat faeces.

250 In the greenhouse experiment, AF- and F-plants had significantly higher foliar nitrogen
251 contents compared to random distributions while they were reduced in A- and C-plants after
252 feeding period one (Table 1). The phosphorus and potassium content did not differ significantly
253 between treatments (Table 1), but feeding significantly influenced the plants' stoichiometry of
254 nitrogen, phosphorus, and potassium as the N:K and the N:P ratios of AF-plants were shifted to
255 higher values compared to random distributions (Table 1). Thus, nitrogen limitation ($N < 2.0\%$
256 d.wt, $N:P < 14.5$, $N:K < 2.1$; Olde Venterink *et. al.* 2003; Supporting Information) was
257 significantly reduced in these plants. Nitrogen from bat faeces was efficiently absorbed because
258 $\delta^{15}N$ values in AF- and F-plants were significantly higher compared to random distributions and
259 significantly lower in the other two treatments after feeding period one (Fig. 3; also see
260 Supporting Information). Most importantly, a mean of $95.4 \pm 5.1\%$ (MixSIAR GUI) of the foliar
261 nitrogen in the long-term (4 months) nutrient-deprived AF-plants derived from bat faeces even
262 though arthropods contained on average more nitrogen ($N = 8.19 (5.09-9.09)\%$ d.wt) than faeces
263 ($N = 7.51 (7.32-7.62)\%$ d.wt, $P = 0.006$; Table S1 in Supporting Information).

264

265 EFFECTS ON PHOTOSYNTHESIS

266 In the greenhouse experiment, AF- and F-plants had higher foliar contents of photosynthetic
267 pigments compared to random distributions while A- and C-plants had significantly lower ones
268 after feeding period one (Table 1). During that time period, only AF- and F-plants were released
269 from nutrient stress as indicated by a maximum photochemical quantum yield (F_v/F_m) of at least

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270 0.83 (Table 1) (Parkhill, Maillet & Cullen 2001). Mean Φ_{PSII} values in Rapid Light Curves
271 indicated higher photosynthetic performance in AF- and F-plants than in the other two treatments
272 (Fig. 4a). Non-photochemical quenching (NPQ) decreased in AF- and F-plants (irradiances > 758
273 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) while it was still increasing in the other treatments (Fig. 4c). After feeding
274 period two, there was no longer a significant F_v/F_m difference between F- (0.84 (0.82-0.84)) and
275 A-plants (0.83 (0.75-0.84); $P = 0.10$). Thus, A-plants had overcome nutrient stress as well. Rapid
276 Light Curves also converged in F- and A-plants (Fig. 4), which suggests similar photosynthetic
277 performances at the end of feeding period two.

278

279 **Discussion**

280 *NEPENTHES HEMSLEYANA* BENEFITS FROM OUTSOURCING ITS PREY CAPTURE
281 AND DIGESTIVE MECHANISMS

282 Carnivory has evolved on a number of occasions in the plant kingdom, in disparate orders
283 (Ellison & Gotelli 2009). The carnivorous syndrome is generally only of benefit to plants that
284 grow in moist, sunny sites with soils that are deficient in key plant macronutrients, particularly N
285 and P (Givnish *et al.* 1984). The benefits of capture and digestion of arthropod prey, such as
286 increased growth and/or nutrient content in plant tissues, have been demonstrated for various
287 carnivorous plants (Aldenius, Carlsson & Karlsson 1983; Chapin & Pastor 1995; Pavlovič *et al.*
288 2013). However, it is also thought that some taxa have evolved away from carnivory (e.g.,
289 *Ancistrocladus*) (Stevens 2001), while in others, carnivorous traits are present only for a short
290 part of the plants' life cycle (e.g., *Triphyophyllum*) (Green, Green & Heslop-Harrison 1979).
291 Within *Nepenthes*, several species (*Nepenthes lowii*, *Nepenthes macrophylla* and *Nepenthes*
292 *rajah*) acquire a significant proportion of N from the capture of tree shrew faeces (Clarke *et al.*
293 2009; Chin, Moran & Clarke 2010), while *Nepenthes ampullaria* obtains almost one third of

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294 foliar N from the capture of leaf litter (Moran, Clarke & Hawkins 2003; Pavlovič, Slovákova &
295 Šantrucek 2011). This demonstrates that the loss of carnivorous traits in this genus is not unique
296 to *N. hemsleyana*. All of these species grow in habitats that are colonized by other carnivorous
297 plant species that trap only arthropods. This raises a fundamental question: why do some
298 carnivorous plants partially or wholly abandon carnivory, particularly when the syndrome is
299 successfully deployed by other congeners in the same habitats?

300 One possible explanation is that co-occurring *Nepenthes* species are engaged in resource
301 partitioning. By outsourcing its prey capture mechanism to *K. hardwickii*, *N. hemsleyana* may
302 avoid direct competition, e.g., with the closely related *N. rafflesiana* for the same arthropod prey.
303 Some empirical evidence to support this hypothesis exists for other *Nepenthes* species (Chin,
304 Chung & Clarke 2014), but *N. hemsleyana* and *N. rafflesiana* do not commonly co-occur in
305 mixed populations. In sites where levels of anthropogenic disturbance are minor or absent, *N.*
306 *hemsleyana* tends to occur in closed forests, whereas *N. rafflesiana* is generally found in open
307 sites (Clarke 2006). Alternatively, direct competition with *N. rafflesiana* could be avoided by a
308 spatial habitat shift. As with other mutualisms (Mazancourt, Loreau & Dieckmann 2005), the
309 interaction with the bats could enable *N. hemsleyana* to occur in more uncommon habitats for
310 carnivorous plants such as shady, closed forests (Givnish *et al.* 1984).

311 A second explanation could be that *N. hemsleyana* has evolved away from carnivory
312 because the benefits derived from “capturing” bat faeces outweigh those of arthropod capture,
313 resulting in selective pressure for the loss of some carnivorous traits such as the ability to
314 effectively assimilate nutrients from the direct capture and digestion of arthropods. Under this
315 scenario, plants that have been starved of nutrients would suffer from nutrient stress for longer
316 time periods accompanied by low photosynthesis and growth. Moreover, their chances to recover
317 would be lower if arthropods were their only source of supplementary nutrition. Our findings

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318 provide strong support for this hypothesis, and indicate that the mutualism with *K. hardwickii* is
319 likely to become an obligate one for *N. hemsleyana* (Ellers *et al.* 2012).

320 In our greenhouse experiment, feeding the average quantity of arthropod prey that *N.*
321 *hemsleyana* capture in their natural habitat was ineffective in preventing plants from long-term
322 nutrient stress. Plants that either received only arthropods or were unfed (A- and C-plants) both
323 showed severe signs of nutrient stress while this was not the case in the AF- and F-plants that
324 both received bat faeces (Table 1). Compared to the protein-bound nitrogen in arthropods,
325 nitrogen uptake from faeces should be faster as nitrogen in faeces is typically bound in urea
326 molecules that can be easier assimilated by plants (McFarlane, Keeler & Mizutani 1995). Urea
327 molecules easily dissociate into ammonium molecules (Kumar & Wagenet 1984) that are
328 preferentially absorbed by *Nepenthes* (Schulze, Frommer & Ward 1999). This mechanism can
329 explain our findings that AF-plants in the greenhouse gained 95.4% of their nitrogen from faeces
330 although they had arthropods with even higher nitrogen contents available for digestion at the
331 same time.

332 The results of the greenhouse experiment also showed that after a prolonged period of
333 feeding (after period two) the majority of *N. hemsleyana* plants were able to overcome the initial
334 nutrient stress by utilizing arthropod prey alone. However, 21.4% of the A-plants did not survive
335 the experiment while none of the faeces-fed plants died. This indicates that by relying on
336 arthropod prey alone *N. hemsleyana* faces a reduction in survival probability.

337 Nutrient contents and ratios between nitrogen, phosphorus, and potassium can be used to
338 detect limiting nutrients for plant growth (Olde Venterink *et al.* 2003). The tissue of *N.*
339 *hemsleyana* plants in the greenhouse and in the field experiment was nitrogen deprived (e.g.
340 nitrogen content < 2%). However, similar to other *Nepenthes* species (Osunkoya *et al.* 2007) and
341 in contrast to many other carnivorous plants (Ellison 2006), *N. hemsleyana* was generally not

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342 phosphorus limited (e.g. phosphorus content < 0.1%; Table 1). Feeding faeces contributed
343 significantly to reducing nitrogen deficiency in the greenhouse plants as indicated by the
344 increased N:P and N:K ratios and by increased foliar nitrogen contents in the AF- and F-plants
345 compared to the A- and C-plants. On the contrary, foliar nutrient contents did not differ between
346 the feeding treatments in the field experiment (Table 1). Moran & Moran (1998) assumed that the
347 closely related *N. rafflesiana* maintains nutrient concentrations in their leaves by retaining limited
348 nutrients, which results in reduced growth rates. Similarly, C- plants in our field experiment
349 primarily grew less than AF- and A-plants but retained similar nutrient concentrations. Only
350 when nutrient stress was very pronounced in the greenhouse experiment, foliar nutrient
351 concentrations were lowered in the C-plants.

352

353 EVOLUTIONARY IMPLICATIONS

354 The key finding of this study is that *Nepenthes hemsleyana* strongly profits from
355 outsourcing prey capture to *K. hardwickii*, which provides the plants supplementary nutrition in
356 the form of pre-digested arthropods. Arthropods that passed through the bats' digestive tract
357 appear to facilitate accelerated nutrient assimilation by the plant. This is indicated by a faster
358 recovery from nutrient stress in AF- and F-plants after a four months starvation period.
359 From an evolutionary perspective, outsourcing of prey capture and digestion is so beneficial that
360 *N. hemsleyana* could reduce its investment in arthropod attraction (Moran 1996) and instead
361 invests in traits that make its pitchers attractive to bats (Grafe *et al.* 2011; Schöner, M.G. *et al.*
362 2015). These changes are reinforced by the fact that the interaction with the bats seems to incur
363 nearly no costs for the plants, which further strengthens the interaction (Schöner, C.R. *et al.*
364 2015). The fact that some important traits for prey capture, e.g., viscoelastic pitcher fluid, can still
365 be found in *N. hemsleyana* (Bazile *et al.* 2015) does not contradict our findings. Such traits may

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366 be important in early stages of the plants' ontogeny as the bats only use pitchers after the plants
367 reached a minimum height of 1.2 m (Schöner *et al.* 2013).

368 It is notable that all known coprophagous *Nepenthes* species that have outsourced prey
369 capture to mammals, such as *Tupaia montana*, *Rattus baluensis*, or *K. hardwickii*, exploit their
370 partners' sensory system to attract them to their pitchers (Wells *et al.* 2011; Moran *et al.* 2014;
371 Schöner, M.G. *et al.* 2015). In exchange, these pitcher plants receive multiple benefits: The
372 associated mammals all have foraging areas that most likely exceed the catchment area of the
373 plants' arthropod attractants by far. Moreover, some of the involved mammals feed on a variety
374 of food items, such as animal and plant material, that otherwise are not accessible for the plants
375 (Payne, Francis & Phillipps 1985). Finally, all of the mammals involved predigest the food, and
376 deliver it via their faeces to the plants (Clarke *et al.* 2009; Chin, Moran & Clarke 2010; Grafe *et*
377 *al.* 2011) in a readily assimilated format, as shown in this study.

378 The phenomenon of outsourcing crucial performances to mutualistic partners,
379 accompanied with trait loss, is widespread in a huge variety of mutualisms also outside the
380 carnivorous plant context, e.g., in fungi that make use of the mobility and the nutrimental
381 processing of leaf cutter ants (Weber 1966). In turn, the ant partners that are characterized by a
382 reduction of nutrient acquisition genes rely on food production by the fungi (Suen *et al.* 2011).
383 Similarly, *Acacia* species that are protected by ants have reduced chemical defence mechanisms
384 (Rehr, Feeny & Janzen 1973). We propose that benefits from ecological outsourcing are one of
385 the major driving forces in the evolution and maintenance of mutualisms that allow the involved
386 species to occupy new niches where none of them could have persisted on its own.

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396

397 **Data accessibility**

398 All data used in the analyses presented here are available in the Dryad Digital Repository.

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536 **Tables**

537 **Table 1.** Effects of feeding treatments on nutrient content and photosynthesis of *Nepenthes*
538 *hemsleyana* plants in the field experiment (tree symbol) and the greenhouse experiment (house
539 symbol). Median and range of each parameter are shown. Arrows indicate significant positive (↑)
540 or negative (↓) differences of treatments from random distributions (permutation tests):
541 significance level: ↑,↓: $P < 0.05$; ↑↑,↓↓: $P < 0.01$. Abbreviations: O = pitcher with natural capture
542 rate that was left open; AF = plants fed with arthropods and faeces; F = fed with faeces; A = fed
543 with arthropods; C = unfed control; d. wt = dry weight

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545 **Figure Legends**

546 **Fig. 1.** Influence of feeding treatments on the four tested growth parameters (number of newly
547 developed leaves and pitchers, increase of the leaf area, and weight of the newly developed plant
548 parts). In the first row results of the field experiment are shown (tree symbol), in second row the
549 results of the greenhouse experiment (house symbol). Significant positive (↑) or negative (↓)
550 differences of treatments from random distributions (permutation tests) are shown below each
551 boxplot: significance level: ↑, ↓: $P < 0.05$; ↑↑, ↓↓: $P < 0.01$.

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553 **Fig. 2.** Increase in the leaf area of the youngest fully developed leaf at the start of the greenhouse
554 experiment (start), after feeding period one (end1) and after feeding period two (end2). (a)
555 *Nepenthes hemsleyana* plants fed with faeces. (b) *Nepenthes hemsleyana* plants fed with
556 arthropods.
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558 **Fig. 3.** *Nepenthes hemsleyana*'s foliar $\delta^{15}\text{N}$ values of the youngest leaf in the field experiment
559 (tree symbol) and in the greenhouse experiment (house symbol) after feeding period one.
560 Significant positive (\uparrow) or negative (\downarrow) differences of treatments from random distributions
561 (permutation tests) are shown below each boxplot: significance level: $\uparrow\uparrow$, $\downarrow\downarrow$: $P < 0.01$.
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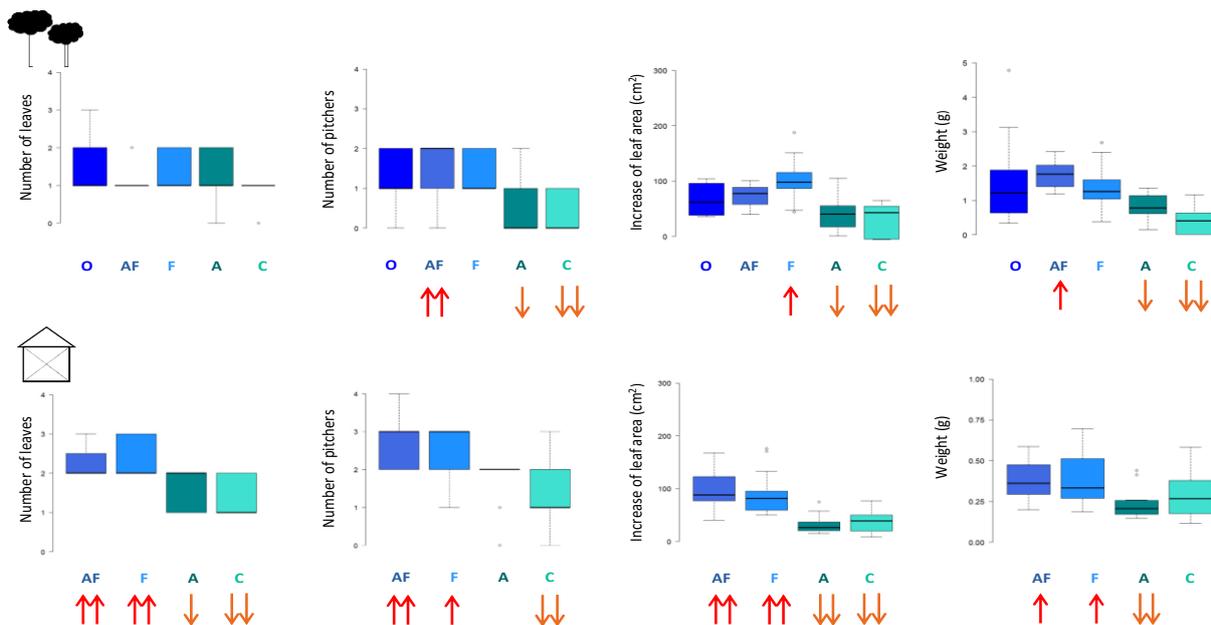
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563 **Fig. 4.** Results of the Rapid Light Curves of the *N. hemsleyana* plants in the greenhouse:
564 photosynthetic quantum yield (Φ_{PSII}) (a), (b) and non-photochemical quenching (NPQ) (c), (d)
565 were determined for increasing irradiances after feeding period one (a), (c) and feeding period
566 two (b), (d). Values are means \pm s.e.; Colour code: dark blue = plants fed with faeces and
567 arthropods, light blue = fed with faeces, dark green = fed with arthropods, light green = unfed
568 control; Abbreviation: PAR = photosynthetic active radiation.

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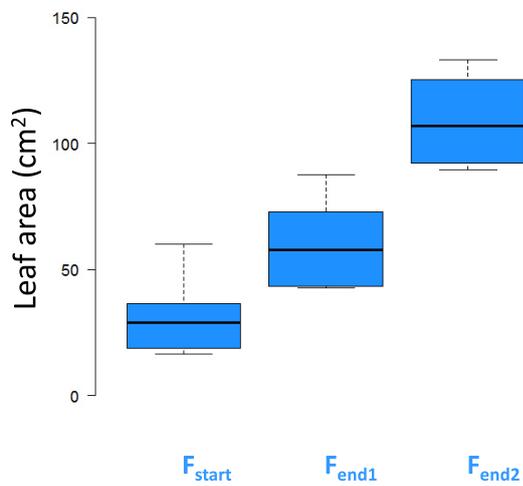
Treatments	O	AF	F	A	C
	Nutrient content				
N (% d. wt)	1.70 (0.55-2.65)	1.38 (1.10-1.83)	1.69 (1.30-1.90)	1.54 (1.14-1.89)	1.70 (1.42-3.41)
P (% d. wt)	0.09 (0.03-0.23)	0.11 (0.07-0.20)	0.12 (0.06-0.17)	0.07 (0.06-0.13)	0.08 (0.04-0.14)
K (% d. wt)	2.18 (0.81-4.50)	2.20 (1.46-3.54)	3.06 (1.95-3.46)	2.13 (1.82-3.05)	2.82 (1.65-3.48)
	Nutrient content				
N (% d. wt)	-	1.12 (0.63-1.44) ↑↑	1.05 (0.77-1.67) ↑↑	0.70 (0.47-1.02) ↓↓	0.69 (0.51-0.91) ↓↓
P (% d. wt)	-	0.25 (0.16-0.29)	0.27 (0.20-0.60) ↑↑	0.22 (0.19-0.48)	0.21 (0.14-0.26) ↓↓
K (% d. wt)	-	1.76 (1.05-2.71)	2.03 (1.64-5.58)	1.88 (1.55-3.37)	2.34 (1.30-3.25)
N:P (%/%)	-	4.14 (2.93-8.16) ↑↑	3.43 (1.71-6.72)	3.00 (1.47-4.94) ↓↓	3.56 (2.53-5.00)
N:K (%/%)	-	0.60 (0.45-0.90) ↑↑	0.51 (0.17-0.66)	0.32 (0.26-0.38) ↓↓	0.34 (0.20-0.40) ↓↓
K:P (%/%)	-	6.41 (4.89-13.44)	8.39 (3.20-20.47)	8.79 (5.42-16.28)	11.49 (7.34-16.02)
	Photosynthesis				
F_v/F_m	-	0.84 (0.80-0.84) ↑↑	0.83 (0.81-0.84) ↑↑	0.81 (0.76-0.84) ↓↓	0.79 (0.77-0.81) ↓↓
Chl <i>a</i> (mg g ⁻¹ d. wt)	-	3.51 (1.97-3.94) ↑↑	2.95 (2.23-4.13) ↑	1.87 (1.21-2.94) ↓↓	1.60 (1.04-2.82) ↓↓
Chl <i>b</i> (mg g ⁻¹ d. wt)	-	1.69 (0.99-1.88) ↑↑	1.49 (1.04-2.02) ↑	0.90 (0.57-1.47) ↓	0.79 (0.52-1.38) ↓↓
Carotenoids (mg g ⁻¹ d. wt)	-	0.45 (0.29-0.51) ↑	0.40 (0.29-0.65) ↑	0.31 (0.25-0.44)	0.28 (0.21-0.41) ↓



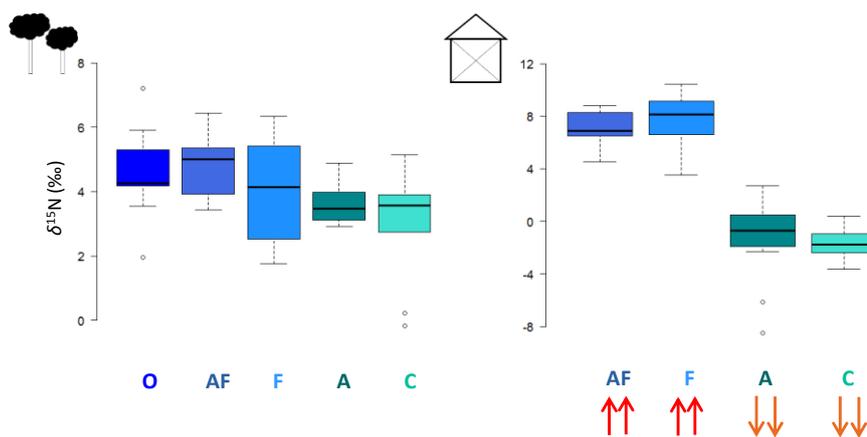
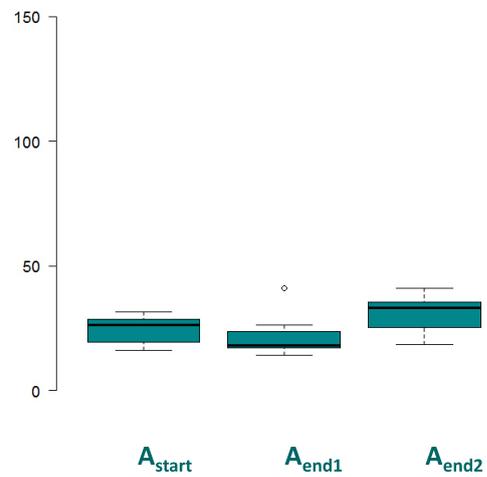
Costs and benefits in a bat-pitcher plant mutualism

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(a)



(b)



Supporting Information

Schöner *et al.*: Ecological outsourcing: a pitcher plant benefits from transferring pre-digestion of prey to a bat mutualist

Spent prey in pitchers

We determined the total nitrogen, potassium, and phosphorus content and the stable isotope ratio of nitrogen of the plants' youngest leaves (field and greenhouse experiments). The same was done for the spent prey (arthropods and/or faeces) that was left in five randomly selected pitchers of AF-, F-, and A-plants, respectively, two weeks after the last prey addition of feeding period one to see how efficiently the plants absorb different nutrients.

After two weeks of digestion the total nitrogen content of the spent faeces inside *N. hemsleyana* pitchers was significantly increased compared to fresh faeces ($P = 0.004$; Table S1). At the same time, $\delta^{15}\text{N}$ values of the spent faeces were significantly lower than those of the fresh faeces ($P = 0.004$; Table S1). This indicates nitrogen addition from alternative nitrogen sources such as nitrogen fixing bacteria in faeces-fed plants. In contrast, *N. hemsleyana* did not efficiently absorb nitrogen from fed arthropods as the total nitrogen content and the $\delta^{15}\text{N}$ values of the spent arthropods did not differ from that of the originally fed prey (N: $P = 0.42$; $\delta^{15}\text{N}$: $P = 0.13$; Table S1).

One explanation for this pattern could be that nitrogen fixing bacteria lowered $\delta^{15}\text{N}$ values in the pitcher fluid (by addition of ^{14}N). The trapping organs of other carnivorous plants, e.g., *Sarracenia purpurea* are inhabited by bacterial dinitrogen fixators (Prankevičius

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& Cameron 1991). Schulze *et al.* (1997) also assumed nitrogen fixation processes in old *Nepenthes* pitchers due to microbial activities. Our results suggest that faecal enrichment in the digestive fluid could provide an optimal habitat for nitrogen fixing bacteria. Further studies should therefore investigate if *N. hemsleyana* gains further benefits from the microbial community inside its pitchers.

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Full description of methods

Determination of *Nepenthes hemsleyana*'s nutrient income

To determine the natural arthropod capture rate of *N. hemsleyana* pitchers, we selected 44 aerial pitchers (11 pitchers per day), emptied and flushed them, refilled them with water to the original level following Moran (1996) and checked the capture success after 24 hours.

Captured arthropods were counted and weighed. Furthermore, we determined the order of the captured insects whenever possible. *Nepenthes hemsleyana* pitchers caught 1.68 ± 0.82 (mean \pm SD) arthropods per day with a weight of 4.98 ± 6.18 mg.

Furthermore, we determined the average amount of faeces *N. hemsleyana* receives from *Kerivoula hardwickii*. Bats stay on average 0.57 ± 0.41 days per week in *N. hemsleyana*'s aerial pitchers (Schöner *et al.* 2013). To determine the amount of faeces produced by the bats per day, we radio-tracked 6 bats (3 males, 3 females; transmitters: mass: 0.3 g; Holohil Systems, Ontario, Canada; fixed with skin bond adhesive (Manfred Sauer GmbH, Lobbach, Germany); TRX-1000S Wildlife Materials receiver fitted with a Yagi three-element antenna; Carbondale, IL, USA) in the very early morning hours when they were still hunting. We followed them to their roost (between 5:30 and 6:00 am) and put them into *Drosophila* cultivation tubes (28 x 85 mm) where they rested till they became active again (between 6:30 and 7:00 pm). Faeces produced per day weighed 74.24 ± 16.29 mg. Given the fact that pitchers were occupied on average 0.57 ± 0.41 days per week, this equates to a weight of 42.33 ± 9.29 mg to be fed to experimental pitchers per week.

Choice of arthropods and faeces for feeding experiment

For the field experiment, we chose termites of the genus *Hospitalitermes* as these arthropods were the fourth most common prey type accounting for 5.41% of the entire prey spectrum of *N. hemsleyana* (see above). Moreover, they were easy to capture in sufficient numbers to run

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the experiment. Termites were collected and deep-frozen until they were fed to the pitcher. Faeces were collected from different *Kerivoula* bat species as we could not collect enough faeces from the small populations of *K. hardwickii* without keeping them in captivity, which would have caused undue stress to the bats. To determine the nutrient content of the bat faeces, which was necessary for designing the field experiment, we first collected faeces of *K. hardwickii* (4 individuals) as well as of *Kerivoula papillosa* (1 individual), *Kerivoula intermedia* (3 individuals) and *Kerivoula minuta* (3 individuals). The faeces of *K. intermedia* and *K. minuta* were pooled within the species. We dried the faeces on silica gel (Silica Gel Orange, Carl Roth GmbH, Karlsruhe, Germany) and sent them to Eurofins Umwelt Ost GmbH (Freiberg, Germany) to analyse their nutrient content (nitrogen: combustion analysis DIN ISO 13878, 10-1995; phosphorus and potassium: microwave decomposition DIN EN ISO 11885, DIN EN ISO 11885). As the mean nitrogen content of the faecal samples of the different *Kerivoula* species were within or close to the range of *K. hardwickii*'s faecal nitrogen content (*K. hardwickii*: median N = 9.28 (7.82 – 9.65)% d.wt; pooled samples of the other species *K. papillosa*: 7.79%, *K. intermedia* 8.57% and *K. minuta* 8.79%), we used a mix of deep-frozen faeces from different *Kerivoula* species for the field experiments.

For the greenhouse experiment we chose *Drosophila hydei* as arthropod prey for the greenhouse experiment, as Diptera made up 66.22% of *N. hemsleyana*'s prey. The flies were cultivated on a medium of apple-oat mix. We fed the flies to the pitchers after they had been deep-frozen. For the greenhouse experiment we also used a deep-frozen faeces mixture of three European insectivorous bat species (*Myotis bechsteinii*, *Myotis nattereri*, and *Plecotus auritus*) after their nutrient content had been analysed (Centre for Stable Isotope Research and Analysis, Göttingen, Germany (Rapid Communication Mass Spectrometry 13, 1237–1241 (1999)). The nitrogen content of the 20 analysed faeces samples (median N = 7.51 (7.32–7.62)% d.wt) was lower than that of *K. hardwickii* (median N = 9.28 (7.82 – 9.65)% d.wt;

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Wilcoxon rank sum test: $W = 0$, $P < 0.0001$). Thus, our approach in the greenhouse experiment is conservative and nutrient gain by bat faeces could be undervalued.

Experimental plants in the greenhouse

Nepenthes hemsleyana plants were 4-5 years old, 10-30 cm tall and had 2.55 ± 0.93 lower pitchers on average in the beginning of the experiment. The plants derived from seeds that had originally been collected in the Belait District of Brunei Darussalam (pers. communication R. Cantley). During summer time natural sunlight was sufficient but during winter time, plants were additionally irradiated by fluorescent tubes for 12 hours per day (Lumilux cool white 58 Watt, Osram, Munich, Germany). Most *Nepenthes* species undergo an ontogenic shift in pitcher structure, with immature plants producing so-called “lower” or “terrestrial” pitchers that tend to be broadest at the base and rest on or near the ground. In contrast, mature plants produce “upper” or “aerial” pitchers that tend to have narrow, funnel shaped bases and are generally produced when the plant’s stems climb into the surrounding vegetation. As the cultivated plants were too small to produce aerial pitchers, we used lower pitchers for the feeding experiments in the greenhouse. However, the use of aerial pitchers during the field experiment enabled us to compare the results of both experiments. Furthermore, we consider the use of lower pitchers in the greenhouse experiment to be conservative: *N. hemsleyana*’s aerial pitchers show several traits that are likely to be adaptations to the bats. Thus, if there is a difference in the efficiency of taking up nutrients from bat faeces, the effect should even be stronger in aerial pitchers compared to ground pitchers.

Measurements during the experiments

We fixed leaves on a paper with adhesive tape so that the leaf surface was maximized and drew the contour of each leaf. Leaf area was then calculated by ImageJ (Rasband, W.S.,

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ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, <http://imagej.nih.gov/ij/>, 1997-2014). Areas of all leaves measured at the end of the experiment were summed and leaf areas measured at the beginning of the experiment were subtracted. We also compared leaf size of the latest leaf at the beginning of the experiment with the latest fully developed leaf at the end of feeding period one and two in the greenhouse experiment for all 13 plants of the F- and A-treatment that were fed during both feeding periods. This analysis was not possible for the field experiment as many of the experimental plants just developed one leaf that was not fully grown by the end of the experiment.

Elemental analysis

The collected leaves and the remaining arthropods and faeces were dried on Silica gel. Afterwards, the leaves were ground to fine powder. We packed 2 mg of each sample in tin capsules. Leaf samples, dried insects/faeces used for feeding and the spent prey in the pitchers were analysed in the Centre for Stable Isotope Research and Analysis in Göttingen with an elemental analyser (NA1110, CE-Instruments, Rodano, Milano, Italy) and a mass spectrometer (Delta Plus, Finnigan MAT, Bremen, Germany). The primary reference scale for $\delta^{15}\text{N}$ was atmospheric air. An internal standard (Acetanilide: $\text{C}_6\text{H}_5\text{NH}(\text{COCH}_3)$) was measured twice every ten samples for accuracy verification (s.d. = 0.1 ‰ for $\delta^{15}\text{N}$).

For the determination of the potassium content 100 to 300 mg of the remaining samples were used for a microwave decomposition (5 ml HNO_3 (65%) + 2 ml H_2O_2 (30%) + 4 ml H_2O) and a subsequent flame atomic absorption spectroscopy (contraAA 300, Analytik Jena, Jena, Germany). For the phosphorus content determination 100 μl of the microwave digestion were used for UV/VIS photometry (CE 1021, CECIL Instruments Ltd, Cambridge, UK). Analyses were conducted in the Institute of Botany and Landscape Ecology of the University of Greifswald, Germany.

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To calculate if feeding also influenced the plants' stoichiometry of nitrogen, phosphorus and potassium, we used the referring ratios to determine which nutrients are limiting plant growth (Olde Venterink *et al.* 2003). Nitrogen limitation is assumed if $N < 2.0\%$ d.wt ($N:P < 14.5$, $N:K < 2.1$), phosphorus limitation if $P < 0.1\%$ d.wt ($N:P > 14.5$, $K:P > 3.4$) and K limitation if $K < 0.8\%$ d.wt ($N:K > 3.1$, $K:P < 3.4$).

Measurements of chlorophyll *a* fluorescence and chlorophyll and carotenoid content

Chlorophyll fluorescence analysis is based on the three possible ways plants can use absorbed light energy: as a driver of photosynthesis, dissipated as heat, or re-emitted as fluorescence. Measuring fluorescence emission provides information about the other two processes (Maxwell & Johnson 2000). To measure if different nutrient sources affect the plants' photosynthetic activity, we measured chlorophyll *a* fluorescence (MINI-PAM Fluorometer, Walz GmbH, Effeltrich, Germany) of the youngest fully developed leaf of the greenhouse plants after feeding period one and two with a PAM fluorometer. All tested leaves were dark adapted (Dark Leaf Clip DLC-8, Walz GmbH, Effeltrich Germany) for 12 hours. First, minimal fluorescence (F_0) was measured ($\sim 1.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR). By applying a saturation pulse ($4000 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, 800 ms duration) we determined maximum fluorescence (F_m) and maximum yield $(F_m - F_0)/F_m$. We followed Maxwell and Johnson (2000) in their calculation of the effective photochemical quantum yield of photosystem II (Φ_{PSII}) and the non-photochemical quenching (NPQ).

For the analyses of the chlorophyll and carotenoid content we randomly selected nine greenhouse plants of each treatment and punched a circular area (diameter of 1 cm^2) out of the youngest fully developed leaf of each of these plants. The samples were immediately stored in liquid nitrogen and then ground in a mortar. The extractions were conducted with methanol and MgCO_3 . Samples were centrifuged at 3000 g (20°C) for 20 min. We determined the

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supernatants' concentration of assimilation pigments spectrophotometrically (Evolution 200, Thermo Scientific, Waltham, USA; $\lambda = 666$ nm and $\lambda = 653$ nm for chlorophyll *a* and *b*, additionally $\lambda = 470$ for carotenoids). Chlorophyll and carotenoid concentrations were calculated according to Wellburn (1994).

Statistical analysis

For the permutation tests, we tested the null hypothesis that the feeding treatments did not affect the plants' growth, nutrient content or photosynthesis. To exclude effects of group size in the different treatments, we adjusted group sizes to that of the smallest group by a random selection of samples. We first calculated the plants' mean performance in each treatment, which were then compared to the distribution of values expected under the null hypothesis. The null hypothesis distribution was obtained by permuting the plants' performances between treatments. In the case of the field experiment with set-dependent data, the plants' performances were permuted for each set of plants. Afterwards, we calculated the plants' mean performance per treatment. This procedure was repeated 10,000 times. The *P*-value was then calculated by comparing the plants' mean performance in the considered treatment to the null distribution. Tests were two-sided for the comparisons of more than two groups but one-sided for pairwise comparisons.

To compute Bayesian stable isotope mixing models (MixSIAR GUI; Parnell *et al.* 2013), discrimination factors ($\Delta^{15}\text{N}$) have to be calculated, which describe the differences in isotopic composition between an organism and its diet:

$$\Delta^{15}\text{N} = \delta^{15}\text{N}_{\text{plant tissue}} - \delta^{15}\text{N}_{\text{prey}}$$

We used $\delta^{15}\text{N}$ values of *N. hemsleyana* leaves in the F- and A-treatment (Figure 3) for $\delta^{15}\text{N}_{\text{plant tissue}}$ and $\delta^{15}\text{N}$ values of the fed prey types were used for $\delta^{15}\text{N}_{\text{prey}}$ (see Table S1).

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Table S1. Median nutrient content (and range) of faeces and arthropods fed to the plants during the field (☛) and greenhouse experiment (☞) are shown. Moreover, we analysed the nutrient content of the digested prey collected after two weeks of digestion. One pooled sample (~ 0.2 g) was used for the analysis of the phosphorus and potassium content of the arthropod prey and the spent prey. (* Data already published in Grafe *et al.* (2011))

	$\delta^{15}\text{N}$ (‰)	N (% d. wt)	P (% d. wt)	K (% d. wt)
	Fresh prey			
Faeces	1.83* (0.64-3.87)	9.28 (7.82-9.65)	0.29 (0.08-0.46)	0.25 (0.15-0.59)
Arthropods	-5.74 (-0.07-(-0.74))	7.78 (5.11-9.69)	0.36	1.12
	Fresh prey			
Faeces	4.97 (4.56-5.15)	7.51 (7.32-7.62)	1.39 (0.35-2.44)	1.57 (0.50-3.42)
Arthropods	5.51 (5.10-5.87)	8.19 (5.09-9.09)	1.04	1.05
	Spent prey			
Faeces	0.96 (0.70-2.62)	8.19 (7.66-9.21)	0.21	-
Arthropods	6.77 (4.99-7.63)	8.12 (5.04-10.81)	0.26	-

Journal of Ecology**References**

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4.2 Costs in a Bat-Pitcher Plant Mutualism



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Low costs reinforce the mutualism between bats and pitcher plants

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abstract

Whether mutualisms persist or vanish during the course of evolution depends on the cost-benefit ratio for the species involved. In the interaction between the pitcher plant *Nepenthes hemsleyana* and the bat *Kerivoula hardwickii* both partners mutually benefit: *N. hemsleyana* offers high quality roosts in exchange for nutrients from the bats' faeces. Here, we tested the hypothesis that the partners should also incur costs from their interaction. In the field, we examined potential costs that are likely to occur in our system and that are already known to be present in other bat-plant interactions. Regarding the plants, the bats could injure the tissue of the fragile pitchers and thus affect the pitchers' longevity. Regarding the bats, the ephemeral nature of *N. hemsleyana* pitchers and incoming rainwater could force them to switch these roosts more frequently than if using an alternative roost type. Our results suggest that neither the pitcher plants nor the bats accrue substantial costs from their interaction. We conclude that the ratio of high benefits to low costs strengthens this mutualism and promotes reciprocal adaptations and specialisations.

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1. Introduction

Mutualisms are key interactions in nature that occur in all ecosystems (Bronstein, 1994 and Bronstein, 2001). To maintain a mutualism, benefits gained by the species must outweigh the costs, otherwise the partners become autonomous again or one partner parasitises the other (Sachs and Simms 2006). For a full understanding of the ecology and evolution of mutualisms, the evaluation of both benefits and costs is therefore important (Bronstein, 2001). However, because costs in mutualisms are difficult to identify and in particular to quantify they are less studied than the benefits (Bronstein, 2001).

Carnivorous plants occur in nutrient-deprived habitats and typically compensate the lack of nutrients by capturing arthropods (Givnish et al., 1984). However, the pitcher plant *Nepenthes hemsleyana* (Macfarlane, 1908) is an inefficient arthropod trap catching seven times less prey than closely related species (Moran, 1996). Instead of capturing large amounts of arthropods, the plant attracts and hosts bats of the species *Kerivoula hardwickii* (Horsfield, 1824)

in its pitchers whose faeces contribute substantially to the plant's foliar nitrogen (Grafe et al., 2011). *N. hemsleyana* is the only known pitcher plant species that benefits from bats. Occasionally, the bats use other *Nepenthes* (Linnaeus, 1753a) species (*Nepenthes bicalcarata* (Hooker, 1873), *Nepenthes ampullaria* (Jack, 1835)) but only if their pitchers are damaged or dead. Intact pitchers of these species contain too much digestive fluid to allow bats to roost in them (Schöner et al., 2013).

The bats profit from roosting in *N. hemsleyana* pitchers because those pitchers are roosts of higher quality than the damaged or dead pitchers of the other two *Nepenthes* species. Only *N. hemsleyana* pitchers provide the bats with a stable microclimate with respect to humidity, which is more variable inside *N. bicalcarata* and *N. ampullaria* pitchers. Moreover, bats roosting in the latter species are exposed to a higher parasite infestation risk. Only bats roosting in *N. hemsleyana* pitchers are free of parasites that depend on the bats' roost for reproduction (Schöner et al., 2013 and Schöner, unpublished data).

Currently, nothing is known about whether *N. hemsleyana* and *K. hardwickii* also incur costs from their interaction. Potentially, a variety of different costs would be possible. For example, bats could reduce *N. hemsleyana*'s arthropod capture rate when blocking the pitchers' entrance. The bats themselves may suffer from an increased predation risk when they use weakly pigmented pitchers because they are more visible from outside. Here, we investigated if

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factors that have already been shown to be costly in other bat-plant interactions also negatively affect either the bats or the pitcher plants in the currently studied mutualism.

Firstly, we wanted to test the hypothesis that *K. hardwickii* injure the pitchers with their claws when they enter or leave these roosts. From some other bat species it is known that they modify and damage the plant material they are roosting in (Kunz and McCracken, 1996). *N. hemsleyana* pitchers have a low lignin content (Osunkoya et al., 2008), thin walls and are very delicate. Thus, bat occupancy could decrease the pitchers' longevity.

Secondly, roosting in *N. hemsleyana* pitchers may also accrue costs for *K. hardwickii*. We hypothesised that the bats have to regularly invest considerable time in finding new and suitable *N. hemsleyana* pitchers, which then only provide short-lived roosts. Locating highly ephemeral roosts is a challenging task for bats, as has been shown for example in *Thyroptera tricolor* (Spix von, 1823) (Chaverri et al., 2010). *N. hemsleyana* pitchers collapse when they start senescing after a lifespan of a few weeks (Osunkoya et al., 2008). In contrast, the dead pitchers of *N. bicalcarata* and *N. ampullaria* are not only more abundant than the rare *N. hemsleyana* pitchers but they also maintain their shape because of their high lignin content (Osunkoya et al., 2008 and Schöner et al., 2013). Thus, bats should have to invest more energy for searching for suitable *N. hemsleyana* roosts than for the less suitable dead pitchers of *N. bicalcarata* and *N. ampullaria*, which we expected to be longer lasting. Additionally, it is unknown so far if *N. hemsleyana* pitchers are continuously provided at the same plant like in the case of *N. bicalcarata* and *N. ampullaria* plants. *N. bicalcarata* and *N. ampullaria* plants used by *K. hardwickii* always contain one to 30 additional habitable roost pitchers. In contrast, *N. hemsleyana* plants rarely offer more than four pitchers to the bats (personal observation).

Moreover, we hypothesised that heavy rainfall could temporarily fill up *N. hemsleyana* pitchers with water. Pitchers of this species are usually completely intact when used by the bats, and without holes in the bottom. This would lead to further costs for *K. hardwickii* using *N. hemsleyana* pitchers as incoming water could expel bats from their current roost immediately. If such an emergency event happens during daylight hours and in rainy conditions, the bats' predation risk and energy loss would be increased (Rydell et al., 1996 and Voigt et al., 2011).

Assessing these potential costs for the involved partners and the resulting cost-benefit ratio will shed light on the interaction strength in the mutualism between *N. hemsleyana* and *K. hardwickii*.

2. Methods

Field studies were conducted at three sites (referred to here as "Labi", "Saw Mill", "Badas") in the Belait district/Brunei Darussalam from 20 June to 3 December 2012 and from 14 April to 1 September 2014.

To determine whether bats damage the pitcher tissue and thus shorten the pitchers' longevity, we monitored 25 *N. hemsleyana* pitchers from the day they opened their lid until they started to decay and could no longer be occupied as the pitchers collapsed. Pitchers were checked daily for roosting bats. After the bats had left the pitchers we visually inspected if they had injured the inner pitcher tissue and/or peristome. With a Welch Two Sample *t*-test we tested whether pitchers occupied or not occupied by bats differ in their longevity. We computed a Pearson's product-moment correlation to investigate if the time period pitchers are occupied by bats negatively correlates with the pitchers' longevity.

The above-described measurements also provided data about how long *N. hemsleyana* pitchers in general are suitable roosts for bats. We additionally tested the hypothesis that any given plant of *N. hemsleyana* has at least one pitcher that is suitable for *K. hard-*

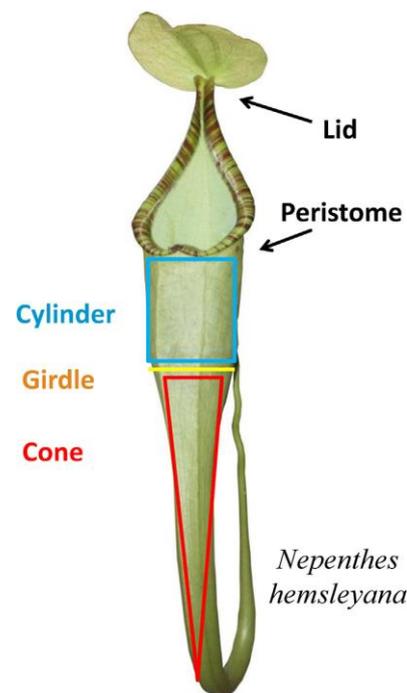


Fig. 1. Morphology of a *N. hemsleyana* pitcher.

wickii as a roost at any given time. Every day we monitored the 25 *N. hemsleyana* plants from 20 June to 3 December 2012 and checked if at least one pitcher is available for the bats to roost in. We investigated if the amount of plants that continuously provides pitchers differs from that with intermittent pitcher production using a Chi-squared test for given probabilities. We also determined how long the monitored *N. hemsleyana* plants did not offer a suitable pitcher to the bats. Moreover, we investigated how long it takes until a pitcher fully develops and if the development is completed before older pitchers of the same plant decay. Thus, we measured the time period from the day a pitcher opened its lid until the next pitcher of the same plant opened its lid. With a paired *t*-test we tested if the time *N. hemsleyana* plants need to produce new pitchers differs from the time periods the preceding pitcher is suitable as a bat roost.

To find out how long *N. bicalcarata* and *N. ampullaria* pitchers can be used by the bats, we marked 48 *N. bicalcarata* and 40 *N. ampullaria* pitchers that had already lost their digestive fluid and had just started to senesce in July 2012. We checked their condition in November 2012 (after four months) and again in April and August 2014 (after 21 and 25 months). We assumed that *N. bicalcarata* and *N. ampullaria* pitchers stop being suitable bat roosts when pitchers fell off the plants or when the bottom of the pitchers was destroyed. With Pearson's Chi-squared tests we compared if there were interspecific differences in the pitchers' suitability as bat roost after an observation period of four months.

Bats mainly use the cylindrical space above the girdle of *N. hemsleyana* pitchers for roosting (Grafe et al., 2011) (Fig. 1). If the fluid level reaches this roosting space, the bats are forced to search for a new roost. To find out if rainwater regularly floods *N. hemsleyana* pitchers, we checked and counted all *N. hemsleyana* pitchers after heavy rain in the three study sites in July and November 2012. We noted when the fluid level reached the pitcher's girdle. Moreover, we determined the canopy cover above the pitchers with a spher-

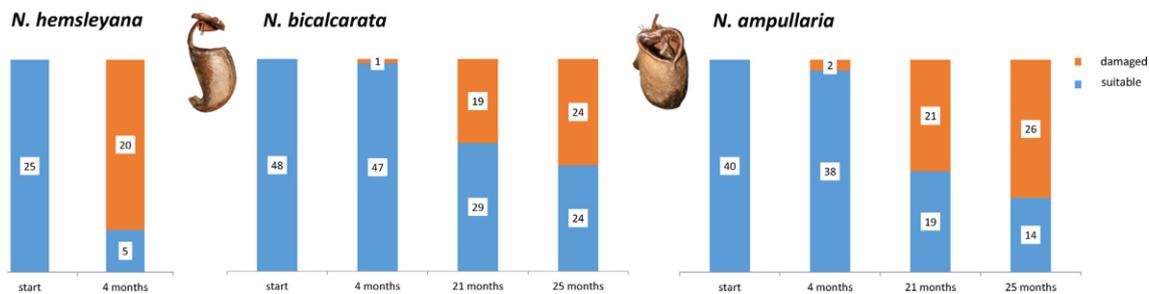


Fig. 2. Proportion of *N. hemsleyana*, *N. bicalcarata* and *N. ampullaria* pitchers suitable for bats to roost in after a time period of 4, 21 and 25 months. No *N. hemsleyana* pitcher lasted 21 months or longer.

ical densitometer (Convex Model A, Forestry Suppliers, Jackson, Canada) to find out whether pitchers with increased fluid levels mainly occur in areas with low canopy cover that are avoided by the bats (Schöner et al., 2013).

Data are presented as mean \pm standard deviation. We tested our data for normality (Shapiro test), homogeneity of variance (Bartlett test), and equality of distribution (Kolmogorov-Smirnov test) before applying parametric or non-parametric tests. Tests were conducted with R(v.3.1.1; R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Costs for *N. hemsleyana*

Ten of the 25 monitored *N. hemsleyana* pitchers harboured bats for a mean time period of 4.40 ± 2.62 days (range: 1–9 days) during the pitcher's lifetime. We could not find any injuries in the pitcher wall tissue after bats had roosted inside the pitchers. Moreover, the longevity of pitchers did not differ between pitchers that were not used by *K. hardwickii* (mean = 91.50 ± 28.76 days) and those used by the bats (mean = 88.33 ± 30.84 days; Welch Two Sample *t*-test: $t = 0.25$, $p = 0.80$). The number of days in which pitchers were occupied by the bats had no influence on the pitchers' lifespan (Pearson's product-moment correlation: $r = 0.50$, $p = 0.63$).

3.2. Costs for *K. hardwickii*

Within the study period, 19 of the 25 monitored *N. hemsleyana* plants continuously offered at least one pitcher to the bats. Thus, significantly more plants continuously provided at least one pitcher to the bats as only the remaining six *N. hemsleyana* plants had no pitcher (Chi-squared test for given probabilities: $\chi^2 = 6.76$, $df = 1$, $p = 0.009$) for a mean duration of 28.17 ± 17.02 days. On average, the monitored *N. hemsleyana* plants required 76.32 ± 13.65 days (range: 55–112 days) to produce a new pitcher, which was significantly shorter than the pitchers' lifespans (89.60 ± 30.07 days, range: 29–151 days; Paired *t*-test: $t = -2.21$, $p = 0.04$).

Both *N. bicalcarata* and *N. ampullaria* pitchers are long-term roosts as even after 25 months 50% of the 48 *N. bicalcarata* pitchers and 35% of the 40 *N. ampullaria* could still be occupied by the bats (Fig. 2). In contrast, *N. hemsleyana* pitchers had a significantly shorter lifespan: after four months, only 20% of the 25 monitored *N. hemsleyana* pitchers were still suitable bat roosts whilst in *N. bicalcarata* and *N. ampullaria* 98% and 95%, of the pitchers could still host bats after that time period (Pearson's Chi-squared test; comparing all species: $\chi^2 = 70.56$, $df = 2$, $p < 0.001$; *N. hemsleyana* vs. *N. bicalcarata*: $\chi^2 = 44.97$, $df = 1$, $p < 0.001$; *N. hemsleyana* vs. *N. ampullaria*: $\chi^2 = 35.37$, $df = 1$, $p < 0.001$; *N. bicalcarata* vs. *N. ampullaria*: $\chi^2 = 0.03$, $df = 1$, $p = 0.87$; Fig. 2).

Only in 4.82% ($\pm 1.13\%$) of the 413 monitored *N. hemsleyana* pitchers in the three study sites, fluid levels rose higher than the pitchers' girdle after heavy rainfall. Of these pitchers $83.73 \pm 18.19\%$ occurred at locations with a canopy cover of less than 50%. Bats usually avoid such open habitats (Schöner et al., 2013).

4. Discussion

4.1. Limited costs to the interaction between bats and pitcher plants

To test whether the plants and the bats experience negative effects from their interaction, we had selected factors that incur costs in other bat-plant interactions (e.g., Kunz and McCracken 1996; Chaverri et al., 2010). However, neither of the investigated potential costs appears to be significant in the mutualism between *N. hemsleyana* and *K. hardwickii*. This is in strong contrast to the benefits for both interacting species. Benefits for the bats include, e.g., a parasite-free roost with favourable microclimatic conditions, and the fact that there is no interspecific competition for pitchers as roosts. The pitcher plants are able to gain important nutrients although they grow on nutrient deprived habitats and are poor insect traps (Moran, 1996; Grafe et al., 2011 and Schöner et al., 2013). All these benefits should outweigh the low costs, if any are present at all.

N. hemsleyana pitchers are very thin and delicate due to their low lignin content (Osunkoya et al., 2008) and even small injuries in the pitchers' tissue can reduce their lifespan (Schöner, personal observation). However, during our observations, roosting bats did not cause any visible damage to the *N. hemsleyana* pitchers. Moreover, the bats had no influence on a pitcher's lifespan. Even repeated bat occupations did not cause untimely senescing. It could be that the low lignin content (Osunkoya et al., 2008) and the concomitant high elasticity of *N. hemsleyana* facilitate bat roosting. The flexible pitcher tissue adjusts itself to the bats' body shape and movements and could prevent the pitchers from damage by its inhabitants. Moreover, our results suggest that bats do not use their claws when entering and leaving the pitchers. Future studies have to clarify if these bats have adhesive skin structures at their thumbs and feet similar to those of other bat species roosting in plant structures (Riskin and Racey, 2010). Despite the rarity of *N. hemsleyana* pitchers as well as the slow production of new pitchers and their concealment in the vegetation, *K. hardwickii* preferentially seeks out and uses such roosts (Schöner et al., 2013) suggesting that these pitchers are a resource greatly valued by the bats. Therefore, it is reasonable to expect that the bats should avoid damaging these roosts if possible, as appears to be the case. Similarly, in tent roosting bats it is known that bats do not damage the leaves completely when modifying them into a tent so that the roost is available for long time periods (Cholewa et al., 2001).

N. hemsleyana pitchers have shorter lifespans than pitchers of *N. bicalcarata* and *N. ampullaria*. However, the lifespan of *N. hemsleyana* pitchers is still considerably longer (mean for all 25 pitchers = 89.60 ± 30.07 days) than that of highly ephemeral bat roosts such as developing tubular leaves of plants in the order Zingiberales (e.g., *Heliconia* (Linnaeus, 1753b)) that force their inhabitants to search for new roosts every day due to a rapid unfurling process (Vonhof and Fenton, 2004; Chaverri et al., 2010). Individuals of *K. hardwickii* normally switch *N. hemsleyana* pitchers after 1.77 ± 1.72 days (Schöner et al., 2013) but they often return to the same pitchers. In this study four of the eleven bats that had used one of the monitored *N. hemsleyana* pitchers returned to these pitchers after a time period of 36.75 ± 17.92 days. As the majority of *N. hemsleyana* plants continuously provide at least one mature pitcher suitable for roosting, bats are usually able to return to pitchers of the same plant and thus do not have to search elsewhere. This is especially important given the low number of available *N. hemsleyana* plants in most study sites (Schöner et al., 2013). In fact, we found *K. hardwickii* individuals roosting in different pitchers of the same plant after time periods of more than two years.

The vast majority of *N. hemsleyana* pitchers are a safe roost for bats since the fluid levels did not rise above the girdle (Fig. 1), even after heavy rain. Most of the pitchers with higher fluid levels were located in very open areas that are avoided by the bats probably due to extremely high daytime temperatures (Schöner et al., 2013). The lid of *Nepenthes* pitchers is known to minimise rainwater entry into the pitcher (Bauer et al., 2012). Incoming water would not only threaten the bats but also dilute the digestive fluid and decrease the pitcher's digestive capabilities (Clarke, 2006).

4.2. Implication for the mutualistic interaction

The mutualism between *K. hardwickii* and *N. hemsleyana* has most likely developed recently after the last glacial period (Meimberg and Heubl, 2006 and Chin et al., 2010). Our results suggest that not only high benefits for the involved species but also low costs (Connor, 1995) have promoted this mutualism (Grafe et al., 2011 and Schöner et al., 2013) within such a short time period. Further costs cannot be excluded but like in other close mutualisms, e.g., between yucca moths and yuccas or between ants and *Acacia* (Miller, 1754) plants, it is highly likely that the interacting partners limit and regulate the costs induced by the partner species (Addicott and Bao, 1999 and Raine et al., 2002). Future investigations are needed to show if, for example, plants with intermittent pitcher production are at risk of bats visiting them less frequently, leading to a significant reduction in the benefits delivered by the mutualism. Similar observations have been made in the mutualism between the tree shrew *Tupaia montana* (Thomas, 1892) that feeds on nectar produced by *Nepenthes rajah* (Hooker, 1859). Here, the plants produced less pitchers and secreted less nectar during times of limited rainfall. At the same time fewer faecal pellets of the tree shrews could be found in *N. rajah* pitchers possibly due to altered foraging strategies of the tree shrews (Clarke et al., 2010). Considering climate change will lead to prolonged drought periods, reductions in pitcher production may be the consequence and this again may undermine various animal–pitcher plant interactions including the mutualism between *N. hemsleyana* and *K. hardwickii*.

In conclusion, this study showed that the mutualism between bats and *N. hemsleyana* is characterised by a low cost to benefit ratio. This should further strengthen and stabilise their interaction (Foster and Wenseleers, 2006). Environmental change, however, may have a weakening effect on this and similar mutualisms.

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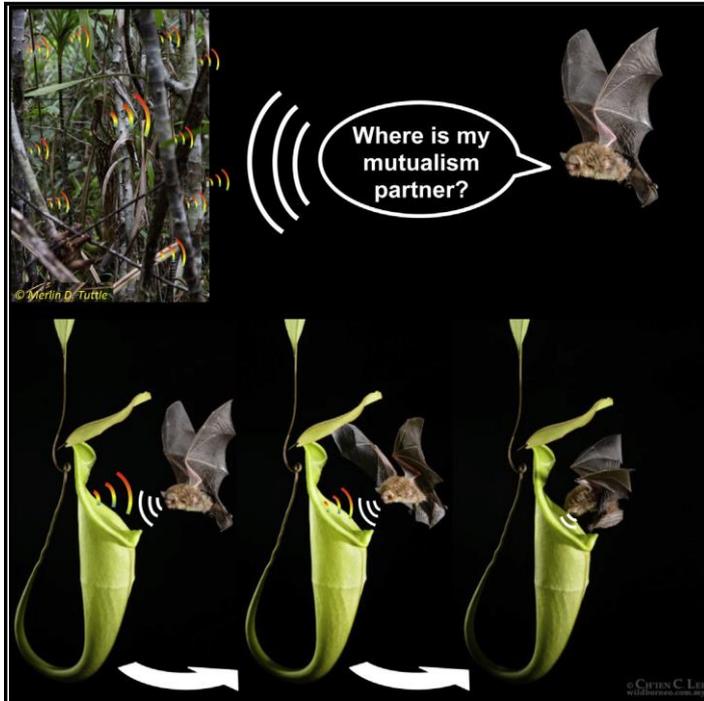
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Current Biology

Bats Are Acoustically Attracted to Mutualistic Carnivorous Plants

Graphical Abstract



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In Brief

To maintain mutualisms, plants specifically appeal to their animal partners' perception. Schöner et al. now show that Paleotropical carnivorous plants have reflective structures that are acoustically attractive for mutualistic bats. This phenomenon can similarly be found in a few Neotropical bat-pollinated flowers.

Highlights

- A carnivorous plant features an ultrasound reflector attractive for mutualistic bats
- This reflector enables the bats to easily find and identify the plant's pitchers
- The bats fertilize these Paleotropical plants with feces in exchange for roosts
- Such reflectors were convergently acquired in Neotropical bat-pollinated plants

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Bats Are Acoustically Attracted to Mutualistic Carnivorous Plants

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SUMMARY

Mutualisms between plants and animals shape the world's ecosystems [1, 2]. In such interactions, achieving contact with the partner species is imperative. Plants regularly advertise themselves with signals that specifically appeal to the partner's perceptual preferences [3–5]. For example, many plants have acquired traits such as brightly colored, fragrant flowers that attract pollinators with visual, olfactory, or—in the case of a few bat-pollinated flowers—even acoustic stimuli in the form of echo-reflecting structures [6–9]. However, acoustic attraction in plants is rare compared to other advertisements and has never been found outside the pollination context and only in the Neotropics. We hypothesized that this phenomenon is more widespread and more diverse as plant-bat interactions also occur in the Paleotropics. In Borneo, mutualistic bats fertilize a carnivorous pitcher plant while roosting in its pitchers [10, 11]. The pitcher's orifice features a prolonged concave structure, which we predicted to distinctively reflect the bats' echolocation calls for a wide range of angles. This structure should facilitate the location and identification of pitchers even within highly cluttered surroundings. Pitchers lacking this structure should be less attractive for the bats. Ensonifications of the pitchers around their orifice revealed that this structure indeed acts as a multidirectional ultrasound reflector. In behavioral experiments where bats were confronted with differently modified pitchers, the reflector's presence clearly facilitated the finding and identification of pitchers. These results suggest that plants have convergently acquired reflectors in the Paleotropics and the Neotropics to acoustically attract bats, albeit for completely different ecological reasons.

RESULTS AND DISCUSSION

How mutualisms evolve or how these interactions are maintained is still not sufficiently understood [12]. Particularly, if

partners regularly separate, they require species-specific mechanisms to find each other again. This is also true for the carnivorous pitcher plant *Nepenthes hemsleyana* (Nepenthaceae), which recently was reported to have a mutualistic interaction with the insectivorous bat *Kerivoula hardwickii* (Vespertilionidae). This bat fertilizes the plant with its feces while roosting inside the pitchers. The bat droppings enhance the nitrogen intake of *N. hemsleyana* by 34% on average [10]. In turn, the pitcher plants provide the bats with roosts that are free of parasites, have a stable microclimate, and offer enough roosting space for one or two bats while at the same time preventing the bats from falling into the digestive fluid due to their unique morphological shape and low fluid level [11]. Finding and identifying *N. hemsleyana* pitchers that grow in the dense Bornean peat swamp forests, however, is a challenging task for echolocating bats: they have to distinguish echoes of the pitchers from those of the cluttered surroundings [13, 14]. The situation is further complicated by the fact that the bats need to distinguish the rare [11, 15] *N. hemsleyana* pitchers from the more common and similarly shaped pitchers of sympatric *Nepenthes* species, which are unsuitable for roosting [10].

In the Neotropics, a few bat-pollinated plants found an efficient solution to attract bats by developing floral ultrasound reflectors [7, 9], which enabled them to exploit the bats' echolocation system. However, such reflectors have never been described for plants outside the Neotropics, probably because in the Paleotropics, chiropterophilous plants are pollinated by fruit bats (Pteropodidae) that are unlikely to use echolocation for foraging [16, 17]. We hypothesized that this phenomenon can also be found in the Paleotropics. If so, bat-dependent plants such as *N. hemsleyana* should have echo-reflecting structures making it easier for bats to localize and identify pitchers. Pitchers lacking such reflectors should be more difficult to find. Additionally, the bats should have echolocation calls that facilitate the finding of targets even within highly cluttered surroundings.

Do Pitcher Plants Have Ultrasound Reflectors?

To test whether a certain pitcher structure serves as an effective reflector that acoustically stands out in cluttered environments and guides the bats to their target, we measured ultrasound echoes of pitchers from different angles using a biomimetic sonar head. We sampled pitchers of both *N. hemsleyana* and its closest relative, *Nepenthes rafflesiana* (Figure S1), which does not host bats, and ensonified them in the elevation plane



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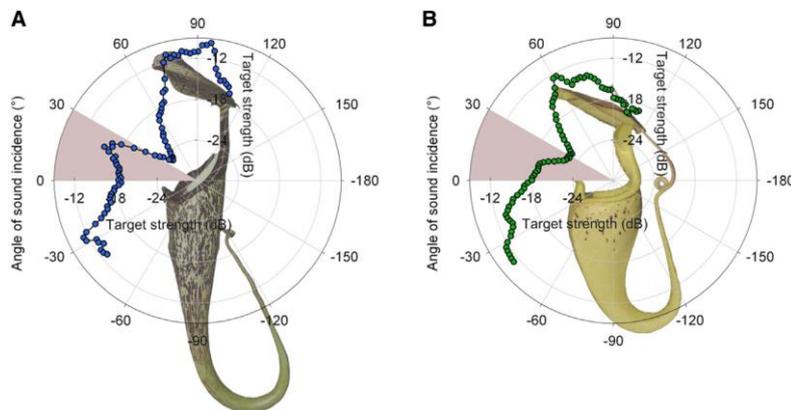


Figure 1. Echo Reflectance of *Nepenthes hemsleyana* and *Nepenthes rafflesiana* Pitchers for the Elevation Plane Given in Spectral Target Strength

(A and B) Target strength (TS; mean from 40 to 160 kHz) of *N. hemsleyana* (A) and *N. rafflesiana* (B) pitchers ($n = 9$), respectively, for different angles of sound incidence in the elevation plane. The reddish areas indicate where bats typically approach (see also Figures S1, S2, and S3A). Note that within this area (0° to 30°), the TSs of *N. hemsleyana* pitchers significantly exceed the TSs of *N. rafflesiana* pitchers.

(from -40° to 110° ; each species $n = 9$; Figure 1) and the azimuth (horizontal circular) plane (90° on either side of the pitchers' orifice; each species $n = 8$; Figure 2A).

We analyzed the mean spectral target strength (TS), which is a measure of acoustic backscattering of an object, for the whole frequency area of 40–160 kHz. For the measurements in the elevation plane, we found a clear peak for *N. hemsleyana* pitchers (Figure 1) for angles where the sonar beam ensonified the exposed and prolonged inner back wall at the pitcher's orifice. This concave structure is lacking in *N. rafflesiana* (Figure S1A) and other sympatric *Nepenthes* species (e.g., *N. ampullaria*, *N. bicalcarata*; Figure S1B). Consequently, within this area, *N. hemsleyana* pitchers have significantly higher TS than *N. rafflesiana* pitchers (Wilcoxon signed-rank test: $V = 11.0$, $p < 0.001$; compare Figures 1A, 1B, and S3A). Interestingly, this was also the area where the bats usually approached the pitchers (0° to 30° , data not shown; Figure S2). When ensonifying the pitchers from steeper angles ($>30^\circ$), the sonar beam pointed into the pitcher's cavity, resulting in a strong decline in TS for both species due to sound energy loss by multiple reflections. As *N. hemsleyana* pitchers are elongated compared to those of *N. rafflesiana*, the TS changed more abruptly and reached much lower values above 30° angles in the former species. This pattern of a very loud reflector echo followed by a weak echo of the pitcher's cavity can be seen as a contrast enhancement mechanism, which facilitates the recognition of the orifice.

Ensonifying *N. hemsleyana*'s orifice in the complete azimuth plane (180°) around the exposed inner pitcher surface showed that the TS for the area between -50° and $+50^\circ$ is significantly higher than in *N. rafflesiana* pitchers (Wilcoxon signed-rank test: $V = 0.0$, $p < 0.001$; Figures 2A and S3B). Thus, echoes from *N. hemsleyana* are reflected with higher intensity across a wide angle. As a result, the catchment area, which is the area where the bats are able to detect an object by echolocation, is also significantly larger for *N. hemsleyana* pitchers ($13.0 \pm 1.5 \text{ m}^2$, mean \pm SD) than for *N. rafflesiana* pitchers ($11.2 \pm 0.6 \text{ m}^2$; one-sided Welch two-sample t test: $t = -2.98$, $p = 0.007$). Such an increased catchment area can also be found in reflectors of bat-pollinated flowers in the Neotropics [7, 9].

One of these bat-pollinated plants (*Marcgravia evenia*) not only features an increased catchment area but additionally shows characteristic spectral signatures [9]. We therefore also analyzed

the spectral contents of the pitchers' echoes and found that directional spectral information of *N. hemsleyana* pitchers

clearly differs from that of *N. rafflesiana* (as exemplarily shown in the spectral directional plots in Figures 2B and 2C). Sliding-window comparisons (27°) of the spectra of *N. hemsleyana* and *N. rafflesiana* pitchers ($n = 8$ each) revealed significant spectral differences between the species within an angular range of 20° to 25° on either side of the pitcher's orifice, angles at which the back wall is ensonified (Figure 2D; see Supplemental Information). Thus, the bats could use the pitchers' species-specific spectral pattern to identify them, especially during lateral approaches, while the significantly increased TS of *N. hemsleyana* pitchers helps the pitchers to acoustically stand out in cluttered surroundings.

Are the Bats' Echolocation Calls Suited to Detect Pitchers in Highly Cluttered Space?

Bats in the genus *Kerivoula* generally have relatively short, high-pitched calls [18] covering a very large bandwidth, which further increases when they approach an object [19]. Such a call design is typical for the guild of narrow-space gleaning foragers [20] as it facilitates hunting in dense vegetation [19, 20]. Calls of *Kerivoula* have also been proposed to facilitate detection of fluttering prey [21].

To examine whether the bats' call design is also suitable for the detection of pitchers, we recorded the echolocation calls of five *K. hardwickii* individuals upon their approach toward pitchers, selected the last five calls, and analyzed their starting, peak, and end frequency, bandwidth, duration, and pulse interval [19] as well as directionality [22]. The analyzed calls consisted of only the first harmonic with a very short duration, broad bandwidth, and exceptionally high starting frequencies of up to 292 kHz (Figures 3A and 3B). To our knowledge, these are the highest frequencies ever recorded in bats. These high-pitched calls result in a very high call directionality [20, 23–25] (Figures 3A and 3C), which facilitate localization and classification of targets in cluttered surroundings as only the object of interest is ensonified while clutter echoes are blended out [23]. Thus, these calls are well suited to detect targets in highly cluttered space, including pitchers that are partially hidden in vegetation. Interestingly, other bat species interacting with plants that offer reflectors, e.g., *Glossophaga soricina*, have similar echolocation calls. They are also broadband and high pitched [25], except that Glossophagine calls often consist of multiple harmonics and are

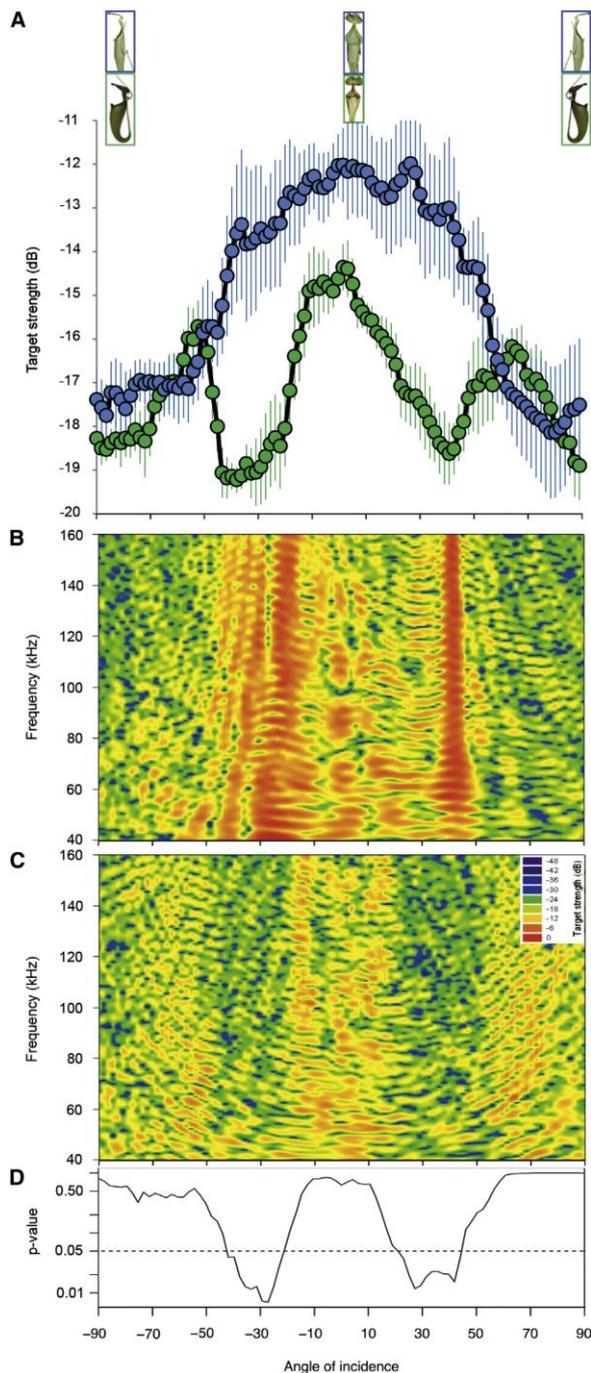


Figure 2. Echo Reflectance of *N. hemsleyana* and *N. rafflesiana* Pitchers in the Azimuth Plane

The *N. hemsleyana* and *N. rafflesiana* pitchers shown at the top of the figure indicate the different angles.

(A) Mean spectral TS (40–160 kHz) of *N. hemsleyana* (blue dots) and *N. rafflesiana* (green dots) pitchers ($n = 8$), respectively, for different angles of sound incidence in the azimuth plane (error bars show SE; see also Figure S3B).

slightly shorter. Generally, such calls should enable the bats to get a highly resolved acoustic image of targets and facilitate recognition of floral reflectors [25–27] or, in the case of *N. hemsleyana*, species-specific spectral signatures of the pitchers.

How Do the Bats React to the Ultrasound Reflector of *Nepenthes hemsleyana*?

To test the efficacy of the reflector of *N. hemsleyana* in attracting bats, we conducted a series of behavioral experiments with wild *K. hardwickii* in a flight tent. In the first experiment, we tested whether the reflector helps the bats to find pitchers faster in a cluttered environment. We measured the time until the bats ($n = 24$) approached a single pitcher hidden within shrubbery. In this experiment, the pitchers' reflector was either unmodified or enlarged or completely removed ($n = 8$ individual bats per type of pitcher; Table S1A; Movie S1). Bats needed significantly less time to approach enlarged (92.4 ± 58.5 s; $W = 2$; $p < 0.001$) and unmodified (182.1 ± 111.0 s; exact Wilcoxon rank-sum test: $W = 10$; $p = 0.02$) pitchers than those with removed reflectors (408.8 ± 228.1 s; Figure 4A).

In a second experiment, we tested whether the reflector is decisive for roost identification: we simultaneously confronted a single bat ($n = 18$) with three types of *N. hemsleyana* pitchers with modified reflectors (enlarged, partly or completely removed; Table S1B) and an unmodified *N. hemsleyana* pitcher as control (Movie S2). Bats approached enlarged pitchers significantly more often than expected by chance (number of approaches per bat = 3.1 ± 3.6 ; permutation tests, $p = 0.005$; for explanations, see Supplemental Experimental Procedures), whereas pitchers with reduced reflectors were approached significantly less frequently than expected (1.0 ± 1.3 ; $p = 0.03$; Table S2). The number of approaches to unmodified control pitchers did not differ from random expectations (2.1 ± 2.1 ; $p = 0.26$). These results confirm that the reflector is crucial for attracting the bats to the pitchers. When it came to the final roost selection, bats predominantly entered pitchers with unmodified reflectors and avoided those that had been enlarged or reduced ($p < 0.001$; Figure 4B; Table S1B). These results suggest that bats are initially attracted by the enlarged reflectors but then do not identify them as *N. hemsleyana*, possibly because such artificial reflectors do not contain the typical *N. hemsleyana* spectral cues.

To assess the importance of the reflector over other structures of the pitcher in attracting bats and to exclude the possibility that the bats generally avoided roosting in modified pitchers, we conducted further choice experiments. This time, we modified lids or peristomes of *N. hemsleyana* pitchers but kept the reflectors intact. The bats' roost choice was not influenced by such modifications (Table S1C), demonstrating that bats did not generally

(B and C) Exemplary spectral directional pattern of one *N. hemsleyana* pitcher (B) and one *N. rafflesiana* pitcher (C) for different angles of sound incidence (angular resolution 1.8°) in the azimuth plane.

(D) Results of the permutation testing the null hypothesis that *N. hemsleyana* and *N. rafflesiana* ($n = 8$ pitchers per species) did not differ in spectral content. p values (y axis) lower than 0.05 indicate significant differences in spectral content between the two species. Comparisons were conducted by calculating the mean log-spectral distance of 27° sliding windows in a pairwise manner (see Supplemental Information for further details).

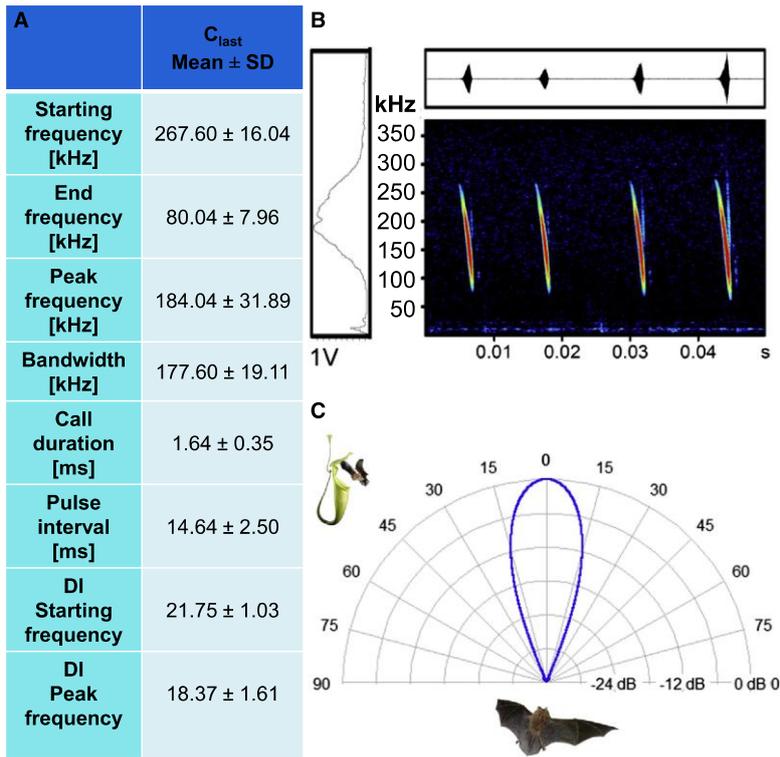


Figure 3. Echolocation Calls and Call Directionality of *Kerivoula hardwickii*

(A) Call parameters (n of all analyzed calls = 25) of the last five calls of a pitcher approach (C_{last}) and the referring call directionality (measured as directivity index [DI]). (B) Spectrogram, power spectrum, and oscillogram of the echolocation calls of *K. hardwickii*. (C) Beam shape of the calls of *K. hardwickii*. The high mean peak frequencies in C_{last} resulted in a very high call directionality (blue line; half-amplitude angle = 11°; photographs provided by C.C. Lee).

avoid roosting in modified pitchers and that other structures of the pitcher were not important compared to the reflector.

Taken together, the results of the ensonification measurements and the behavioral experiments provide strong support that the reflector of *N. hemsleyana* is crucial for the bats to find, identify, and finally enter pitchers.

Conclusions

As predicted, we found that bats are attracted to echo-reflective structures in a Paleotropical plant. Ensonifications revealed that

the exposed back wall of *N. hemsleyana* efficiently reflects acoustic signals over a wide range of angles of sound incidence. Additionally, the pitchers are characterized by a species-specific spectral pattern facilitating echo-acoustic recognition of *N. hemsleyana* pitchers by the bats. We confirmed the importance of the reflector for the detection and identification of suitable roost pitchers with behavioral experiments. According to our predictions, bats had a high affinity to pitchers with intact reflectors. They needed more time to find pitchers where the reflector was missing, and they subsequently rejected them as roosts. Interestingly, pitchers with enlarged reflectors were found faster in the cluttered environment and were approached more often. This suggests that natural selection could act on pitchers to develop larger reflectors, leading to more bat visits and hence a higher nutrient intake. Finally, due to the narrow beam width of their calls, the bats should easily recognize *N. hemsleyana* pitchers with a reflector, even within the typically cluttered environment they occur.

Overall, our findings suggest that *N. hemsleyana* exploits the bats' perceptual bias to attract them echo-acoustically. This helps the bats to quickly find and enter suitable day roosts and

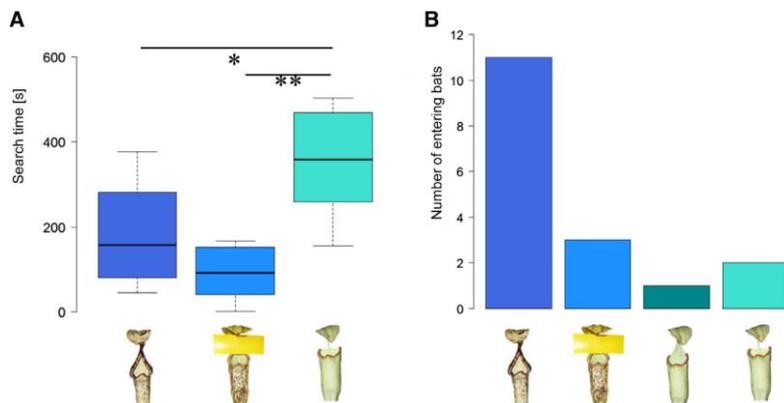


Figure 4. Behavioral Responses of *K. hardwickii* to Reflector Modifications

During behavioral experiments, bats could choose between pitchers whose reflectors were unmodified, enlarged, or (partly or completely) reduced (Wilcoxon rank-sum tests: *p < 0.05; **p < 0.01). (A) Search time for a single pitcher hidden in shrubbery. (B) Final choice of the bats between four simultaneously offered pitchers (see also Tables S1 and S2).

the plants to benefit from higher nitrogen intakes [10]. Our study provides the first example of a plant structure allowing bats to find it and identify it for reasons other than pollination. From an evolutionary point of view, our findings support the hypothesis that unrelated Neotropical bat-pollinated angiosperms and Asian carnivorous plants have convergent structures that specifically reflect bats' echolocation calls. Further studies will be necessary to infer whether structures involved in such complex plant-animal interactions primarily evolved by natural selection for their current use (adaptations to the bats) or were coopted for their current use (exaptations, probably followed by secondary adaptation), either from adaptations to other functions or from non-adaptive structures [28].

EXPERIMENTAL PROCEDURES

Experimental Ensonifications

We used a biomimetic sonar head with a ¼" free-field microphone (G.R.A.S. Sound & Vibration) and a custom-built condenser speaker (Sensory Technology, University of Erlangen). This setup allowed measurements within a frequency range of 40 to 160 kHz. Measurements were taken at a distance of 20 cm and from different angles around the pitcher's orifice (defined as 0°) for the elevation plane (−40° to +60°) and azimuth (±90°, Figure S1A) in increments of 1.8° [9].

Echolocation Call Analyses

Bats were caught in harp traps or in *Nepenthes* pitchers [11]. Call recordings and experiments were filmed in a flight arena (3.5 m × 3.5 m, height 2.5 m). Echolocation calls of five *K. hardwickii* were recorded (Avisoft UltraSoundGate 116 Hn; sampling rate 750 kHz) during approaches to pitchers. The microphone (CM16/CMPA) was placed 5 cm laterally behind the pitcher. Recordings were analyzed with SASLab Pro (256 FFT, FlatTop window, 87.5% overlap; threshold element separation of −30 dB relative to maximum) and were high pass filtered (30 kHz), and the noise was manually removed. We followed former approaches for the directivity index and half-amplitude angle calculations [22]. Immediately after the experiments, all bats were released into their original habitat.

Behavioral Experiments

Each bat was tested once in the flight tent while searching for a pitcher inside vegetation (for unmodified, enlarged or reduced reflectors: n = 8 bats each) or while choosing between randomly arranged unmodified and modified pitchers (reflector modification: n = 18; lid modification: n = 11; peristome modification: n = 10). We defined an approach as hovering flight in front of an object within a distance of 10 cm. Videos were analyzed by individuals without knowledge of the experimental design. Statistically, we compared the observed approach distribution to permuted datasets in which observed approach numbers were randomly allocated to the four provided pitchers (10,000 permutations).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, three figures, two tables, and two movies and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2015.05.054>.

AUTHOR CONTRIBUTIONS

M.G.S., C.R.S., and R.S. contributed equally to this manuscript. M.G.S., C.R.S., R.S., T.U.G., and G.K. developed the concept of the study. R.S. conducted the ensonification. C.R.S., M.G.S., and L.L.J. performed the behavioral experiments and call recordings. M.G.S., C.R.S., R.S., and S.J.P. analyzed the data. M.G.S., C.R.S., R.S., G.K., S.J.P., T.U.G., and L.L.J. wrote the paper.

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Supplemental figures

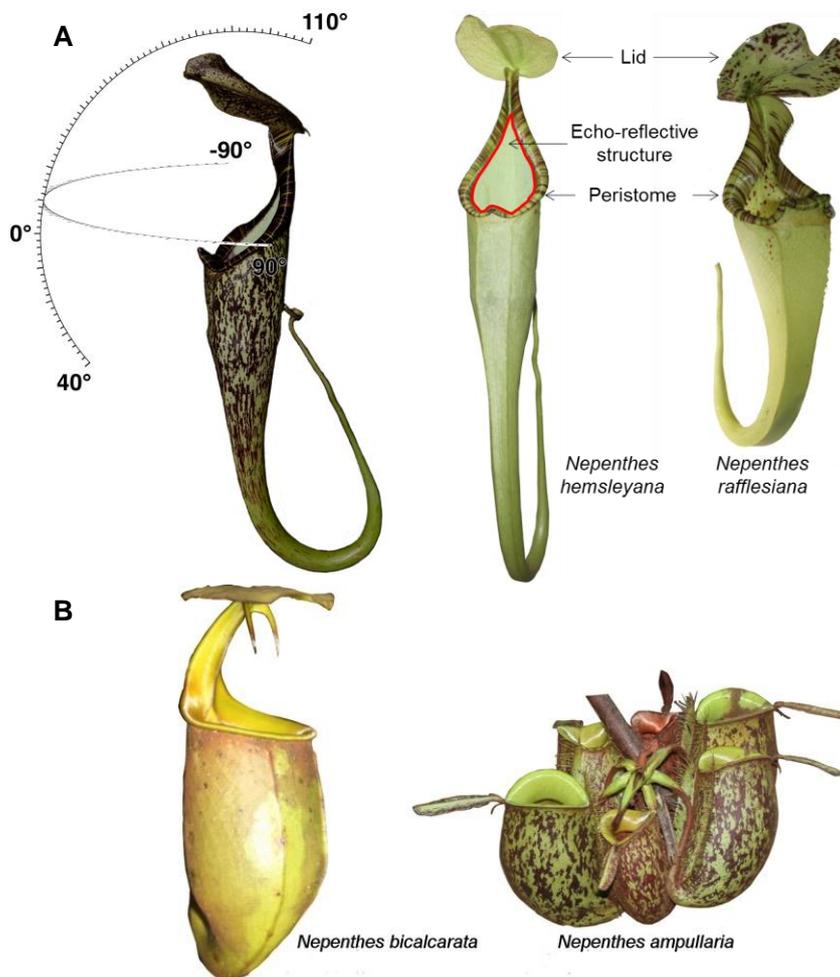


Figure S1, Related to Figure 1 and 2. *Nepenthes hemsleyana* and other sympatric *Nepenthes* species.

(A) Measurement planes of the ensonification exemplarily shown for one *N. hemsleyana* pitcher. Pitchers of *N. hemsleyana* and *N. rafflesiana* were ensonified from different directions in the elevation plane (9 pitchers per species) and in the azimuth plane around the pitchers' orifice (8 pitchers per species). In contrast to *N. rafflesiana*, *N. hemsleyana* has an exposed and echo-reflective inner backwall (indicated by the red marking). (B) The echo-reflective inner backwall is also missing in other sympatric *Nepenthes* species.



Figure S2, Related to Figure 1 and 2. Bat approach towards a *N. hemsleyana* pitcher.

(A) Approaching bats are initially echolocating towards the reflector, which directly leads them to the pitcher's orifice. (B), (C) Once the bats have reached this orifice they direct their calls into the pitchers (photographs provided by C. C. Lee).

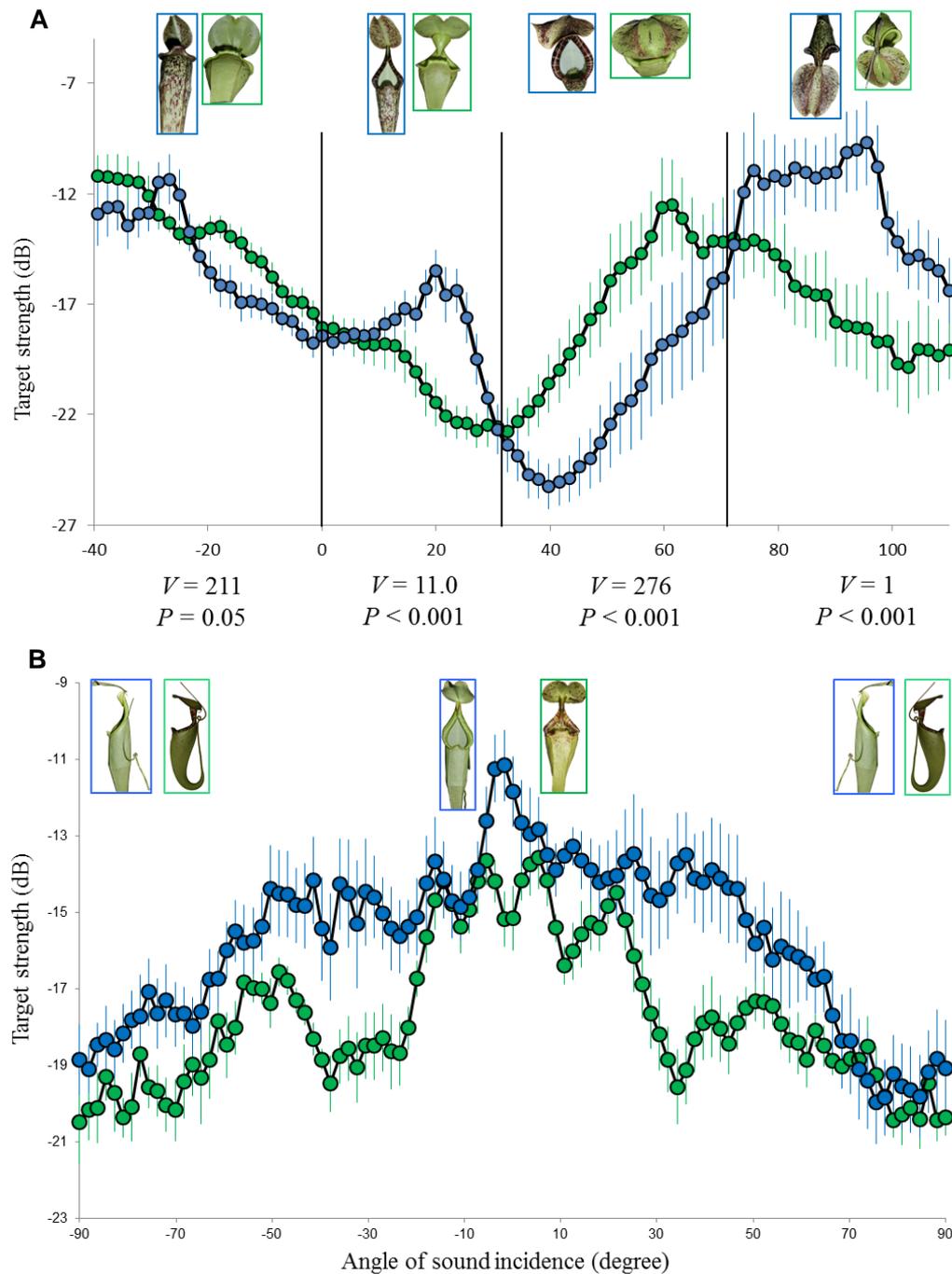


Figure S3, Related to Figure 1 and 2. Target strength of *N. hemsleyana* and *N. rafflesiana*.

The graph shows the direct comparison between *N. hemsleyana* (blue curve) and *N. rafflesiana* (green curve) for different angles of sound incidence (error bars show SE) in **(A)** the elevation plane ($n = 9$ pitchers per species) and **(B)** the azimuth plane ($n = 8$ pitchers per species) with the sonar head tilted 5° downwards.

Supplemental tables

Table S1. Modifications of *Nepenthes hemsleyana* pitchers in three behavioral experiments and experimental set ups.

(A) In initial experiments we measured the time until a bat approached the offered but partly hidden pitcher whose reflector was unmodified, enlarged or completely reduced. In further experiments we compared the bats' roost selection when we offered *N. hemsleyana* pitchers with a modified (B) reflector, (C) lid, or peristome. We simultaneously provided bats with one unmodified and three modified pitchers. Numbers indicate how many bats entered the four different *N. hemsleyana* pitchers in each choice experiment. Both in the lid and the peristome experiments bats randomly selected potential roosting pitchers independent of their degree of modification (lid: $P = 0.63$; peristome: $P = 0.94$). Thus, pitcher modifications (apart from modifications of the reflector) had no influence on the bats' roost choice.

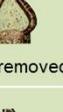
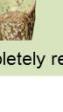
A Experiment 1		B Experiment 2		C Experiment 3	
 Shrubbery with pitcher  Shrubbery without pitcher  Camera		 1 unmodified and 3 modified pitchers  Camera			
Reflector	Reflector	Lid	Peristome	Lid	Peristome
					
unmodified	unmodified 11	unmodified 3	unmodified 2	unmodified 3	unmodified 2
	enlarged 3			1/3 removed 4	1/3 removed 3
enlarged	partly removed 1			1/2 removed 2	1/2 removed 2
	completely removed 2			completely removed 1	completely removed 1
completely removed	completely removed	completely removed	completely removed	completely removed	completely removed

Table S2. Approaches of bats to simultaneously offered *N. hemsleyana* pitchers with different reflectors.

The left column shows the total number of each bat's approaches towards the four pitchers (unmodified, enlarged, partially and completely removed reflector, respectively) in the reflector choice experiment. In the right column only approaches of *K. hardwickii* individuals (Kh) that landed on the pitchers are shown. Bats landed more often on unmodified reflectors than expected by chance (mean = 1.2 ± 1.0 ; $P < 0.001$), whereas there was no difference in enlarged reflectors (mean = 0.5 ± 0.7 ; $P = 0.48$). Pitchers with reduced reflectors were disfavored (mean = 0.2 ± 0.4 ; $P = 0.01$).

Bat-ID	Number of all approaches:				Number of approaches with landing:			
								
Kh01	3	9	0	4	1	2	0	1
Kh02	4	3	0	1	2	1	0	0
Kh03	3	0	0	1	2	0	0	0
Kh04	3	13	0	2	2	1	1	0
Kh05	1	2	2	4	1	0	0	1
Kh06	6	7	0	0	3	2	0	0
Kh07	1	0	0	0	1	0	0	0
Kh08	0	3	0	3	0	1	0	1
Kh09	0	0	2	3	0	0	0	1
Kh10	8	5	0	0	4	1	0	0
Kh11	1	0	0	0	1	0	0	0
Kh12	1	6	3	3	0	0	0	1
Kh13	1	0	0	0	1	0	0	0
Kh14	1	0	0	0	1	0	0	0
Kh15	2	4	3	2	1	0	0	0
Kh16	0	1	0	0	0	1	0	0
Kh17	1	1	0	1	1	0	0	0
Kh18	1	1	1	1	1	0	1	0
Total	37	55	11	25	22	9	2	5

Supplemental Experimental Procedures

Permits: Capturing and handling of the bats was conducted with permission of the University Brunei Darussalam Research Committee (UBD/PNC2/2/RG105 &193) adhering to the Animal Behavior Society Guidelines [S1] and the Forest Department Sarawak (NCCD.907.4.4(JLD.10)-207).

Description of study site and time: From 20 June to 3 December 2012 and from 5 April to 10 September 2014 we conducted field studies in the peat swamp and heath forests of the Belait district of Brunei Darussalam [S2] and in the Gunung Mulu National park, Sarawak, Malaysia.

Experimental ensonifications: We ensonified *N. hemsleyana* and *N. rafflesiana* pitchers with a biomimetic sonar head consisting of a custom built condenser speaker with a membrane made of Electro Mechanical Film and a $\frac{1}{4}$ " free-field microphone Type 40BF in combination with the preamplifier 26AB, which was connected to the power module 12AA (all from G.R.A.S. Sound & Vibration, Denmark). Using a continuously replayed MLS (Maximum Length Sequence) for ensonification allowed us to retrieve IR (impulse responses) through deconvolution of echo and original MLS. The frequency response of the speaker allowed measurements between 40-160 kHz (sound pressure levels at 1 m distance: approximately 95 ± 6 dB) [S3] covering *K. hardwickii*'s peak and end frequency range. We measured from a distance of 20 cm from different angles (1.8° /step) around the pitcher's orifice (defined as 0°) for the elevation (-40 to $+60^\circ$) and the azimuthal plane ($\pm 90^\circ$; Figure S1A). For the azimuthal measurement the sonar head was directly ensonifying the backwall structure between the lid and the pitcher's orifice (Figure 2A). During a further azimuthal measurement the sonar head was tilted 5° downwards pointing into the pitchers cavity (results

of this measurements are shown in Figure S3B). For the calculation of the detection distances we used the sonar equation [S4]: $DT = SL + TLA + TLS + TS$ (dB), where DT is the detection threshold, SL is the source level of the bat's call, TLA is the transmission loss owing to absorption, TLS is the transmission loss owing to spherical spreading and TS is the target strength of the pitcher. TLA and TLS are functions of distance. We calculated detection distances for a source level of 90 dB SPL (which is a conservative estimate for the echolocation call intensity of *Kerivoula*) and assumed a detection threshold of 0 dB [S5]. TLA and TLS were calculated for a frequency of 80 kHz, a temperature of 20°C and 97% humidity. To deduce the catchment area for every pitcher, we calculated the detection distance for every measurement. From these distances we extrapolated the catchment area. As data were normally distributed (Shapiro test), a one-sided Welch two-sample *t* test was applied to test if there were differences in the catchment area between *N. hemsleyana* and *N. rafflesiana*. To compare results of the ensonifications' azimuth and elevation plane of the two pitcher plant species, we used Wilcoxon signed rank tests as these data were not normally distributed. These and all following tests were conducted with R (v.2.15.2; R Foundation for Statistical Computing, Vienna, Austria).

Spectral comparison: To find out if echoes reflected from *N. hemsleyana* and *N. rafflesiana* pitchers have species-specific spectral features, we compared spectra of the azimuthal measurement (see Fig 2B and 2C). We computed intra- and inter-specific pairwise comparisons of spectra from 8 pitchers from each species (*N. hemsleyana* and *N. rafflesiana*). For each comparison, we compared spectral content using a 27° angle sliding window (step=1.8°). Within each sliding window, the Log-spectral distance D between the two pitchers was calculated for each measurement (every 1.8°) and then averaged (arithmetic mean). As the spectra of the different species had different overall TS levels (see Fig. 2A) and

we only wanted to deduce the spectral difference (e.g., different frequency of notches), we centralized the data of each spectrum to the mean energy prior to calculating distances between pitchers. The following formula was used to calculate Log-spectral distance D :

$$D_{\text{spectrum1} | \text{spectrum2}} = D_{\text{spectrum2} | \text{spectrum1}} = \sqrt{\sum \left[10 \times \log_{10} \left(\frac{\text{spectrum1}}{\text{spectrum2}} \right) \right]^2}$$

These comparisons were done with a custom written LabView code (LabView, National Instruments, Austin, Texas, United States).

With permutations we tested the null hypothesis that species did not differ in spectral content. The following statistic [mean (diff)] was used as an estimate of the distance between the two species that is not due to within species variability:

$$\text{mean(diff)} = \text{mean(diff Nh/Nr)} - \frac{\text{mean(diff Nh)} + \text{mean(diff Nr)}}{2}$$

with 'mean(diff Nh/Nr)' being the mean inter-specific Log-spectral difference, 'mean(diff Nh)' and 'mean(diff Nr)' the mean intra-specific Log-spectral difference for *N. hemsleyana* and *N. rafflesiana*, respectively. We compared the observed (mean(diff)) value to the distribution of values expected under the null hypothesis. To obtain the null hypothesis distribution we randomly assigned species status and then calculated each intra- and the inter-specific mean difference. This procedure was repeated 10,000 times. Then we calculated the P -values by comparing the observed mean inter-specific differences 'mean(diff)' to the null distributions.

Echolocation call recording and analyses: In the flight arena we recorded echolocation calls of five female *K. hardwickii* during approaches to pitchers with an Avisoft UltraSoundGate 116Hn (sampling rate 750 kHz). We directly placed the microphone (CM16/CMPA condenser microphone; frequency range 10 to 250 kHz) laterally behind the focal pitcher's entrance (distance: 5 cm). For the analyses we used SASLab Pro (256 FFT, FlatTop window, 87.5 % overlap). We set a threshold element separation of -30 dB relative to maximum.

Noise-induced errors were avoided by a high-pass filter (30 kHz) and manual background noise removal. We analyzed the last five calls (C_{last} ; $n = 25$) of a bat approaching a pitcher within a maximal distance of 20 cm to exclude atmospheric damping. To avoid pseudo-replication due to the presence of more than one call per individual, we generated 10,000 data sets by randomly selecting one call per individual bat and then ran 10,000 tests resulting in 10,000 P -values from which we calculated the median.

Following Jakobsen et al. (2013) [S6] we calculated the intensity of a signal at different angles from the source by using a Piston model

$$R_p(\theta) = \frac{2 \times J_1(k \times a \times \sin(\theta))}{k \times a \times \sin(\theta)}$$

(with $R_p(\theta)$ = ratio between the pressure on-axis and at a given angle θ ; J_1 = a first-order Bessel function of the first kind; $k = 2\pi/\lambda$; λ = wavelength; a = piston radius), and the directivity index ($DI = 20\log_{10}(2\pi a/\lambda)$). To estimate DIs, we used a constant gape assumption for which we measured the gape height (0.0025 ± 0.004 m) from five living *K. hardwickii*. Atmospheric attenuation was accounted for a relative humidity of 97%.

Behavioral Experiments: We caught bats in harp traps or *Nepenthes* pitchers and marked them with PIT-tags for individual identification [S3] to ensure that each bat was tested only once. Experiments were filmed (Sony HDR-CX560VE) in a flight arena (3.5 m \times 3.5 m, height 2.5 m) and conducted in the early morning hours around dawn (5:00 to 7:00), which is the normal time when bats are searching for new roosts (personal observation during radio-tracking studies). We fed and released the bats within 12 hours of capture into their original habitat. Pregnant and lactating females as well as juveniles were excluded from the experiments. To be sure that fragrance definitely has no influence on the bats' choice, we had emptied all experimental pitchers and washed them before starting the experiment.

To find out if the reflector reduces the time a bat needs to find a pitcher in cluttered habitat (Experiment 1, Table S1A), we placed shrubbery of plants that naturally occur close to pitcher plants (e.g., *Macaranga bancana*) in each of two corners of the flight arena. Then we randomly placed an unmodified pitcher or one where the reflector had been enlarged or removed in one of the two shrubberies so that leaves surrounded around 40% of a pitcher. Importantly, the potential reflector part was freely accessible. We tested each bat (19 males, 5 females) once randomly with only one of the three pitcher types. For the randomizations we used the “sample” function in R. To limit the number of pitchers that we had to remove from the field, we tested up to two different bats with the same pitcher (in total 18 pitchers) for this experiment. We released each bat in the flight arena in front of the camera and stopped the time when the bat first approached a pitcher. We defined an approach as frontal flight towards an object within a distance of 10 cm for at least 0.2 s. Using Kruskal-Wallis and Wilcoxon rank sum tests we compared search times for the different treatments.

Furthermore, we conducted experiments in which each bat could choose between one unmodified and three pitchers with modified reflector (6 male, 12 female bats; 44 different pitchers; Experiment 2, Table S1B), the lid (5 males, 6 females; 30 pitchers), or the peristome (5 males, 5 females; 25 pitchers; Experiment 3, Table S1C). For these experiments we used each pitcher to test up to three different bats (1.41 ± 0.62 mean \pm s.d.). We randomly arranged the unmodified and the three modified ($1/3$, $1/2$ or complete lid or peristome removed) pitchers within the flight arena (distance to each other = 0.5 m; height = 1.5 m). Each bat was tested only once per type of experiment but due to the limitation of individuals 12 of the 25 bats were tested in different types of experiments. One bat in each of the experiments regarding the reflector and the lid and two bats in the peristome experiment made no choice within the maximum time span of 20 min per trial so that we had to exclude them from the analyses of the bats' final pitcher choices. Individuals without background knowledge on the experiments

analyzed the videos. Because of camera problems two experiments had to be analyzed based on direct observations during their performance. For the statistical analysis we pooled the approaches to the partly and completely removed reflectors into a 'modified reflector' treatment as there was no difference between them ($P = 0.15$).

For the permutation tests, we tested the null hypothesis that the treatments did not affect the number of approaches. We first calculated each treatment's mean number of approaches, which we then compared to the distribution of values expected under the null hypothesis. To obtain the null hypothesis distribution we permuted the number of approaches between treatments for each tested animal and then calculated the mean number of approaches per treatment. We repeated this procedure 10,000 times from which the null distribution of the mean number of approaches was obtained. Then we calculated the P -value by comparing the mean number of approaches for the considered treatment to the null distribution.

Supplemental References

- S1. (2012). Guidelines for the Treatment of Animals in Behavioural Research and Teaching. *Anim. Behav.* 83, 301–309.
- S2. Schöner, C.R., Schöner, M.G., Kerth, G., and Grafe, T.U. (2013). Supply Determines Demand: Influence of Partner Quality and Quantity on the Interactions between Bats and Pitcher Plants. *Oecologia* 173, 191–202.
- S3. Simon, R., Holderied, M.W., Koch, C.U., and Helversen, O. von (2011). Floral Acoustics: Conspicuous Echoes of a Dish-Shaped Leaf Attract Bat Pollinators. *Science* 333, 631–633.
- S4. Nachtigall, P.E., and Moore, P.W.B. (1988). *Animal Sonar* (Boston, MA: Springer US).
- S5. Goerlitz, H.R., ter Hofstede, H.M., Zeale, M.R., Jones, G., and Holderied, M.W. (2010). An Aerial-Hawking Bat Uses Stealth Echolocation to Counter Moth Hearing. *Curr. Biol.* 20, 1568–1572.
- S6. Jakobsen, L., Ratcliffe, J.M., and Surlykke, A. (2013). Convergent Acoustic Field of View in Echolocating Bats. *Nature* 493, 93–96.

4.3 Contribution to Publications

Manuscript 1

Grafe, T.U., **Schöner, C.R.**, Kerth, G., Junaidi, A. & Schöner, M.G. (2011). A novel resource-service mutualism between bats and pitcher plants. *Biol. Lett.*, 7, 436–439.

In collaboration with Michael G. Schöner I contributed to the study design and was responsible for data acquisition and data analysis including the statistics. Together with all co-authors I wrote and edited the manuscript.

Manuscript 2

Schöner, C.R., Schöner, M.G., Kerth, G. & Grafe, T.U. (2013). Supply determines demand: influence of partner quality and quantity on the interactions between bats and pitcher plants. *Oecologia*, 173, 191–202.

Under the guidance of Gerald Kerth and T. Ulmar Grafe I was in charge of experimental design, and the main part of data acquisition and analysis. Moreover, I was primarily responsible for writing the manuscript and for correspondence with the journal and public media.

Manuscript 3

Lim, Y.S., **Schöner, C.R.**, Schöner, M.G., Kerth, G., Thornham, D.G., Scharmann, M. & Grafe, T.U. (2015). How a pitcher plant facilitates roosting of mutualistic woolly bats. *Evol. Ecol. Res.*, 16, 581–591.

I collected and analysed all data on the bats and their roosts relevant for the bat-related section of the publication. Moreover, I was involved in the writing and editing process of the manuscript.

Manuscript 4

Costs and benefits in a bat-pitcher plant mutualism

Schöner, C.R., Schöner, M.G., Grafe, T.U., Clarke, C.M., Dombrowski, L., Tan, M.C. & Kerth, G. (submitted). Ecological outsourcing: a pitcher plant benefits from transferring pre-digestion of prey to a bat mutualist.

Together with Gerald Kerth and Michael Schöner I developed the concept of the study. I was the main person responsible for data acquisition, analysis, the writing process and was also the corresponding author.

Manuscript 5

Schöner, C.R., Schöner, M.G., Kerth, G., Suhaini, S.N. & Grafe T.U. (2015). Low costs reinforce the mutualism between bats and pitcher plants. *Zool. Anz.*, 258, 1-5.

I was the main person responsible for the concept of the study, for data acquisition,

Manuscript 6

Schöner, M.G., **Schöner, C.R.**, Simon, R., Grafe, T.U., Puechmaille, S.J., Ji, L.L. & Kerth, G. (2015). Bats are acoustically attracted to mutualistic carnivorous plants. *Curr. Biol.*, 25, 1911-1916.

On this paper I was co-first author with Michael G. Schöner and Ralph Simon. Together with Michael G. Schöner and under the guidance of Gerald Kerth I developed the experimental design of the study. I collected data regarding the behavioural experiments and was responsible for data analysis. Moreover, I was involved in the writing and editing process of the paper.

Prof. Dr. Gerald Kerth

Caroline Regina Schöner

EIGENSTÄNDIGKEITSERKLÄRUNG

EIGENSTÄNDIGKEITSERKLÄRUNG

Hiermit erkläre ich, dass diese Arbeit bisher von mir weder an der Mathematisch-Naturwissenschaftlichen Fakultät der Ernst-Moritz-Arndt-Universität Greifswald noch einer anderen wissenschaftlichen Einrichtung zum Zwecke der Promotion eingereicht wurde.

Ferner erkläre ich, dass ich diese Arbeit selbständig verfasst und keine anderen als die darin angegebenen Hilfsmittel und Hilfen benutzt und keine Textabschnitte eines Dritten ohne Kennzeichnung übernommen habe.

Unterschrift des Promovenden

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2007 – 2011	Teaching degree: German
2004 – 2011	Teaching degree: Biology, Chemistry
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Received awards:

15/5/2015	2. Student poster award at the 4 th International Berlin Bat Meeting
15/8/2013	Karl F. Koopman Award for oral presentation at the 16 th International Bat Research Conference, Costa Rica
03/3/2013	1. Student poster award at the 3 rd International Berlin Bat Meeting
28/7/2011	Honours for best teaching degree

Publications:

Peer reviewed articles:	Schöner, C.R., Schöner, M.G., Grafe, T.U., Clarke, C.M., Dombrowski, L., Tan, M.C. & Kerth, G. (submitted). Ecological outsourcing: a pitcher plant benefits from transferring pre-digestion of prey to a bat mutualist.
	Lim, Y.S., Schöner, C.R., Schöner, M.G., Kerth, G., Thornham, D.G., Scharmann, M. & Grafe, T.U. (2015). How a pitcher plant facilitates roosting of mutualistic woolly bats. <i>Evol. Ecol. Res.</i> , 16, 581–591.
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Biological field research:

04/2014 – 09/2014	Biological field research in Brunei and Malaysia
06/2012 – 12/2012	Biological field research in Brunei and Malaysia
08/2011 – 01/2012	Biological field research in Brunei
06/2009 – 08/2009	Biological field research in Brunei
08/2009 – 10/2010	Monitoring of colonies of Bechstein's bats
05/2008 – 10/2008	Diploma thesis, topic: The colony is more than the sum of its members: Influence of individual behavior and information transfer on the organization of a Bechstein's bats' colony (<i>Myotis bechsteinii</i>)
04/2008 – 10/2008	Internship: Long-term conservation of black adders

Skills and further expertise, advanced trainings, memberships:

Language skills:	English (fluent), French (very good command), Malay, Spanish (good command), Latin
Academic skills:	University teaching, supervision of internships, and graduate and master students
Ecological skills:	Radio-telemetry, habitat analyses, experimental setups, bioacoustics, population genetics, PAM-fluorometry
Advanced trainings:	E.g., project management; research ethics; university didactics; analysis u. visualizations of ecological data with GIS, statistics with R
Scientific Memberships:	Since 2014: IUCN Red List Group for Carnivorous Plants Since 2012: South East Asian Bat Conservation Research Unit (SEABCRU) Since 2010: Mediävistenverband e.V. (Medievalists' Society) Since 2010: Association for Tropical Biology and Conservation (ATBC) Since 2009: DZG - Deutsche Zoologische Gesellschaft (German Zoological Society)

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