Stabilization of a bat-pitcher plant mutualism

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“We cannot force the world to conserve tropical nature;

we must seduce it, and the bait is intellectual mutualism”.

Daniel H. Janzen

To Caroline,

my best friend, love, and wise colleague,

and to Sophia Charlotte,

may you always discover the world with your own eyes.

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ABSTRACT

Abstract

Presumably every organism on earth is involved in at least one mutualistic interaction with one or several other species. To interact with each other, the species need traits that provide benefits to the partner species. Surprisingly, the function of traits for the stabilization of mutualisms has rarely been investigated, despite of a general lack of knowledge how mutualisms are maintained.

The aim of this work was to find functional traits, which stabilize the mutualism between a bat species and a carnivorous pitcher plant in Northern Borneo. *Kerivoula hardwickii* is the only bat species known to roost in pitcher-shaped trapping organs of Palaeotropical pitcher plants (*Nepenthes*). These bats fertilize the pitcher plant *Nepenthes hemsleyana* with their nutritious nitrogen-rich faeces while roosting inside the pitchers. The plants have outsourced capture and digestion of arthropod prey to the bats on which they strongly rely for nutrient acquisition. The bats in contrast are less dependent on their mutualism partner as they also roost in pitchers of two further *Nepenthes* species as well as in developing furled leaves of various plant species in the order Zingiberales. In earlier studies, we found that *N. hemsleyana* outcompetes alternative roosts by providing high-quality roosts for the bats. However, which traits exactly stabilize the mutualism between *K. hardwickii* and *N. hemsleyana* was still unclear.

I found that both the bats and the pitcher plants show traits, which have the potential to stabilize their interaction. On the level of morphological traits, I found that the pitchers have a low fluid level and a particular shape that provide just enough roosting space for one individual of the solitary *K. hardwickii*, a mother with juvenile or a mating couple. The bats have enlarged thumb and foot pads that enable them to cling to the smooth surfaces of their roosts without using their claws. This avoids damage to the sensitive *N. hemsleyana* pitchers. On the level of communicational traits, again *N. hemsleyana* acquired morphological structures that act as effective ultrasound-reflectors, which guide the echo-orientating bats to the opening of the pitchers and help the bats to identify their mutualism partner. The bats’ calls on the other hand are characterized by extraordinary high starting frequencies and broad bandwidths, which enable *K. hardwickii* to easily locate pitchers of *N. hemsleyana* and other *Nepenthes* species in their dense habitats. Finally, on the level of behavioural traits the bats often but not always prefer their mutualism partner to other roosts when they can select roosts in their natural environment or in behavioural experiments. The reason for this behaviour seems to be a combination of 1) *N. hemsleyana*’s superior quality compared to alternative roosts and 2) different roosting traditions of the bats.

In conclusion, the mutualism between bats and pitcher plants is asymmetric as *N. hemsleyana* is more dependent on *K. hardwickii* than vice versa. For the plants bat faeces present their most important nutrient source. In contrast, *K. hardwickii* can select between alternative roosting plants. This asymmetric dependency is reflected in the specifity and function of the traits that stabilize the mutualism in each of the two involved species. Especially on the morphological level, *N. hemsleyana* seems to have evolved several traits that perfectly fit to *K. hardwickii*. In contrast, the bats’ traits more generally facilitate their roosting in funnel-shaped plant structures and their occurrence in cluttered habitats. Thus, they are probably exaptations (i.e. traits that evolved for another reason) that are nevertheless functional and stabilize the mutualism with *N. hemsleyana*. This plant‘s superior roost quality is likely a consequence of the competition with alternative roosting plants and is a pre-requisite for the bats to prefer *N. hemsleyana*. Moreover, my study confirms earlier findings that asymmetric dependencies support the stabilization of mutualistic interactions. Finally, my work indicates that the specifity of functional traits can be used as a measure to determine mutual dependencies of mutualistic partners.

Zusammenfassung

Buchstäblich jeder Organismus der Erde, so vermutet man, interagiert auf mutualistische Weise mit einer oder mehreren anderen Arten. Um miteinander interagieren zu können, benötigen die betroffenen Spezies Merkmale, die für den Partner gewinnbringend sind. Überraschenderweise wurden solche Merkmale auf ihre Funktion für die Stabilisierung von Mutualismen eher selten untersucht und dies, obwohl noch immer unklar ist, welche Mechanismen Mutualismen erhalten.

Ziel dieser Arbeit war es, funktionelle Merkmale zu finden, die den Mutualismus zwischen einer Fledermausart und einer fleischfressenden Kannenpflanze auf Nord-Borneo stabilisieren. Als einzig bekannte Fledermausart übertagt *Kerivoula hardwickii* in den kannenförmigen Fangorganen paläotropischer Kannenpflanzen (*Nepenthes*). Dabei düngen die Fledermäuse die Kannenpflanzenart *Nepenthes hemsleyana* mit ihrem stickstoffhaltigen, nährstoffreichen Kot. Die Pflanzen haben den Fang und die Verdauung ihrer Beute, die vor allem aus Arthropoden besteht, auf die Fledermäuse ausgelagert. Von diesen sind *N. hemsleyana* daher stark abhängig, um ihren Nährstoffbedarf decken zu können. Im Gegensatz dazu hängen die Fledermäuse weniger von *N. hemsleyana* ab, da sie zusätzlich die Kannen anderer *Nepenthes*-Arten sowie sich entwickelnde gedrehte Blätter einer Reihe von unterschiedlichen Pflanzenarten der Ordnung Zingiberales als Tagesquartier nutzen. In früheren Studien konnten wir zeigen, dass *N. hemsleyana* aufgrund ihrer Qualität als Fledermausquartier wettbewerbsstärker ist als diese (unfreiwillig) konkurrierenden Pflanzenquartiere. Welche funktionellen Merkmale jedoch den Mutualismus zwischen *N. hemsleyana* und *K. hardwickii* stabilisieren, war bisher weitgehend unklar.

Sowohl für die Pflanzen wie für die Fledermäuse konnte ich Merkmale bestimmen, die potentiell stabilisierend auf die Interaktion der beiden Partner wirken. Auf morphologischer Merkmalsebene fand ich heraus, dass die Pflanzen einen niedrigen Flüssigkeitsstand und eine spezielle Form haben, die gerade genügend Platz für ein einzelnes Individuum, eine Mutter mit Jungtier oder ein sich fortpflanzendes *K. hardwickii*-Pärchen bereitstellt. Die Fledermäuse haben vergrößerte Daumen- und Fußflächen, die es ihnen erlauben, sich an die glatten Oberflächen ihrer Tagesquartiere zu hängen, ohne ihre Krallen einsetzen zu müssen. Dadurch vermeiden sie Beschädigungen an der sensiblen Oberfläche der *N. hemsleyana*-Kannen. Auf der kommunikativen Merkmalsebene konnte ich zeigen, dass *N. hemsleyana* morphologische Strukturen aufweist, die als effektive Ultraschallreflektoren wirken. Diese weisen den echoortenden Fledermäusen den Weg zur Kannenöffnung und helfen den Tieren, ihren Mutualismuspartner zu erkennen. Die Ultraschallrufe der Fledermäuse wiederum sind gekennzeichnet durch ungewöhnlich hohe Startfrequenzen und enorme Bandbreiten, die es *K. hardwickii* erlauben, die Kannen von *N. hemsleyana* und anderer *Nepenthes*-Arten in ihren dichtbewachsenen Habitaten aufzufinden. Auf Ebene der Verhaltensmerkmale schließlich bevorzugen die Fledermäuse häufig, jedoch nicht ausschließlich, die Kannen ihres Mutualismuspartners gegenüber alternativen Tagesquartieren und zwar sowohl im Freiland als auch bei Verhaltensexperimenten. Der Grund für dieses Verhalten ist vermutlich eine Kombination aus 1) der überragenden Qualität als Tagesquartier verglichen zu anderen Pflanzen seitens *N. hemsleyana* und 2) unterschiedlichen Traditionen bezüglich der Tagesquartierwahl seitens der Fledermäuse.

Zusammenfassend lässt sich feststellen, dass der Mutualismus zwischen Fledermäusen und Kannenpflanzen asymmetrisch ist, wobei *N. hemsleyana* stärker von *K. hardwickii* abhängig ist als umgekehrt. Dagegen können die Fledermäuse zwischen unterschiedlichen Tagesquartieren wählen. Bei beiden Partnern spiegelt sich diese asymmetrische Abhängigkeit in den Merkmalen wider, welche den Mutualismus stabilisieren, und zwar sowohl bezüglich ihrer Spezifität auf den Partner hin als auch in ihrer Funktion. Besonders auf morphologischer Ebene scheint *N. hemsleyana* Merkmale entwickelt zu haben, die perfekt auf *K. hardwickii* angepasst sind. Die Merkmale der Fledermäuse dagegen sind unspezifischer und unterstützen generell das Übertagen in tunnelförmigen Pflanzenstrukturen und ihr Vorkommen in dichtbewachsenen Habitaten. Daher sind die Fledermausmerkmale vermutlich als Präadaptionen zu sehen, als Merkmale also, die zu einem anderen Zweck evolvierten, aber dennoch funktionell sind und den Mutualismus mit *N. hemsleyana* stabilisieren. Die überragende Tagesquartierqualität von *N. hemsleyana* resultiert vermutlich aus dem Wettbewerb mit alternativen quartierbietenden Pflanzen und hilft *N. hemsleyana*, die Fledermäuse stärker an sich zu binden. Darüber hinaus bestätigt meine Studie frühere Erkenntnisse, dass asymmetrische Abhängigkeiten die Stabilisierung mutualistischer Interaktionen unterstützen. Letztendlich gibt meine Arbeit Hinweise darauf, dass die Spezifität der funktionellen Merkmale als Maß für die gegenseitige Abhängigkeit mutualistischer Partner verwendet werden kann.

1 INTRODUCTION

1 INTRODUCTION

> *"Is there anything left to say about mutualisms, something that would not be said by working on seed dispersal or gut floras or extra-floral nectaries? (...) Mutualism is not a complex subject and is easily explored through the application of common sense and natural history knowledge. (...) I wonder if we are not beating a dead horse (...) mutualism has been thought to death (...)."*
>
> Daniel H. Janzen, 1985

1.1 Stabilization of mutualisms – what do we know so far?

In a provoking and thereafter controversially discussed chapter on the natural history of mutualisms, tropical ecologist D. H. Janzen stated that mutualistic research per se is obsolete and could be completely covered by more basic research questions (Janzen 1985). Although mutualistic research as a biological discipline was still in its infancy when Janzen wrote this in 1985, he was already aware of the importance of mutualisms themselves. Later research confirmed many of his early statements about mutualisms:

- that they are omnipresent in nature, occur in all ecosystems and affect almost every organism (Bronstein 2015),
- that the cooperation between different organisms has resulted in key innovations, which had far-reaching consequences for the course of evolution on earth (Bronstein 2001a; Bronstein 2001b),
- that mutualisms facilitated the colonization of new habitats (e.g., during the settlement of land by plants; Brundrett 2002), and
- that some of these interactions have been so successful that they entailed adaptive radiations (e.g., the co-radiation of insects and flowering plants; van der Niet & Johnson 2012).

In short, Janzen already knew that **mutualisms are drivers of evolution and stabilize whole ecosystems**. Despite Janzen's scepticism, mutualistic research broadly developed in the last 30 years (Akcay 2015). However, the most intriguing questions in mutualistic research still remain (Bronstein 2001a; Bronstein 2001b; Jander & Herre 2010; Akcay 2015): **How did mutualisms themselves evolve? How are they stabilized?** In my thesis, I will focus on functional traits that stabilize interspecific interactions (☞ Box 1). Once such traits are identified, they can build the fundament for future studies on how these traits (and thus the mutualism itself) evolved.

Box 1: Glossary *Interspecific Interactions*

Mutualism: first defined within a biological context in 1873 by Pierre van Beneden as “mutual aid” among species (c.f. Bronstein 2015). More precisely defined by Mazancourt *et al.* (2005) as “a mutually beneficial interaction between individuals of two species“ (Mazancourt *et al.* 2005). Bronstein (Bronstein 1994a, 1994b) took the costs for the involved species into account: “Mutualisms are interspecific interactions in which both partners experience a net benefit” (Bronstein 1994a). As in all interspecific interactions, one should keep in mind that organisms engaged in mutualisms act to increase their benefit: “Mutualisms are best viewed as reciprocal exploitations that nonetheless provide net benefits to each partner“ (Herre *et al.* 1999).

Symbiosis is often used as a synonym for the term *mutualism* or, more specifically, to describe an intimate mutualism where partners physically stay together for their whole lifetime. However, early definitions, e.g., that of Anton de Bary (1879) used the term as an expression for “unlike organisms living together”. Costs or benefits of the involved species were not accounted so that these interactions could range from mutualism to parasitism (Bronstein 2009, 2015).

Parasitism refers to an interaction where one organism, the parasite, harms another organism, the host, which is utilized as habitat by the parasite (Raffel *et al.* 2008).

Facilitation is a broader term than *mutualism* (Pugnaire *et al.* 1996; Bronstein 2015). Stachowicz (2001) defined *facilitation* as “encounters between organisms that benefit at least one of the participants and cause harm to neither” (Stachowicz 2001; Bruno *et al.* 2003). Other scientist described *facilitation* as interaction with positive outcome for the facilitated organism but independent of the outcome for the facilitator (Bronstein 2015).

Cooperation means an intraspecific behaviour that is beneficial for another individual in terms of direct fitness and beneficial for the individual performing the behaviour in terms of direct or indirect fitness (intraspecific mutualism/altruism/kin selection) (c.f. Hamilton 1964; Maynard-Smith 1964; Axelrod & Hamilton 1981; Bronstein 1994a; West *et al.* 2007; Clutton-Brock 2009; Carter & Wilkinson 2013). Although *cooperation* and interspecific *mutualism* share many principles and mechanisms such as cheating, there are differences, most importantly, kin selection, which often drive the evolution of intraspecific interactions but are missing in interspecific interactions (Dugatkin 1997; Bronstein 2015).

In fact, it seems there are more **processes counteracting the stabilization of mutualisms** than promoting it. For example, mutualistic partners are on a constant evolutionary run to extend their own benefits (Herre *et al.* 1999; Bronstein 2001b). Mutualisms not only originate from autonomy or even parasitism but can also convert into such forms of interaction (Neuhauser & Fargione 2004; Bronstein 2009; Hibbett *et al.* 2000). Further, when partners in facultative mutualisms can choose alternatives, the mutualism should be destabilized as well. Finally, not only the mutualistic partners but also their environments are subject to on-going short-term and evolutionary changes. These different factors make it difficult for theoretical models to explain how (and why at all) mutualisms become stable interactions (c.f. Akcay 2015; Wang *et al.* 2015). Our current empirical knowledge is largely limited to a small amount of extensively examined systems, mostly obligate mutualisms where one partner cannot survive or reproduce without the other (cf. Bronstein 1994a; Frederickson 2013; Orona-Tamayo & Heil 2013). The much more widespread and complex facultative mutualisms (c.f. Hoeksema & Bruna 2000), where interacting organisms can choose between alternative partners or resources, are thus drastically underrepresented.

We know that for the stabilization of mutualistic interactions, a **high benefit-to-cost ratio and a high fidelity** between species are important factors (Foster & Wenseleers 2006). From this we can make some predictions about the stability of mutualisms: foremost, stabilizing processes should depend on 1) how well organisms can adapt to their partners in order to gain the most benefits at the lowest possible costs for themselves (i.e. the stronger partner species are adapted to each other the more stable their interaction will be) and 2) how easily species can utilize alternative resources that can compensate for the loss of a partner (i.e. the degree of their partner fidelity; Sachs *et al.* 2006). A practical way to test these predictions is to investigate traits that are functional in facilitating mutualistic interactions (see 1.2).

In contrast to their mobile partners, **sedentary species are less flexible** regarding their partner choice; they need communication pathways to advertise themselves and to offer resources as a reward for the service provided by visitors. It has also been discussed that sedentary species have fewer possibilities for cheating because of the risk to lose their interaction partner (c.f. Bronstein *et al.* 2006). This phenomenon can, e.g., be seen in terrestrial plant-animal interactions but is also likely in marine interactions, for example, between anemones and anemonefish (Ollerton *et al.* 2007).

Such **asymmetries** can often be seen in mutualisms (Bascompte 2006; Bascompte & Jordano 2007; Vazquez *et al.* 2009). One of the interacting species stays flexible enough to find alternatives to its mutualism partner, e.g., by living autonomously or switching to another interaction partner. The other more dependent partner will be faced with the selective pressure to outcompete these alternatives, usually by providing a higher quality of resources/services (Noë & Hammerstein 1994, 1995; Guimaraes *et al.* 2006; Vazquez *et al.* 2009). The persistence of mutualistic networks seems to especially rely on a composition of few strong dependencies and many weak interactions, which act as a buffer in case of perturbations. The same is true on an individual basis. For a specialist with high dependency it should make sense to interact with a generalist that is less dependent on one single partner species as mathematical models suggest: If, e.g., a plant and an animal both strongly depend on each other, a decrease in abundance of the plant would cause a decrease of the partner animal, which again would cause a decrease of the plant and so on. Thus, the instability in asymmetric mutualisms itself can ultimately have a stabilizing effect (Bascompte 2006; Bascompte & Jordano 2007). This effect is enforced when the more dependent partner evolves facilitating traits, making itself more attractive than alternatives to the less dependent partner.

To improve our understanding of the underlying mechanisms of the evolution and stabilization of mutualisms, the **functional traits of the involved species** have to be investigated intensively (Bronstein 2009). To do so, it is important to focus both on the proximate mechanisms that these traits fulfil as well as on the ultimate causes of these traits. Knowledge about proximate mechanisms of the traits is necessary to understand how the interaction works while ultimate causes of the traits explain, e.g., why an interaction is stabilized (for further explanations and an historical overview ☞ Box 2).

Box 2: Mayr's proximate and ultimate causes and Tinbergen's four questions

In his publication "Cause and effect in biology" Ernst Mayr described two fundamental causes in biology and classified the research fields, which investigate them: while functional biology focuses on the *proximate causes*, evolutionary biology aims to analyse *ultimate causes* (Mayr 1961). *Proximate causes* deal with the interaction of structural elements and are immediate influences on a trait (Mayr 1961; Laland *et al.* 2011). The critical question for a proximate investigation is: "How does something operate, how does it function?" (Mayr 1961). *Ultimate causes* imply a historical view that involves the question "Why" or more precisely "How come?" (Mayr 1961). Mayr's distinction of causation has influenced the scientific definition of causation and philosophy of science for decades (Laland *et al.* 2011). Nevertheless, there has also been criticism regarding this distinction in causation. One major argument against Mayr's distinction is that the categories are much more strongly interwoven as, for example, a proximate mechanism can influence the course of evolution by influencing selection (Laland *et al.* 2011).

In 1963, only two years after Mayr's publication, Nikolaas Tinbergen specified in his article "On aims and methods in ethology" the four general problems and questions in biology regarding 1) causation (i.e. the mechanistic value), 2) ontogeny (i.e. the developmental value), 3) survival (i.e. the adaptive value), and 4) evolution (i.e. the phylogenetic value) (Tinbergen 1963). Today questions 1) and 2) are typically assigned to the proximate causes, while questions 3) and 4) are assigned to the ultimate functions. Although Tinbergen's aim was to specify typical questions for behavioural studies, his categorization can be applied to a broad variety of organismal traits (Bateson & Laland 2013). This is in accordance with Konrad Lorenz who considered behaviour just as organs (Lorenz 1935, 1937b). Compared to Mayr's dichotomy, Tinbergen's categorization has the advantage that it differentiates more clearly between past and present processes and between cause and function (Bateson & Laland 2013). That is one of the reasons why the concept of Tinbergen was less criticized than that of Mayr (Bateson & Laland 2013).

1.2 The importance of traits for the stabilization of interspecific interactions

Functional traits (☞ Box 3) are probably the most important requisites for the stabilization of interspecific interactions. Functional traits allow the organism to exploit the interaction partner as much as possible while at the same time they limit over-exploitation by the other resulting in the best possible benefit-to-cost ratio (irrelevant of the interaction type; c.f. Kiers *et al.* 2010). They can be found in adaptations but may also be seen in exaptations that evolved without any relation to the interaction but nevertheless are useful (Gould & Vrba 1982). Traits involve morphological, physiological and behavioural components.

Box 3: Glossary (*Functional) Traits*

Traits are „well-defined, measurable properties of organisms“. They are usually measured at the individual level and used comparatively across species. Comparative investigations of traits can lead to generalized and predictable statements (McGill *et al.* 2006).

Functional traits are those that strongly influence organismal performance (McGill *et al.* 2006).

Morphological and physiological traits that stabilize interspecific interactions

Similar to intraspecific cooperation that includes direct and indirect fitness for the partners, traits that stabilize mutualisms may involve **direct benefits for the mutualism partner and indirect benefits for the owner** of the trait (c.f. West *et al.* 2007). For example, as reward for protection, several myrmecophytic plants offer hollow plant structures, so called domatia, as shelter to their ant partners. The domatia thus provide direct benefits for the ants (by gaining shelter), while the plant benefits indirectly (by gaining protection). While the ant colony lives in some of these chambers, they use adjacent chambers to deposit waste. Some plant species have been shown to acquire nutrients from the ant waste. While domatia themselves are morphological plant structures that facilitate the mutualism, the plants' ability to absorb and assimilate nutrients from the ant waste further stabilizes the mutualism but also requires physiological adaptations of the plants (Beattie 1989).

Among the best-examined morphological and physiological traits that facilitate interspecific interactions are those of angiosperm plants (Bronstein 2009). **Interactions between angiosperms and their pollinators** are highly diverse and reach from very generalistic pollinators and plants to highly specialised mutualists, which is also reflected in the traits of the interacting species (Fenster *et al.* 2004; Bascompte & Jordano 2007). Flowering plants have evolved a huge variety of traits to attract their insect pollinators and seed dispersers, e.g., by volatiles or colour patterns of the flowers that in many cases exploit the sensory bias of the insects

(Hossaert-McKey *et al.* 2010). Bat-pollinated flowers, for example, often produce sulphuric substances to attract their mammal pollinators (Helversen *et al.* 2000). Some of these traits have been well investigated (Bronstein 2009), e.g., due to phylogenetic studies (Herre *et al.* 2008) or selection experiments where bee-pollinated plants were brought into novel environments and, among others, their success in attracting pollinators was measured (Geber & Eckhart 2005).

Behavioural traits that stabilize interspecific interactions

Generally, less is known about animal traits that support mutualistic interactions (Bronstein 2009). Reasons for that bias are manifold and can, for example, be found in the **behavioural flexibility** of many animals, which makes fixed morphological or physiological traits often unnecessary (Bronstein 2009). Additionally, in interactions where the partners frequently separate, the mobile partner (usually an animal) is faced with the **challenge to recognize the mutualism partner**. Potentially, animals should be able to solve this problem via 1) individual learning (including imprinting), 2) genetic adaptation, and/or 3) social transmission (Thorpe 1956; Scholz *et al.* 1976; Teuschl *et al.* 1998; Djieto-Lordon & Dejean 1999; Laland & Hoppitt 2003; Dixson *et al.* 2014). While individual learning via trial and error will help to explore new environments and potentially to find novel interaction partners (Brown 2012), it should counteract on the stabilization of existing interactions because individual learning results in heterogeneous and often unpredictable behaviour of individuals (Boyd & Richerson 1988; Laland & Janik 2006). In contrast, the other mechanisms support more homogenous behaviour and thus should be much more efficient to maintain stable interactions.

Communication as a stabilizing trait for interspecific interactions

Partners – irrelevant whether they engage in intra- or interspecific interactions – need communication to maintain their interactions (☞ Box 4). Communication usually involves morphological/physiological/neuroanatomical and behavioural traits of the partners. Traits that enable interspecific communication are not driven by sexual selection of mating partners as it is often the case in intraspecific interactions, but by selection of interaction partners. This perspective has led to a broader application of concepts such as sensory drive or exploitation of sensory bias, which originally were used in the context of sexual selection (Ryan *et al.* 1990; Schaefer & Ruxton 2011; Schöner M.G. *et al.* 2016b). In interspecific interactions, communication enables plants to advertise their flowers to their pollinators (Hossaert-McKey *et al.* 2010) or to indicate that they already have been pollinated (Helversen & Helversen 2003), to attract seed dispersers (Kalko & Condon 1998) or parasitoids of herbivores (Pichersky & Gershenzon 2002), to show their quality to mutualists (Raguso 2004b), or to deter antagonists via honest signals (Maynard Smith & Harper 2003).

As **communication pathways,** the sender can use visual (Schaefer *et al.* 2004), chemical (Raguso 2008; Muhlemann *et al.* 2014), acoustic (Schöner M.G. *et al.* 2016b), or even electric signals (Clarke *et al.* 2013), depending on the preferred sensory channels of the receiver (Raguso 2004b; Schaefer & Ruxton 2011). In many cases, not only one channel but a combination of different signals will be employed (Gonzalez-Terrazas *et al.* 2016). Thus, several traits both in the sender and the receiver are necessary to guarantee successful communication. Note, that in antagonistic or commensalistic interactions, the benefiting organisms often exploit cues of their hosts, that did not evolve to communicate with each other.

Box 4: Glossary Communication

Communication is the act of stimulating a *receiver's* sensory system by *signals* emitted from a *sender*. This stimulation may lead to a change in the receiver's behaviour that often is beneficial for both organisms (Schaefer & Ruxton 2011; Karban 2015).

Signals are "any act or structure which alters the behaviour of other organisms, which evolved because of that effect, and which is effective because the receiver's response has also evolved" (Maynard Smith & Harper 2009).

Cues are "any act or structure that (i) affects the behaviour of other organisms; and (ii) which is effective because the effect has evolved to be affected by the act or structure; but which (iii) did not evolve because of those effects (Scott-Phililpps 2008).

Sensory drive is a model that predicts „how communication systems adapt to local environments“, from the generation of a signal to its perception (Schaefer *et al.* 2004).

Exploitation of sensory bias is another model, which predicts the evolution of traits that exploit a receiver's perceptual biases, i.e. sensory, neuronal and higher cognitive processes (Schaefer & Ruxton 2011).

1.3 The interaction between *Kerivoula hardwickii* and *Nepenthes* pitcher plants – state of knowledge

Compared to other bats, the South-East Asian bat species *Kerivoula hardwickii* (for descriptions of the study species see 1.4) is unique in its roosting behaviour that has led to interspecific interactions with carnivorous plants: it is the only bat species known to roost inside the trapping organs of three *Nepenthes* pitcher plant species (Grafe *et al.* 2011; Schöner C.R. *et al.* 2013). Besides that, we found the bats roosting in furled leaves of different plant species (e.g., in the order Zingiberales). Two of the used pitcher plant species, *Nepenthes bicalcarata* and *Nepenthes ampullaria*, do not benefit from their inhabitants as the bats can only roost in these pitchers when they are damaged or dead. These pitchers contain a high amount of digestive fluid when intact, which makes it impossible for the bats to use the pitchers. It is possible that the bats modify pitchers of *N. bicalcarata* and *N. ampullaria* to make them habitable by nibbling small holes into the bottom through which the digestive fluid is drained off. In this case, the bats would act as parasites (Schöner C.R. *et al.* 2013; Schöner M.G. *et al.* 2016a).

In *N. hemsleyana* the situation is completely different: Compared to other pitcher plant species, *N. hemsleyana* is a poor arthropod trap. Pitchers of this species lack effective traits for arthropod attraction such as sufficient amounts of volatiles or UV-light reflections (Moran 1996). Moreover, *N. hemsleyana* pitchers contain only a small amount of digestive fluid. This enables the bats to use the pitchers as roosts during daytime. While doing so, the bats also defecate into the pitchers (Grafe *et al.* 2011). Bat guano is rich in nitrogen and phosphorous and therefore even harvested as plant fertilizer in caves with large aggregations of cave-dwelling bats (Kingston *et al.* 2006). We found that *N. hemsleyana* plants gain between 34% and 96% of their entire nitrogen from their bat inhabitants (Grafe *et al.* 2011; Schöner C.R. *et al.* 2016).

This nutrient input is **beneficial** for *N. hemsleyana*: Compared to plants without access to bat faeces, individuals supplied with faeces showed increased photosynthesis and growth and their survival probability was higher (Schöner C.R. *et al.* 2016). In turn, *K. hardwickii* benefit from roosting in *N. hemsleyana* pitchers by better microclimatic conditions and a lower parasite infestation compared to individuals roosting in *N. bicalcarata* and *N. ampullaria* pitchers (Schöner C.R. *et al.* 2013; Schöner C.R., unpublished data).

The **costs** for *N. hemsleyana* and *K. hardwickii* seem to be rather low. This is indicated, e.g., by the lifespan of the pitchers, which is not reduced when bats use them. Also, the bats are not faced with the permanent challenge to find new roost plants because individual *N. hemsleyana* plants usually continuously provide at least one suitable pitcher (Schöner C.R. *et al.* 2015). In a detailed cost-benefit analyses, C.R. Schöner could thus show that the interaction between *K. hardwickii* and *N. hemsleyana* is most likely a mutualism (PhD Thesis C.R. Schöner 2015).

1.4 Study organisms

Bats (Chiroptera)

More than 20% of all described mammalian species belong to the order Chiroptera, which make up the second largest mammalian order (Wilson & Reeder 2005; Kunz *et al.* 2011). Bats are not only very species-rich but are also characterized by a huge variety of specific traits. All bat species share the ability of active flight and many species use echolocation for orientation. These echolocating bats are capable to hunt in aerial space during night-time and to roost in caves in complete darkness where visual orientation would not be possible. This helped bats to occupy ecological niches where competition from other species is very limited. Ultrasound calls used for orientation are produced in the larynx of the bats and emitted via their mouth or nose, or by clicking with their tongues or wings as in the case of some Pteropodidae (Schnitzler & Grinnell 1977; Pedersen 2000; Vater 2000; Altringham & Fenton 2005; Jones & Teeling 2006; Metzner 2008; Boonman *et al.* 2014; Racey 2015).

Apart from flight and echolocation, bats show a **broad range of various traits** that characterise their high diversity. Extreme specializations can be found in connection with food search and digestion. For example, nectar-feeding bats often have strongly elongated jaws, brush-like, very long tongues and reduced number and size of teeth. It is broadly assumed that these morphological adaptations have coevolved with corolla lengths of the flowers (Philipps 2000; Muchhala & Thomson 2009). Even more extreme are adaptations of vampire bats that specialised on licking the blood of other mammals or birds. As the water content in blood is very high, the bats have to feed a considerable amount of blood to gain sufficient amounts of nutrients. In order to keep the bloodstream long enough flowing, these bats have substances in their saliva that impede blood coagulation (Fernandez *et al.* 1999). However, the high water content prevents the bats from flying and forces them to stay close to their prey animals. Due to their highly effective renal system, vampire bats are able to quickly excrete high amounts of water and gain mobility again (McFarland & Wimsatt 1965).

Similar adaptations can also be found in connection to the **roosting behaviour** of bats. Bats are known to use an extreme diversity of roosts both on an interspecific level but also within species, e.g., depending on the sex or the reproductive status of the bats (Lewis 1995; Kerth *et al.* 2001). Morphological adaptations to the bats' roosting ecology can, e.g., be seen in the flattened skulls of several bat species, which allow them to occupy narrow crevices and bamboo. The pelage of foliage roosting bats can help them to hide from visually orientated predators. Colour, spots and stripes in fur and skin can be used as disruptive patterns. The yellowish to orange fur of several *Kerivoula* spp. has been discussed to resemble fruits and leaves. The white bat *Ectophylla alba* appears greenish in its tent roosts when light transmits through their roosting leaves (c.f. Kunz & Lumsden 2005). Among the most obvious adaptations are pad-like structures on the palm and feet of bats. These morphological structures are assumed to have convergently evolved in four genera. The referring species use different mechanisms to stick to the slippery surface, such as suction or wet adhesion. The degree of development of these pad structures strongly varies between the species with the highest developed discs to be found in *Thyroptera* (Thewissen & Etnier 1995; Riskin & Fenton 2001; Riskin & Racey 2010).

Several **bats in the genus *Kerivoula*** are also known to roost in plant structures (☞ Box 5). They occur in Africa, South-East Asia and Australia. Typical traits of these species are dense, woolly fur, funnel shaped ears with a long tragus and extremely high and frequency-modulated echolocation calls. These calls are very short but have extremely large bandwidths. The function of these extremely high-pitched calls is currently unknown. Such high frequencies enhance the resolution of the surrounding but have the disadvantage to be attenuated very quickly, which strongly limits the detection distance of the echolocating animal (Siemers & Schnitzler 2004; Brinkløv *et al.* 2008; Schmieder *et al.* 2010).

Box 5: Profile *Kerivoula hardwickii*

Kerivoula hardwickii (Horsfield, 1824) is an insectivorous bat species occurring in large parts of South-East Asia and adjacent areas including Sri Lanka, India, southern China, Philippines, Peninsular Malaysia, Singapore, Borneo and Indonesia (Payne *et al.* 1985; Corbet & Hill 1992; Nowak 1994; Esselstyn *et al.* 2004; Leong & Lim 2009). This small species (forearm length: 32.0 – 34.0 mm; weight: 3.5 – 4.2 g) can be found in the understorey of different forest types (Payne *et al.* 1985; Francis & Barrett 2008). It is characterised by grey-brown fur with dark grey bases on the back and lighter grey on the ventral surface. The taxonomic classification of possible subspecies within *K. hardwickii* is unclear due to controversial results in morphological and multiple genetic datasets (c.f. Douangboubpha *et al.* 2015). For main parts of Borneo a potential subspecies, *K. h. hardwickii*, has been described (Payne *et al.* 1985; Hill & Rozendaal 1989).

Carnivorous Plants

Fig.1. Carnivorous plants are highly diverse and can be found in at least 19 genera (exemplarily shown from left to right and top to bottom): *Cephalotus follicularis*, *Darlingtonia californica*, *Dionaea muscipula*, *Nepenthes tentaculata*, *Heliamphora nutans*, *Sarracenia flava*, *Drosera roraimae*, *Pinguicula alpina*, *Utricularia humboldtii* (credit: Pavlovič & Saganová 2015).

Carnivory is a successful nutrient acquisition strategy convergently evolved in plants that occur in nutrient-deprived habitats with high solar radiation (Givnish *et al.* 1984; Albert *et al.* 1992). So far, carnivory has been described for 12 families and 19 genera (including *Brocchinia*, *Catopsis*, *Cephalotus*, *Darlingtonia*, *Dionaea*, *Drosera*, *Drosophyllum*, *Genlisea*, *Roridula*, *Sarracenia*, *Triphophyllum* and *Utricularia*; Fig. 1). More plants will likely be categorized as carnivorous in the near future.

To be **categorized as carnivorous**, plants (Krol *et al.* 2011) have to fulfil certain criteria: First, they have to attract, capture and digest animal prey, i.e. the plant absorbs and assimilates nutrients from prey items. An important definition criterion for carnivorous plants is that the assimilation of nutrients is connected with a fitness increase for the plants. Thereby, carnivorous plants can be distinguished from other plants that kill animals as a defence strategy but do not benefit from nutrients gained from these animals. Second, the plants need adaptations to actively attract, capture and/or digest prey, which is in contrast to plants benefiting from passively absorbing nutrients from decomposing animals (c.f. Givnish *et al.* 1984; Król *et al.* 2011; Givnish 2015; Pavlovič & Saganová 2015).

One of the larger genera within carnivorous plants is that of the **Palaeotropical *Nepenthes* pitcher plants** (☞ Box 6). The distribution range of *Nepenthes* is unusually large: Diversity centres can be found on the islands of Borneo and Sumatra with generally most species occurring in the Sunda region. Outside South-East Asia, *Nepenthes* spp. also occur in Australia, India, Sri Lanka, Madagascar and the Seychelles but are missing on the African continent (Meimberg *et al.* 2001; Clarke 2006). As typical for carnivorous plants, *Nepenthes* spp. grow on nutrient deprived soils, for example in peat swamp, heath and mountain forests (Bohn 2004).

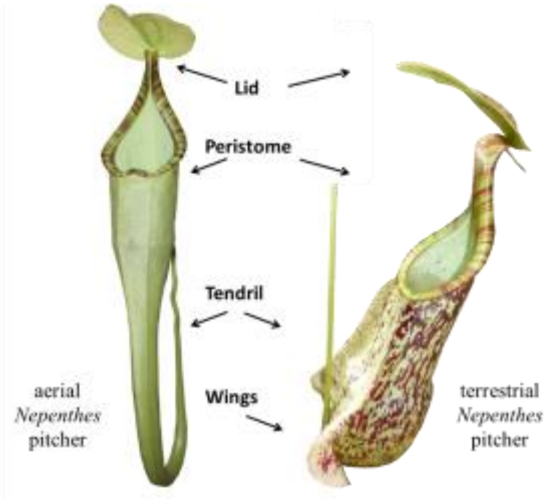


Fig. 2. Pitcher morphology of terrestrial and aerial *Nepenthes* pitchers (Clarke 2006).

The arthropod-trapping organs of these plants, the so-called pitchers, are strongly modified and specialized leaves that contain four functional zones with various **physiological and morphological adaptations** to attract and digest prey (Fig. 2). First, the lid of the pitchers prevents the dilution of the digestive fluid by incoming rainwater and can be important for prey attraction and capture (Clarke 2006; Bauer *et al.* 2012, 2015). The second zone, the peristome, is very slippery, especially when wetted by water or nectar, and increases the plants' capture success as arthropods easily lose their grip there (Bohn 2004). The walls of the upper zone of the pitcher's interior is covered by epicuticular wax crystals or downwards pointing lunate cells (Gaume *et al.* 2002; Bohn 2004), which helps that captured arthropods cannot escape from the pitchers. The lower zone at the bottom of the pitcher is the area where digestive processes take place; here the pitcher walls contain glands that produce and secrete digestive enzymes, such as proteases, lipinases, and chitinases, as well as acids (Tökés *et al.* 1974; Hatano & Hamada 2008; Thornhill *et al.* 2008; Bazile *et al.* 2015). Although all *Nepenthes* species share a similar basic pitcher structure, they strongly vary in details such as colour, size and shape. Additionally, on the intraspecific level a certain diversity can be found as most species produce two types of pitchers: While terrestrial pitchers are produced by young stems and have an ovoid shape, aerial pitchers are more funnel-shaped and can be found on climbing stems. In some species, there are also intermediate forms of the two pitcher types (Clarke 2006).

Excursus: abandonment of carnivory in plants

Several studies showed that some carnivorous plant species secondarily abandoned carnivory again. In some species, carnivory is present only for a certain part of the plants' life (e.g., *Triphyophyllum*; Green 1979). Other species have evolved away from carnivory (e.g., *Ancistrocladus*; Stevens 2001), which is also true for several *Nepenthes* species (Clarke *et al.* 2009; Grafe *et al.* 2011; Pavlovič *et al.* 2011). The fully or partly abandonment of carnivory always seems to be a result of the plants' ability to use alternative nutrient sources, such as leaf litter (Moran *et al.* 2003; Pavlovič *et al.* 2011) or animal faeces (Clarke 2006; Romero *et al.* 2006; Clarke *et al.* 2009; Chin *et al.* 2010; Grafe *et al.* 2011). In the latter case, mammals supply the plants with their nutrient-rich faeces and often get rewarded with nectar or shelter. This can, e.g., be seen in the highland species *Nepenthes lowii*, *Nepenthes macrophylla* and *Nepenthes rajah* that interact with diurnal tree shrews (*Tupaia montana*) and/or nocturnal rats (*Rattus muluensis*; Clarke *et al.* 2009; Chin *et al.* 2010; Wells *et al.* 2011). The plants largely gave up arthropod attraction, capture and digestion and ecologically outsourced these tasks to their more efficient mammal partners (Schöner C.R. *et al.* 2016). In general, such trait loss can typically be observed in at least one (mostly the sedentary) of two mutualistically interacting partners (Ellers *et al.* 2012). Trait loss thus contributes to asymmetric dependencies, which, as mentioned earlier, have stabilizing effects on mutualisms (Bascompte 2006). Nevertheless, most of these plants additionally keep up the strategy of carnivory. This dual strategy results in niche segregation and resource partitioning and probably reduces competition between species (Chin *et al.* 2014; Gaume *et al.* 2016).

Box 6: Profile *Nepenthes hemsleyana*, *Nepenthes bicalcarata*, and *Nepenthes ampullaria*

Nepenthes hemsleyana was previously described as *Nepenthes rafflesiana* var. *elongata* Jack (Cheek & Jebb 2001; Phillipps *et al.* 2008) but recently elevated to species status (Macfarlane 1908; Clarke *et al.* 2011; Scharmann & Grafe 2013). It is a lowland species growing in closed peat swamp and heath forests of North-western Borneo (Clarke 2006; Greenwood *et al.* 2011). Although, its closest relative, *N. rafflesiana*, generally prefers more open sites (Clarke 2006), both species sometimes co-occur in disturbed habitats. Pitchers of *N. hemsleyana* have a relatively low lignin content compared to other *Nepenthes* species (Osunkoya *et al.* 2008). The pitchers show a huge colour variability ranging from green to white to speckled in dark purple (Clarke 2006). The aerial pitchers are characterized by an upper cylindrical and a lower conical part with a total length of up to 25 cm long and an opening width of approximately 5 cm (Grafe *et al.* 2011; Lim *et al.* 2015). Typical traits to attract arthropod prey such as an intense volatile emission and UV light patterns can be observed in the closely related *N. rafflesiana* (Schwallier *et al.* 2016) are missing in *N. hemsleyana* (Moran 1996).

Nepenthes bicalcarata (Hooker, 1873) pitchers vary from those of *N. hemsleyana* by their bulbous or urceolate shape (Clarke 2006) and by the missing wax crystals at the inner pitcher surface of the pitchers (Bohn 2004). The most prominent characteristic of *N. bicalcarata* pitchers are two thorns below the lid of the pitchers that derive from the apical ribs of the peristome (Clarke 2006). Similar to *N. hemsleyana*, the colour spectrum of *N. bicalcarata* pitchers is broad ranging from yellow or orange to green. The species occurs sympatrically with *N. hemsleyana* in the lowland peat and heath forests (Clarke 2006). *Nepenthes bicalcarata* is known for its mutualistic interaction with the ant species *Camponotus schmitzi* that live inside the pitchers and clean them (Thornham *et al.* 2012; Scharmann *et al.* 2013).

Nepenthes ampullaria (Jack, 1835) almost exclusively produces terrestrial pitchers, which can be found as densely clumped carpets that cover the forest floor. These urceolate pitchers' glandular zones covers nearly the entire inner pitcher wall. Compared to other *Nepenthes* the lid is relatively inconspicuous and small and does not cover the pitchers opening (Clarke 2006; McPherson 2009), probably an adaptation to capture dead foliage (Moran *et al.* 2003). The species belongs to the most common *Nepenthes* species in Borneo's heath and peat swamp forests. Despite of a general high endemism of *Nepenthes* that are mostly distributed west of the Wallace line, *N. ampullaria* can also be found in Wallacea (McPherson 2009; Alamsya & Ito 2013). Its distribution range covers Peninsular Malaysia, Sumatra, Borneo, and New Guinea.

Plants with developing furled leaves

Fig. 3. A *Thyroptera tricolor* is leaving its roost, the furled leaf of a *Heliconia* (© Gloriana Chaverri).

Plants in the order Zingiberales contain, among others, the families Zingiberaceae (ginger), Musaceae (banana), Araceae (arum) and Heliconiaceae. This order is widely distributed in Palaeotropical and Neotropical regions (Dahlgren *et al.* 1985). When the species develop new leaves, they produce one narrow longitudinally furled leaf with one half of the blade rolled around the other (Delin & Larsen 2000). Thus, developing leaves form upright tubes before unfurling completely (García-Robledo & Horvitz 2009). Various animals including spiders, ants and grasshoppers seek shelter in such rolled leaves (own observation). Moreover, several bats including species of the genera *Pipistrellus* and *Thyroptera* have specialised on roosting in furled leaves (Fig. 3). These bats are faced with the problem that they frequently need to search for new roosts as leaves unfurl after a few hours to several days and lose their suitability as roosts (Happold & Happold 1996; Riskin & Fenton 2001). So far, it is unknown whether plants with furled leaves benefit from their inhabitants in terms of nutrient gain (pers. communication G. Chaverri).

1.5 Study aim, hypothesis and delimitation from other studies

The **aim of my work** was to investigate the mechanistic value of traits (sensu Tinbergen 1963) of the involved interaction partners for the stabilization of the mutualism between *K. hardwickii* and *N. hemsleyana*. For this aim, I applied comparative approaches with closely related bat and plant species that are not engaged in this particular bat-pitcher plant interaction. Such comparisons potentially indicate which traits might be seen as adaptations and which as exaptations. **I hypothesized that**

1) *K. hardwickii* and *N. hemsleyana* have **morphological traits** by which they directly or indirectly gain benefits in the interaction with the partner and which should thus facilitate the mutualism (this also includes traits for communication);
2) the superior quality of roosts provided by *N. hemsleyana* influences the bats' behaviour. Thus, the bats will contribute to the stabilization of the mutualism via **behavioural traits** (i.e. the bats prefer *N. hemsleyana* to alternative roost plants);
3) overall, the mutualism between *K. hardwickii* and *N. hemsleyana* is **asymmetric**. The plants are more dependent on the bats, which are more flexible and can select alternative roosts. This asymmetry is reflected in the extent of traits, i.e. *N. hemsleyana*'s traits are more likely specifically adapted to *K. hardwickii* whose traits should generally facilitate roosting in plant structures.

Detailed investigations of the partners' ontogeny, their physiological traits or the evolutionary origin of the bat-pitcher plant mutualism were beyond the frame of this thesis. Future studies on various *Nepenthes* spp. that mutualistically interact with mammals will shed light into these questions. However, before such research can be conducted, it is important to understand the adaptive and mechanistic values of this mutualism. While C.R. Schöner assessed the mutualism's ultimate causes in her thesis (PhD Thesis C.R. Schöner 2015), I will focus on the proximate mechanisms. This will allow to draw conclusions about which traits may have stabilizing effects on mutualistic interactions and will allow me to also speculate on the selection pressures imposed on these traits.

2 RESULTS AND DISCUSSION

2 RESULTS AND DISCUSSION

„Certainly inter-specific interactions would be less complex if it were not for mutualisms allowing mutualists to 'have' traits that are incompatible with their primary phenotype.“
Daniel H. Janzen, 1985

2.1 Morphological traits that stabilize the mutualism

2.1.1 Morphological traits of *Nepenthes hemsleyana*

☞ Manuscript 1: Lim *et al.* 2015

In 2011 *Nepenthes hemsleyana* was raised to species status (Clarke *et al.* 2011; Scharmann & Grafe 2013). This was a logical consequence of several preceding studies that had entangled the morphological, physiological and ecological differences to the sister species *Nepenthes rafflesiana* (Moran 1996; Di Giusto *et al.* 2008; Gaume & Di Giusto 2009; Di Giusto *et al.* 2010; Bauer *et al.* 2011; Grafe *et al.* 2011). Above all, several traits (e.g., volatiles, UV-light reflection, etc.) enable *N. rafflesiana* to efficiently capture arthropods. These traits are reduced in *N. hemsleyana* whose capture rate is thus up to seven times lower compared to the sister species (Moran 1996). In earlier studies, we could show that *N. hemsleyana* is able to compensate for this lack of nutrients by interacting with the bats *K. hardwickii* (Grafe *et al.* 2011) and that *N. hemsleyana* pitchers offer roosts of higher quality than that of another *Nepenthes* species (Schöner C.R. *et al.* 2013). However, which morphological traits enable *N. hemsleyana* to harbour bats and profit from them remained to be assessed.

Fig. 4. The shape of *N. hemsleyana* pitchers fits the shape of the bats (Schöner C.R. *et al.* 2013).

In *N. rafflesiana* the level of digestive fluid is so high that there would not be enough space for the bats. This can similarly be seen in *N. bicalcarata* and *N. ampullaria*, which the bats only use, when the fluid has drained off through small holes (Schöner C.R. *et al.* 2013, 2015). This lack of enzymatic digestive fluid however suggests that the aforementioned species cannot take up nutrients from the bat faeces. *Nepenthes hemsleyana* has solved this problem by several morphological traits. First, in *N. hemsleyana* the digestive fluid is only present in the lowest part of the pitchers, which allows the bats to use intact pitchers. Second, the shape of *N. hemsleyana* pitchers is cylindrical in the upper part while highly tapered in the lower part (Fig. 4). Third, compared to *N. raffle-*

siana pitchers, those of *N. hemsleyana* are elongated and have enlarged orifices, which allows the bats to easily enter and leave the pitchers. The combination of the enlarged pitcher size with the low digestive fluid level in *N. hemsleyana* pitchers results in significantly more habitable space to the bats than in pitchers of the sister species. Finally, the size of *N. hemsleyana* pitchers fits well to the body size of *K. hardwickii.* Due to the combination of pitcher shape and fluid level the bats have no risk of falling into the fluid (Lim *et al.* 2015). In conclusion, the unique morphological traits of *N. hemsleyana* enable and facilitate its mutual beneficial interactions with the bats (Lim *et al.* 2015).

2.1.2 Morphological traits of *Kerivoula hardwickii*

☞ Manuscript 2: Schöner M.G. *et al. submitted* a

Morphological traits of *K. hardwickii* that facilitate their mutualism with *N. hemsleyana* are less obvious than those of their interaction partners. However, in a recent study we found that the bats do not cause tissue injuries to their preferred roost type, pitchers of *N. hemsleyana* (Schöner C.R. *et al.* 2015). Usually, these pitchers are available for approximately three months to the bats, but as they have low lignin contents (Osunkoya *et al.* 2008) even small injuries drastically reduce the pitchers' lifespan to few days (own observation). *Kerivoula hardwickii* should thus have a high interest in not injuring the pitchers with their claws while roosting and moving inside. To find out how the bats avoid damaging their roosts, I conducted another study (Schöner M.G. *et al. submitted* a). I tested the idea that the bats have morphological structures, which help them to cling to and move on the slippery surface of their roosts.

There are three ways how animals that need to deal with smooth or slippery surfaces solved the adhesion problem: 1) friction (e.g., by gripping a branch with muscular forces of hands and feet), 2) mechanical interlocking (e.g., when animals use their claws to cling to irregularities of a surface or when they dig their claws into the surface itself), and 3) bonding via pads (c.f. Endlein & Barnes 2014). Several bat species, for example, *Thyroptera tricolor* or *Myzopoda auritus* have evolved pads on their thumbs and feet to adhere to developing furled leaves in which they roost (Riskin & Fenton 2001; Riskin & Racey 2010).

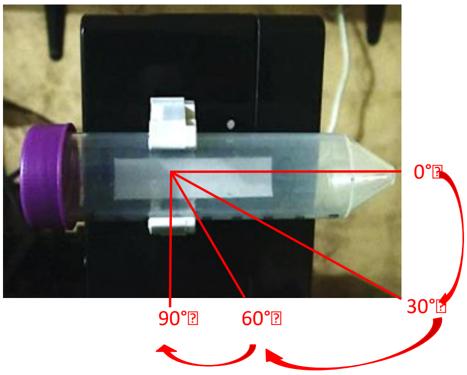


Fig. 5. The rotation device consisted of a motor that clockwise rotated a falcon tube in which we placed the bats. The motor automatically stopped in steps of 30° starting with 0° and ending with 90° with a break of 5 s after each rotation.

With a rotation device (Fig. 5) I experimentally investigated the bats' adhesion capabilities. I compared individuals of *K. hardwickii* with those of their close relatives *K. intermedia* and *K. pellucida*, which roost in wilted leaves. Additionally, I included individuals of the not closely related *Myotis muricola* that, like *K. hardwickii*, roost in developing furled banana leaves (McArthur 2012; Pottie *et al.* 2005; Francis & Barrett 2008; Phillipps 2016; own observation). I hypothesized that species with similar roosting behaviour should have similar adhesion capabilities. Actually, my experiments showed that only *K. hardwickii* were able to cling to smooth surfaces in situations when they could not use their claws. The responsible structures for this ability are pads at *K. hardwickii*'s thumbs and feet that are significantly larger (in relation to body size) than in the other tested bat species. In contrast, during field observations I noticed that individuals of *M. muricola* dig their claws into the surface of banana leaves while moving inside (pers. observation).

Of note, *K. hardwickii* is still able to use its claws. The fact that the bats avoid to use them in the roost involves a morphological and a behavioural component (pers. observation). The enlarged pads thus directly benefit the bats as they can easily cling to the surface of their plant roosts. From a mutualistic point of view, however, these pads also provide a functional trait that stabilizes the mutualism (although the pads are likely exaptations and not adaptations to *N. hemsleyana*): They directly benefit *N. hemsleyana* by avoiding injuries as the pads do not damage the surface, but the claws do. The bats benefit indirectly as their roosts are longer available.

2.2 Communicational traits that stabilize the mutualism

☞ Manuscript 3: Schöner M.G. *et al.* 2015

☞ Manuscript 4: Schöner M.G. *et al.* 2016b

☞ Manuscript 5: Schöner M.G. *et al.* 2016a

In many mutualistic interactions, the partners regularly separate and have to find each other again to maintain their relationship (Bronstein 1994a; Bronstein *et al.* 2006). Obviously, sedentary species such as most plants are not able to search for their mobile interaction partners, which have to manage this task on their own. *Kerivoula hardwickii* is faced with a similar situation; not only are the pitchers of *N. hemsleyana* rare and can be used as roosts only for a few months, they also grow within the dense vegetation of peat swamp forests (Schöner C.R. *et al.* 2015; Schöner M.G. *et al.* 2015). In such cluttered environments, it should be difficult for bats to find structures such as pitchers by means of echolocation (Arlettaz *et al.* 2001).

Other plants that rely on animals, e.g., for pollination, facilitate the search process of their partners by conspicuous traits that specifically appeal to the animals' sensory bias (☞ Box 4; Raguso 2004a). In echolocating bats, the acoustic sensory channel should be preferred for orientation and communication. We hypothesized that *N. hemsleyana* facilitates the bats' search process via acoustic signals. Potentially, this attraction could take place via *active acoustic signalling* where an organism actively emits acoustic signals to attract the partner species, or via *passive acoustic signalling* where plants communicate with a focal animal species by reflecting that animal's emitted sound (☞ Fig. 6). While not much is known about active acoustic signalling in plants, passive acoustic signalling has been shown in few plants that evolved echo-reflectors at their inflorescences or leaves to attract their bat pollinators (Helversen & Helversen 1999; Simon *et al.* 2011; Schöner M.G. *et al.* 2016b).

To get an idea about the echo-acoustic pattern of pitchers, we ensonified pitchers of *N. hemsleyana* and its sister species *N. rafflesiana* with broadband ultrasound similar to the calls of *K. hardwickii* and measured the target strength of the reflected echo. We found that the echo reflected from *N. hemsleyana* pitchers is significantly louder around the orifice of the pitchers than that of *N. rafflesiana* pitchers that do not interact with bats. The reason for this increased target strength is the enlarged concave backwall structure directly at the opening of *N. hemsleyana* pitchers, which serves as an effective and multidirectional echoreflector. Moreover, we found that the spectral pattern of pitchers of the two sister species is species-specific, i.e. *N. hemsleyana* pitchers have a typical sound that significantly differs from that of *N. rafflesiana*.

Behavioural experiments revealed that this echoreflector is highly relevant for the bats: When we positioned unmodified or differently modified *N. hemsleyana* pitchers behind clutter, bats needed significantly longer to locate *N. hemsleyana* pitchers with missing echoreflector compared to pitchers where it was present. In another experiment where we simultaneously presented unmodified and differently modified pitchers, the bats predominantly selected the unmodified pitchers (Schöner M.G. *et al.* 2015). In conclusion, similar to the reflectors of Neotropical bat-pollinated flowers (Schöner M.G. et al. 2016b) the echo-reflective backwall structure of the carnivorous plant *N. hemsleyana* seems to be a trait that is important for *K. hardwickii* to find and identify the plants' pitchers and to stabilize the interaction (Schöner M.G. *et al.* 2015).

We also aimed to assess if the bats show traits that facilitate the search of pitchers and thus the communication process with *N. hemsleyana*. Bat echolocation calls vary strongly between species depending on the bats' hunting habitat. In combination with typical morphological characteristics, bats can be assigned into different guilds. While bats hunting in open space generally have relatively low constant frequency calls that enable the bats to detect targets over larger distances, those hunting in clutter often have calls with high starting frequencies that are strongly frequency-modulated (Jones & Rayner 1991; Kalko *et al*. 1996; Schnitzler & Kalko 1998; Schnitzler & Kalko 2001; Schnitzler *et al.* 2003; Denzinger & Schnitzler 2004, 2013). These calls correspond to the trade-off that high frequencies allow for a high resolution of the bats' environment but are also strongly attenuated (Simmons *et al.* 1974; Lawrence & Simmons 1982; Schnitzler & Kalko 2001). However, so far knowledge is limited whether the structure of echolocation calls has also adapted to the roost search of the bats.

To test the hypothesis that the calls of *K. hardwickii* suit well to find *N. hemsleyana* pitchers in cluttered space, we recorded calls of the bats while they approached to pitchers. We found the highest starting frequencies ever measured in bats which the bats achieve in only one harmonic. These broadband calls with their high starting frequencies result in a high directionality. Highly directional calls facilitate localization and identification of targets in cluttered surroundings as only the object of interest is ensonified while echoes from clutter are blended out (Schöner M.G. *et al.* 2015). We compared *K. hardwickii*'s echolocation calls with those of *Glossophaga soricina*, another bat species that is attracted by plants with echoreflectors (Clare *et al.* 2014; Simon *et al.* 2014). It turned out that *G. soricina*'s calls show similar high-pitched and broadband frequencies but in contrast to *K. hardwickii* they use multi-harmonic calls. Although it is still unclear why such calls originally evolved, they provide a functional trait for bats mutualistically interacting with plants to find and identify their mutualism partner.

Kerivoula hardwickii's ability to identify their plant roosts cannot only be seen when they are interacting with their mutualism partner, *N. hemsleyana.* In a further study, I found that the bats also make use of characteristic cues of the non-mutualistic *N. bicalcarata.* Pitchers of *N. bicalcarata* typically have two sharp and long thorns directly above their opening. I initially hypothesized that these thorns have a deterring effect on the bats, because they could injure their wing membrane while entering or leaving a pitcher. Behavioural experiments where the bats could select between an unmodified and several modified *N. bicalcarata* pitchers with different thorn lengths showed that the opposite is the case: Pitchers with natural or elongated thorns had an attractive effect on the bats while pitchers with reduced or removed thorns were avoided.

Thus, I alternatively hypothesized that the bats exploit the deterrent shape of the thorns to protect themselves against predators. In another choice experiment I provided one unmodified pitcher and one where I had elongated the distance between the thorns and the pitchers' opening to an extreme so that the thorns cannot provide protection anymore. However, the bats did not distinguish between natural and modified pitchers. I conclude that the bats do not exploit the thorns because of their deterrent and potentially protecting effect against predators but as a unique identification cue (Schöner M.G. *et al.* 2016a). The bats are thus able to distinguish pitchers of *N. bicalcarata* from those of other *Nepenthes* species that are not suitable as roosts.

2.3 Behavioural traits that stabilize the mutualism

☞ Manuscript 6: Schöner M.G. *et al. submitted* b

As outlined above (see 1.1), *N. hemsleyana* strongly depends on bat faeces for growth, photosynthesis and survival (Grafe *et al.* 2011; Schöner C.R. *et al.* 2016). However, *K. hardwickii* should be an unreliable mutualism partner as the bats can select between alternative roost possibilities, which are unlikely to benefit from bat faeces (pitchers of *N. bicalcarata* and *N. ampullaria* and developing furled leaves of various Zingiberales plants). According to the biological market model, a species should provide resources of higher quality to its mutualism partner in order to outcompete alternative resources (Noë & Hammerstein 1994, 1995). In fact, *N. hemsleyana* offers roosts to *K. hardwickii* that provide optimal conditions in terms of size, microclimate or search effort (Schöner C.R. *et al.* 2013, 2015; Schöner M.G. *et al.* 2015). We hypothesized that the bats should prefer *N. hemsleyana* whenever possible.

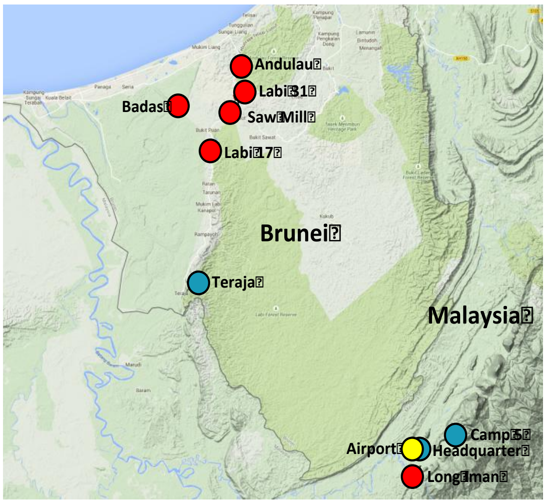
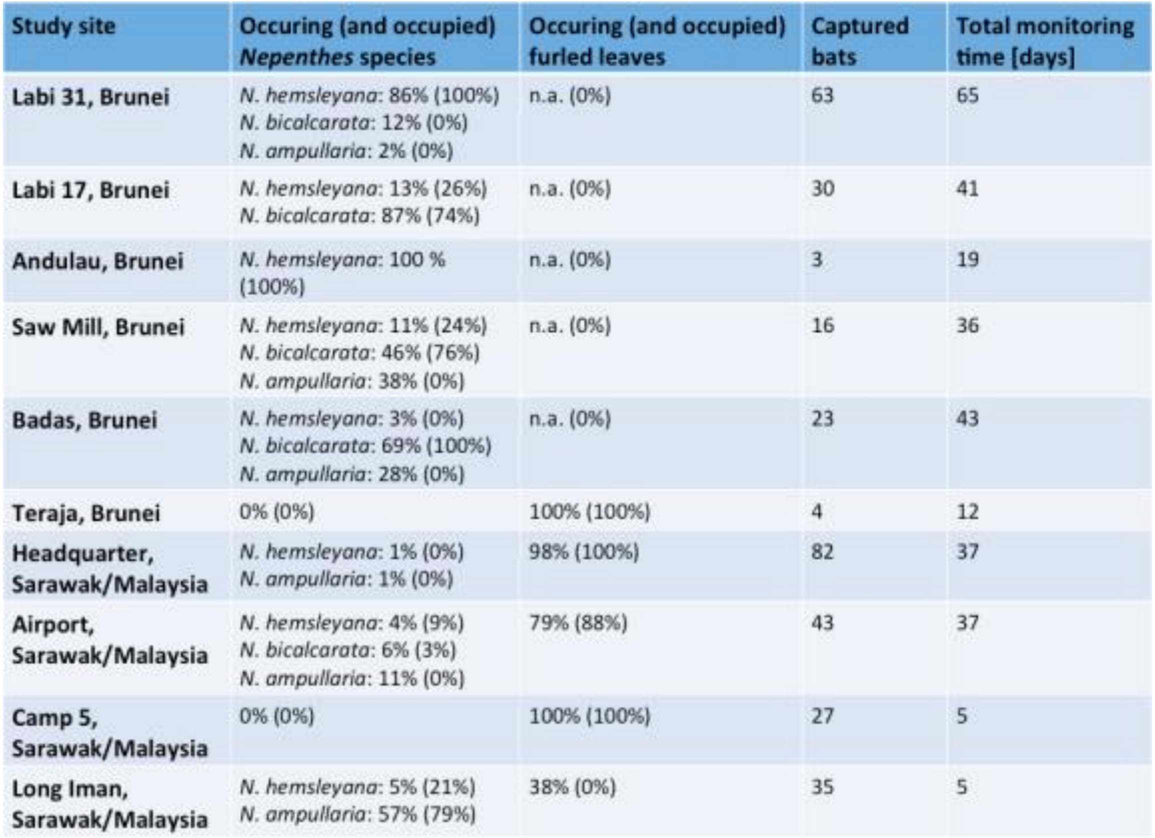


Fig. 3. Study sites with populations of *Kerivoula hardwickii*. Red circles indicate areas where the bats used pitchers, blue circles where they roosted in furled leaves and yellow where both roost types were used (data from 2009 till 2014 included). For details on the used *Nepenthes* species see Table 1 (map modified from PhD Thesis C.R. Schöner 2015).

I investigated the roosting behaviour of *K. hardwickii* in 10 study sites in the Belait District of Brunei Darussalam and in the Gunung Mulu national park in Sarawak/ Malaysia (Fig. 3) during two pre-studies (14 June to 30 July 2009 and 14 August 2011 to 14 January 2012) and during three stays on Borneo in the timeframe of my theses (20 June to 3 December 2012, 14 April to 1 September 2014, 7 to 25 February 2016). In all study sites, one to several *Nepenthes* species and/or plant species that produce furled leaves occurred (Table 1).

Table 1: Study sites, available and occupied roost types. Percentages indicate the share of roosts of a given type/species that were available. Percentages in brackets indicate the share of chosen roosts in a study site. Furled leaves were available in all study sites and checked for bats although we did not quantify them in all sites (indicated by "n.a"). In the table, we did not distinguish between furled leaves of different plant species as the bats showed no significant preferences for one of the furled leaves' species.



Study site	Occuring (and occupied) *Nepenthes* species	Occuring (and occupied) furled leaves	Captured bats	Total monitoring time [days]
Labi 31, Brunei	*N. hemsleyana*: 86% (100%) *N. bicalcarata*: 12% (0%) *N. ampullaria*: 2% (0%)	n.a. (0%)	63	65
Labi 17, Brunei	*N. hemsleyana*: 13% (26%) *N. bicalcarata*: 87% (74%)	n.a. (0%)	30	41
Andulau, Brunei	*N. hemsleyana*: 100 % (100%)	n.a. (0%)	3	19
Saw Mill, Brunei	*N. hemsleyana*: 11% (24%) *N. bicalcarata*: 46% (76%) *N. ampullaria*: 38% (0%)	n.a. (0%)	16	36
Badas, Brunei	*N. hemsleyana*: 3% (0%) *N. bicalcarata*: 69% (100%) *N. ampullaria*: 28% (0%)	n.a. (0%)	23	43
Teraja, Brunei	0% (0%)	100% (100%)	4	12
Headquarter, Sarawak/Malaysia	*N. hemsleyana*: 1% (0%) *N. ampullaria*: 1% (0%)	98% (100%)	82	37
Airport, Sarawak/Malaysia	*N. hemsleyana*: 4% (9%) *N. bicalcarata*: 6% (3%) *N. ampullaria*: 11% (0%)	79% (88%)	43	37
Camp 5, Sarawak/Malaysia	0% (0%)	100% (100%)	27	5
Long Iman, Sarawak/Malaysia	*N. hemsleyana*: 5% (21%) *N. ampullaria*: 57% (79%)	38% (0%)	35	5

In contrast to our initial hypothesis, our field observations showed that the bats did not generally prefer pitchers of *N. hemsleyana* to all other potential roosts (Table 1). Nevertheless, we found that the bats used *N. hemsleyana* pitchers almost always when they were present in a given study site (the only exceptions were the sites "Badas" and "Headquarter" where *N. hemsleyana* only made up 3% or less of all available roost species). The abundance of a given species could not explain the percentage of bats occupying them. Generally, the bats preferred pitchers to furled leaves, which were almost only used in areas without pitchers (again except of study site "Headquarter" where pitchers made up only 2% of the available roosts, which probably explains why they were not used by the bats). However, there was one study site ("Airport") where we found bats roosting in pitchers and furled leaves. Interestingly, all *K. hardwickii* individuals in this study site either used pitchers or furled leaves and never switched between them. In all sites where bats used more than one plant species within the roost type "pitcher" or "furled leaf", individuals switched between the species but always used the same roost type (for example, individuals switched between pitchers of *N. hemsleyana* and *N. bicalcarata* in sites where both were used; similarly, bats that roosted in furled leaves switched between different species with furled leaves).

In a flight tent, we tested whether bats are generally faithful to a roost type (pitchers or furled leaves) or species when they are offered in equal numbers. Again, individuals that roosted in *Nepenthes* pitchers switched between the different pitcher plant species during the behavioural experiments. The same was true for individuals roosting in plants with tubular developing leaves; they switched between different species with furled leaves. When the bats could choose between pitchers and furled leaves, all individuals that we had found in pitchers were absolutely faithful to this roost type. Interestingly, 21% of those bats that had been roosting in furled leaves switched to pitchers during the experiments. This behaviour could provide a unidirectional mechanism that steadily increases the number of bats using pitchers in areas where both pitchers and furled leaves are available.

As *N. hemsleyana*'s distribution range is restricted to Northern Borneo and, as in all pitcher plants, is very patchy (Moran *et al.* 2013) the bats can expand their range by additionally using furled leaves (Schöner C.R. *et al.* 2013). However, this cannot explain, why the majority of *K. hardwickii* was faithful to either pitchers or furled leaves. To find potential answers, we discuss several alternative hypotheses. We assume that imprinting (Lorenz 1937a) can be excluded, as this would imply that the bats' roost selection should be highly specific and stable during an individual's life (Thorpe 1956), which is not the case. Alternatively, the bats using different roost types could belong to different cryptic species. However, our population genetic analyses showed that genetic differentiation was not connected to the individuals' roost type use.

We suggest that the most likely explanation for the bats' faithfulness is provided by different roosting traditions, where juveniles learn from their mothers which roost to use (Laland 2004; Brown 2012). By being faithful to *Nepenthes* and to the more widespread plants with furled leaves, *K. hardwickii* can extend its range (Schöner C.R. *et al.* 2013) and gene flow between the bat populations is maintained. The bats are faced with a lower extinction risk than when they would solely interact with *N. hemsleyana*, which provides an indirect benefit to *N. hemsleyana* due to a reduced risk of co-extinction. Thus, different roosting traditions maintain the asymmetry between *N. hemsleyana* and *K. hardwickii* and stabilize their mutualism (see 2.4; Bascompte & Jordano 2007).

2.4 Conclusion: The mutualism between *Kerivoula hardwickii* and *Nepenthes hemsleyana* is asymmetric

In this work, I determined various functional traits that should effectively stabilize the mutualism between bats and pitcher plants. Especially, on the morphological level I found that *N. hemsleyana* has several traits that ideally fit to *K. hardwickii* and provide them with direct benefits (e.g., shape and size of the pitcher and its echoreflector or level of digestive fluid; Lim *et al.* 2015; Schöner M.G. *et al.* 2015; Schöner M.G. *et al.* 2016b). These traits potentially represent adaptations, which evolved for the interaction with bats. For example, the low level of digestive fluid is only advantageous for *N. hemsleyana* as long as bats are present. In contrast, relevant traits of the bats seem to generally facilitate roosting in slippery funnel-shaped plant structures or orientation in cluttered habitats (i.e. pads, echolocation calls; Schöner M.G. *et al.* 2015; Schöner M.G. *et al.* 2016a; Schöner M.G. *et al. submitted* a, Schöner M.G. *et al. submitted* b,). In contrast, *K. hardwickii* can survive without their mutualism partner. It is thus likely that the bats' functional traits represent exaptations. Future research will need to investigate the evolutionary history of this mutualism more closely.

As outlined above, the mutualism between bats and pitcher plants seems to be asymmetric. *Nepenthes hemsleyana* is obviously more depending on *K. hardwickii* than vice versa, as the bats can select between alternative roosting plants, which should make them an unreliable mutualism partner (Grafe *et al.* 2011; McArthur 2012; Schöner C.R. *et al.* 2013, 2016; Lim *et al.* 2015; Schöner M.G. *et al.* 2015; Schöner M.G. et al. 2016a; Schöner M.G. *et al. submitted* a; Schöner M.G. *et al. submitted* b). This asymmetric dependency of *N. hemsleyana* on its bat mutualism partner is reflected in the specifity and function of traits of the plant (Fig. 7).

The interaction between *N. hemsleyana* and *K. hardwickii* is not the only mutualism that shows an imbalance in the relation between a more dependent sedentary organism and its more flexible mobile partner species. Such asymmetries are especially common in plant-pollinator and plant-seed disperser mutualisms where plant species highly specialised on certain animal partners that interact with various plant species (Bascompte 2006; Bascompte & Jordano 2007). So far, the frequency of visits, e.g., in plant-pollinator networks, has mostly been used as a measure for mutual dependencies (Bascompte 2006) in order to find asymmetries. I suggest that quantification and determination of functional traits regarding their degree of specialisation towards a certain mutualism partner can be used as proxy for mutual dependencies as well.

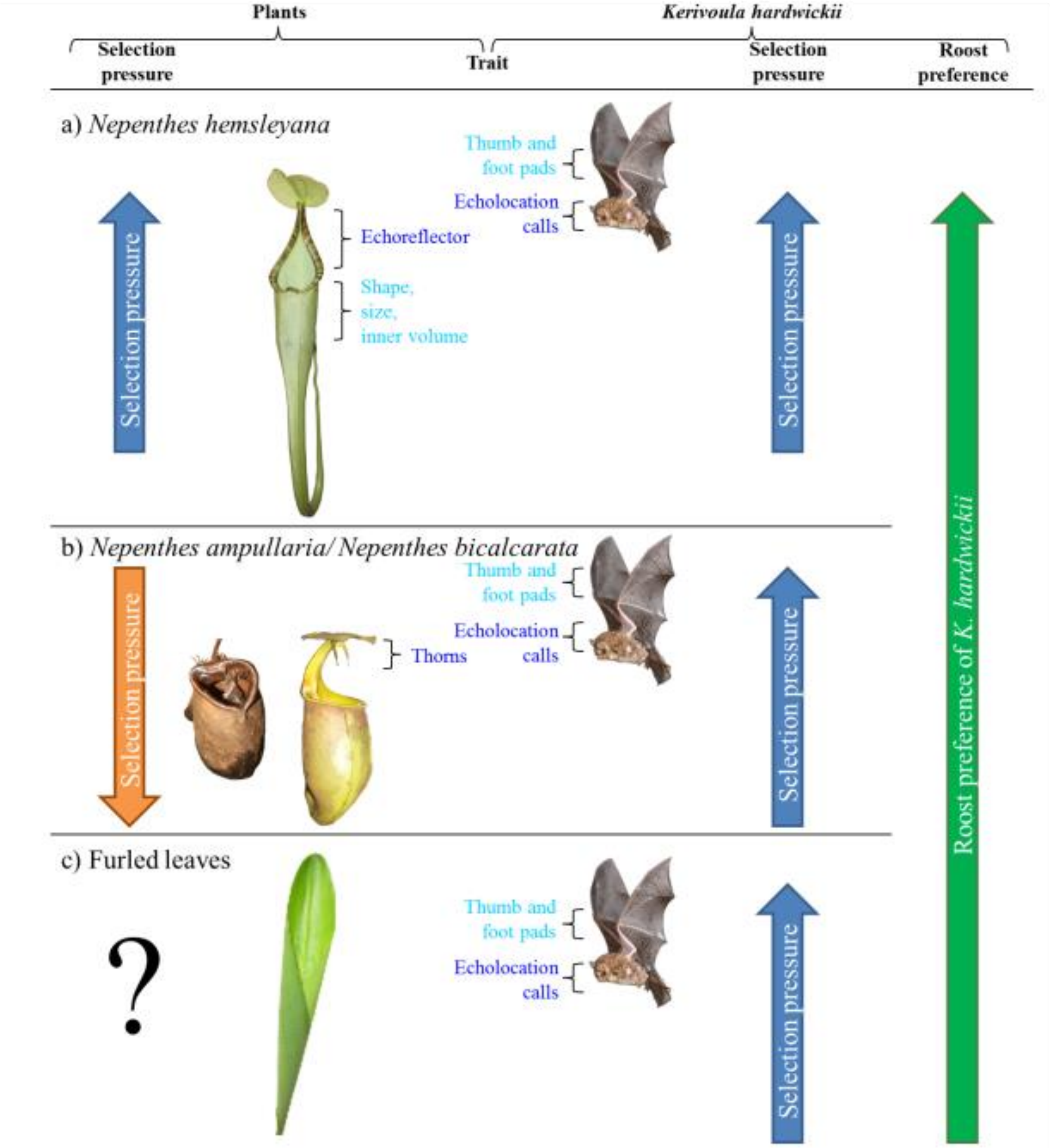


Fig. 7. Functional traits of *Kerivoula hardwickii* facilitating their interaction with a) the mutualistic *Nepenthes hemsleyana*, b) the not benefiting and potentially parasitized *Nepenthes ampullaria* and *Nepenthes bicalcarata*, and c) developing furled leaves. For all these interactions, the bats use the same communicational traits to find plants with funnel-shaped structures (indicated by dark blue) and morphological traits to roost in them (indicated by light blue). Thus, a positive selection pressure should be imposed on traits that generally facilitate roosting in funnel-shaped plant structures (indicated by blue arrow). Overall, the bats prefer *N. hemsleyana* over other pitchers and pitchers in general over furled leaves as roost (indicated by green arrow). a) *N. hemsleyana*'s communicational and morphological traits seem to be specifically adapted to *K. hardwickii*. Due to their strong dependency on the bats, these functional traits should as well be faced with positive selection pressure. b) The characteristic thorns of *N. bicalcarata* have no deterring effect on the bats but are probably exploited as specific cue. If there is a selection pressure at all, it should be negative, i.e. the thorns should become less conspicuous for the bats (indicated by orange arrow). For *N. ampullaria* it is unknown how the bats identify them. c) So far, nothing is known if plants with furled leaves gain benefits from the bats, which functional traits of the plants are used by the bats and which selection pressure is imposed on these traits.

Although more cryptic traits that stabilize the mutualism directly or indirectly are surely present in both partners, it is likely that the majority of these traits will again be found in *N. hemsleyana*. For example, the plants might have adapted the compounds of their digestive fluid to digest bat-faeces, e.g., via special enzymes or microbial interactions (Schöner C.R. *et al.* 2016; but see Yilamujiang *et al.*, *submitted*). Optimized digestion processes could enable the plants to assimilate faecal nutrients more effectively and at lower expenses compared to arthropod prey, and thus function to maintain the mutualism with bats.

Nepenthes hemsleyana's superior roost quality is likely a consequence of this asymmetric dependency. By offering pitchers with high roosting quality, *N. hemsleyana* outcompetes alternative resources of the bats (Noë & Hammerstein 1994, 1995). The combination of *N. hemsleyana*'s high quality and the bats' general faithfulness, which likely results from different roosting traditions, thus supports the stabilization of this mutualism (Schöner M.G. *et al. submitted* b).

Although more detailed investigations should be conducted in future investigations, my results indicate that the asymmetry of this mutualism results in different selection pressures on the stabilizing traits of the partners. The outcome of this mutualism is so beneficial for *N. hemsleyana* that it can be assumed that *N. hemsleyana* will specialize on the bats even stronger. In contrast, the bats should maintain traits that generally enable them to find and use funnel-shaped plant structures as roosts. The use of different roosting plant species is thus important for two reasons: 1) the bats are able to maintain a larger range (Schöner M.G. *et al. submitted* b) and 2) as a consequence the asymmetric dependency of *N. hemsleyana* on *K. hardwickii* is maintained. Due to the weaker dependency of the bats on *N. hemsleyana*, adverse biotic or abiotic influences on one of the two partners are buffered and the risk of co-extinction is reduced (Bascompte *et al.* 2006; Bascompte & Jordano 2007). Thus, the asymmetry itself stabilizes the mutualism between *K. hardwickii* and *N. hemsleyana.*

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4 PUBLICATION LIST

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4.1 Morphological traits that stabilize the mutualism

4.1.1 Morphological traits of *Nepenthes hemsleyana*

Manuscript 1

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Evolutionary Ecology Research, 2014, **16**: 581–591

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 extra-floral 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

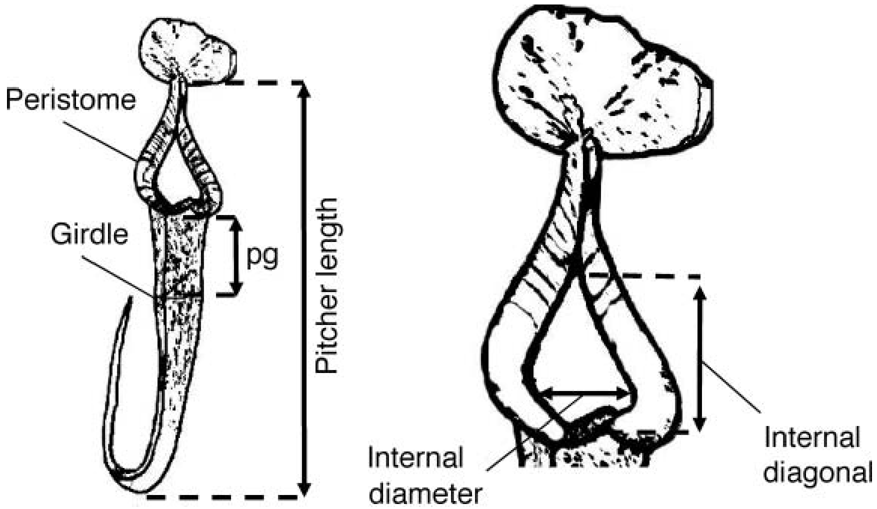

Fig. 1. Measurements undertaken of the aerial pitchers of *N. hemsleyana* and *N. rafflesiana* (pg = distance between peristome and girdle of *N. hemsleyana*; see text for details).

(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 ± standard deviations.

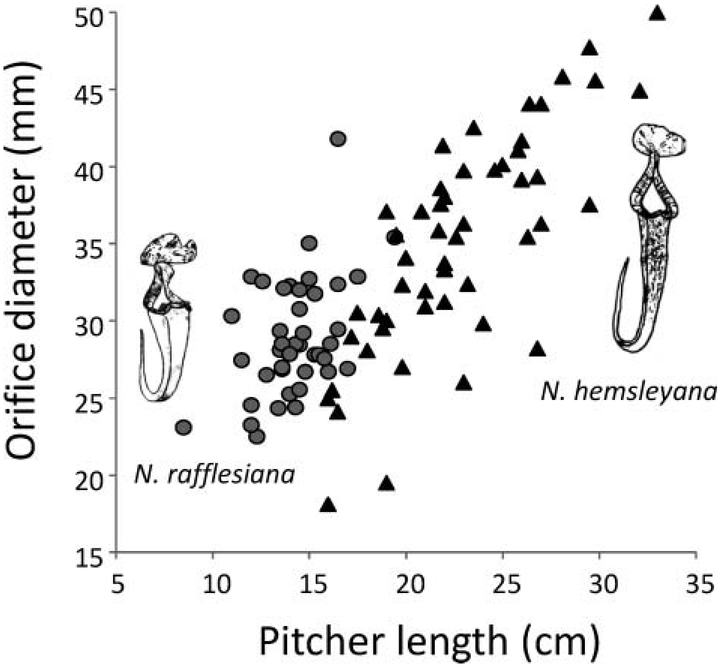

Fig. 2. Relationship between orifice diameter and pitcher length in *N. hemsleyana* (▲) and *N. rafflesiana* (●).

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 mm ± 1.4 mm; $n = 22$), suggesting that bats could manoeuvre into a part of the space below the girdle. Together with their respective calculated cylindrical

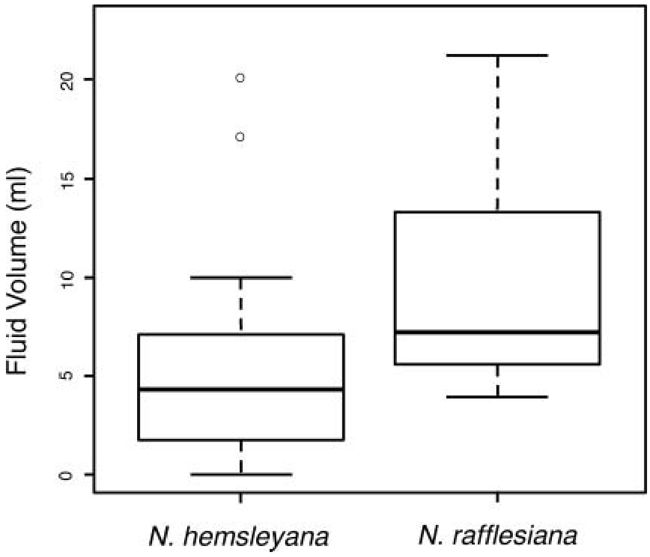

Fig. 3. Fluid volume in *N. hemsleyana* and *N. rafflesiana*. Boxes indicate the 25th and 75th percentiles, the line in the box represents the median, the whiskers are the 10th and 90th percentiles, and the dots show outliers.

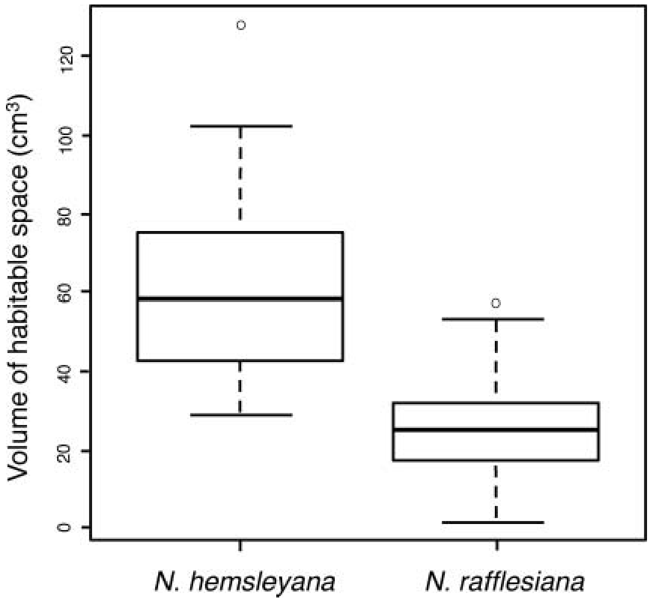

Fig. 4. Volume of habitable space in *N. hemsleyana* ($n = 23$) and *N. rafflesiana* ($n = 37$). See caption to Fig. 3 for further details.

volumes, the total habitable volume of 59.19 ± 25 cm^3 for *N. hemsleyana* pitchers averaged more than twice the habitable volume in *N. rafflesiana* pitchers (27.5 ± 13.25 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).

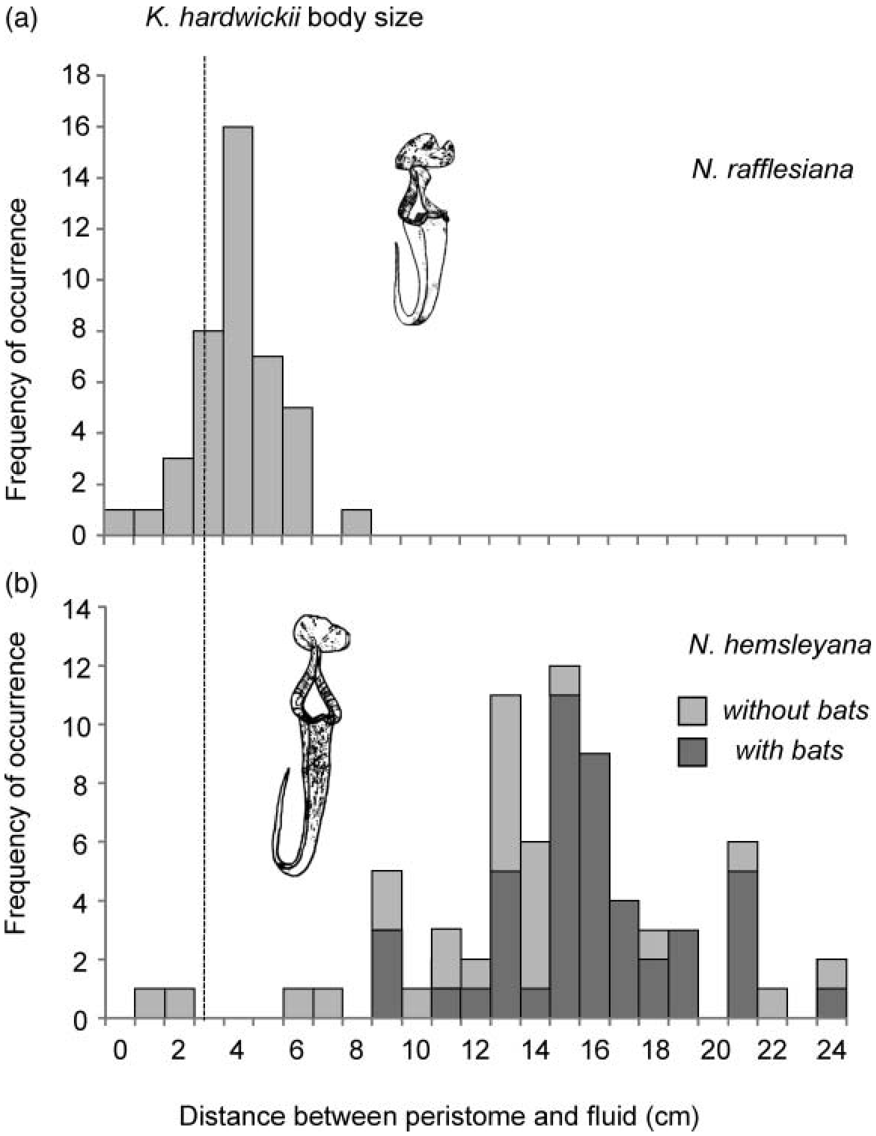

Fig. 5. Size distribution of aerial pitchers of (a) *N. rafflesiana* ($n = 42$) and (b) *N. hemsleyana* ($n = 46$) used as roosts by *K. hardwickii* (dark grey) and not seen to be used by bats ($n = 26$; light grey). Dashed line indicates the average body size of *K. hardwickii.*

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: $U = 523$, $n_1 = 52$, $n_2 = 42$,

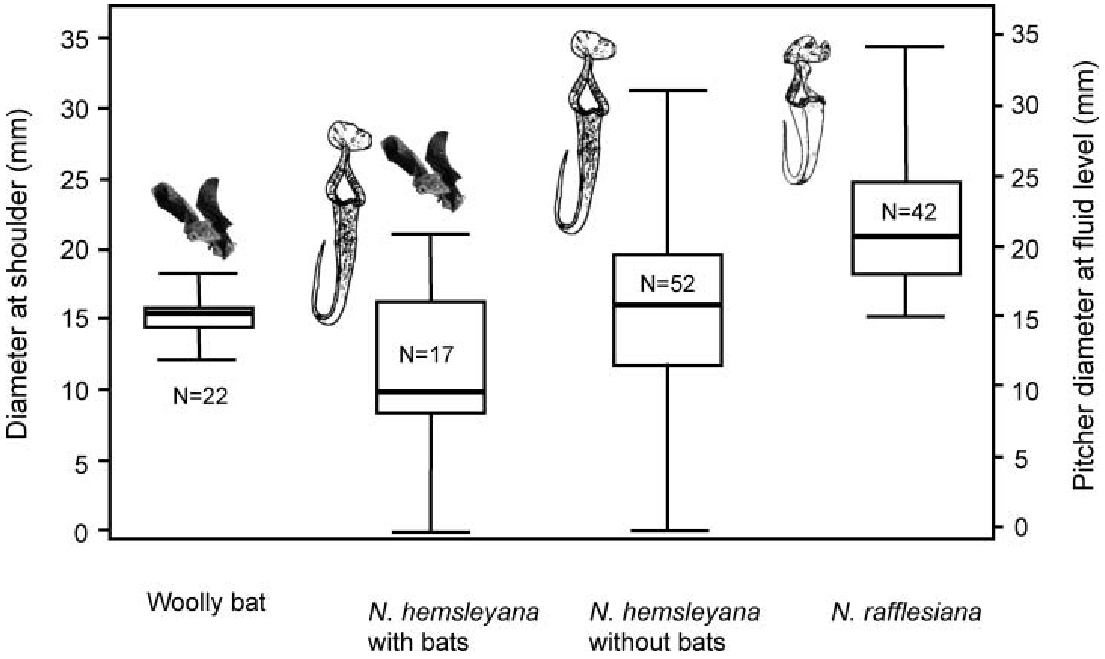

Fig. 6. Diameter of *K. hardwickii* at shoulder height versus pitcher diameter at fluid level in aerial pitchers of *N. hemsleyana* used by bats, aerial pitchers of *N. hemsleyana* not known to be used by bats, and in aerial pitchers of *N. rafflesiana* (photo of bat by C.C. Lee). See caption to Fig.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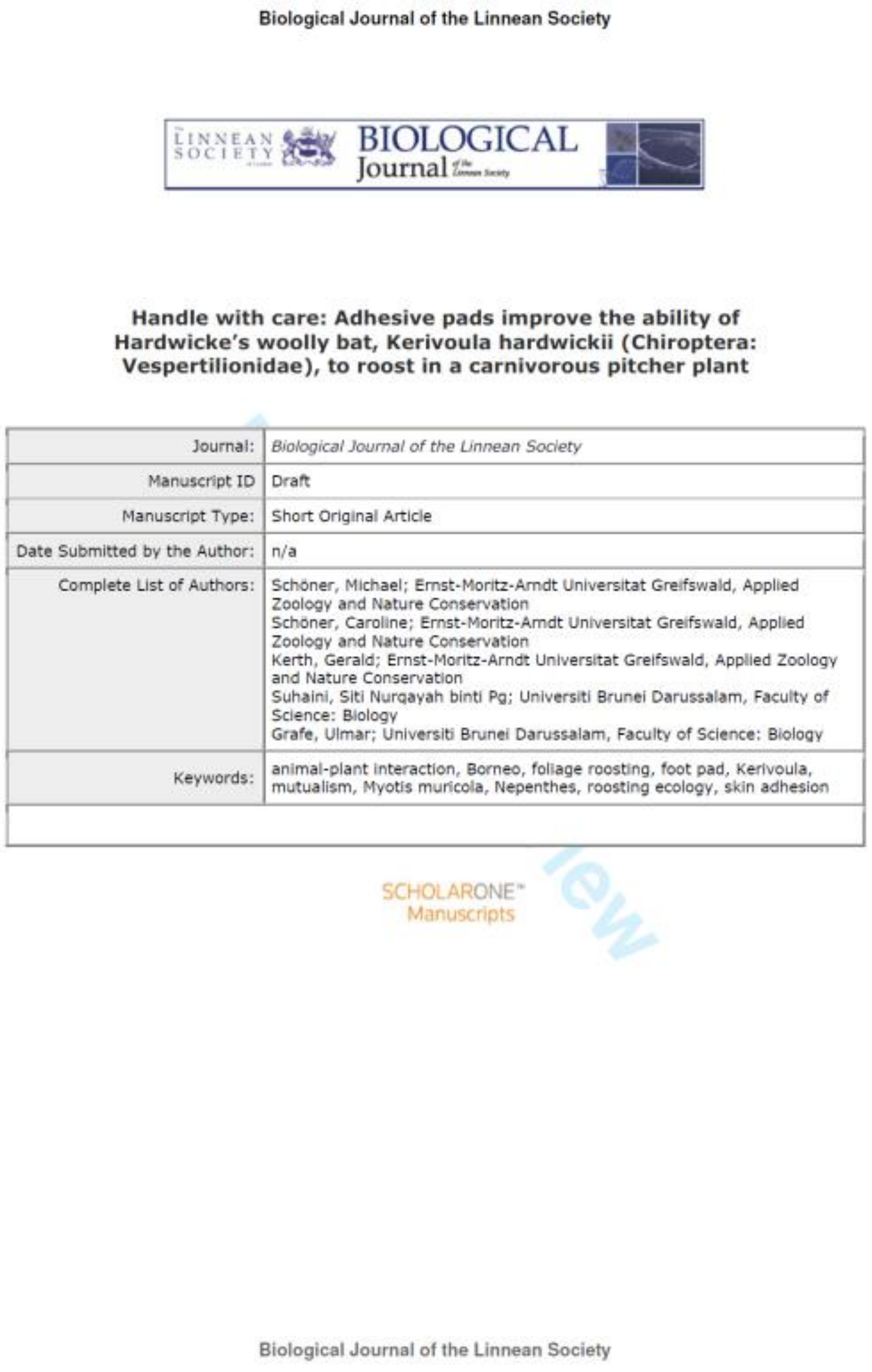
4.1.2 Morphological traits of *Kerivoula hardwickii*

Manuscript 2

Schöner, M.G., Schöner, C.R., Kerth, G., Suhaini, S.N. & Grafe, T.U. (*submitted*): Handle with care: Adhesive pads improve the ability of Hardwicke's woolly bat, *Kerivoula hardwickii* (Chiroptera: Vespertilionidae), to roost in a carnivorous pitcher plant.



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Handle with care: Adhesive pads improve the ability of Hardwicke's woolly bat, Kerivoula hardwickii (Chiroptera: Vespertilionidae), to roost in a carnivorous pitcher plant

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TITLE

Handle with care: Adhesive pads improve the ability of Hardwicke's woolly bat, *Kerivoula hardwickii* (Chiroptera: Vespertilionidae), to roost in a carnivorous pitcher plant

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RUNNING TITLE
Bat adhesive pads

Many animals including several foliage-roosting bats have evolved adhesive pads, which facilitate clinging to and moving on smooth surfaces. In South-East Asia, Hardwicke's woolly bat (*Kerivoula hardwickii*) roosts in furled leaves of different Zingiberales plants and in pitchers of carnivorous *Nepenthes* species. This roosting behaviour led to a mutualism with *Nepenthes hemsleyana*, which provides high-quality roosts in exchange for nitrogen-rich bat faeces. However, even small injuries in *N. hemsleyana*'s soft pitcher tissue lead to accelerated wilting and make pitchers unavailable within only a few days. As we never found injuries in pitchers occupied by bats, we hypothesized that the bats have evolved adhesive pads so that they can avoid using their claws when entering or leaving roosts. We found that *K. hardwickii* has proportionately larger thumb and foot pads and more effectively clings to smooth surfaces than closely related *Kerivoula* species that roost under wilted foliage and – contrary to our prediction – compared to *Myotis muricola*, which also roosts in furled leaves. In conclusion, *K. hardwickii*'s adhesive pads not only facilitate the bats' ability to enter and leave the slippery plant roosts but also prevent damage from pitchers of their mutualism partner *N. hemsleyana*.

KEYWORDS

animal-plant interaction – Borneo – foliage roosting – foot pad – *Kerivoula* – mutualism – *Myotis muricola* – *Nepenthes* – roosting ecology – skin adhesion

The capability to move on slippery surfaces is widespread in arachnids and insects (Federle, Riehle, Curtis & Full, 2002; Peattie, Dirks, Henriques & Federle, 2011). For example, ants of the genus *Crematogaster* are unique in that they can move on the highly slippery waxy layer of *Macaranga* spp. This helps the plants to only host mutualistic ant species, while at the same time benefiting their ant partners by reduced competition with other ant species (Federle *et al.*, 1997; Whitney & Federle, 2013). Adaptations, such as adhesive pads, that facilitate movement on smooth surfaces can also be found in amphibians and reptiles (Endlein & Barnes, 2014). In contrast, only a limited number of mammals shows adhesive structures, e.g., the feathertail glider *Acrobates pygmaeus* Shaw, 1793 (Rosenberg & Rose, 1999; Riskin & Racey, 2010). In bats it is assumed that pad-like structures on the thumbs and feet evolved four times independently in the genera *Thyroptera*, *Myzopoda*, *Pipistrellus* and in the common ancestor of *Tylonycteris* and *Glischropus* (Thewissen & Etnier, 1995). Most of these bat species live in slippery, living plant structures (Feng, Li & Wang, 2008; Chaverri, Gillam & Vonhof, 2010; Ralisata, Rakotondravony & Racey, 2015; Kunz & Fenton, 2005). However, the exact function of these adhesive pads is often unclear (Riskin & Racey, 2010; Riskin & Fenton, 2001), as can be seen in *Pipistrellus nanus* Peters, 1852, where the pad use in the roost is controversially discussed (Thewissen & Etnier, 1995). In contrast, other foliage roosting bat species, which, e.g., use wilted leave structures such as many Paleotropical *Kerivoula* spp. (Francis & Barrett, 2008; Phillipps, 2016) or Neotropical tent-making bats such as *Uroderma bilobatum* Peters, 1866, only use their claws without having evolved adhesive pads (Kunz & Fenton, 2005).

The South-East Asian bat species *Kerivoula hardwickii* Horsfield, 1824, roosts in developing furled leaves of different Zingiberales plant species of ginger (Zingiberaceae), banana (Musaceae), and aroids (Araceae) (McArthur, 2012; own observation), which can be used as roost for only a few hours or days before the leaves open and become unsuitable for the bats

(Happold & Happold, 1996; own observation). On Borneo, *K. hardwickii* additionally roosts in pitcher-shaped trapping organs of three carnivorous pitcher plant species, *Nepenthes ampullaria* Jack, 1835, *Nepenthes bicalcarata* Hook.f., 1873, and *Nepenthes hemsleyana* Macfarl., 1908 (Grafe *et al.*, 2011; McArthur, 2012; Schöner, Schöner, Kerth & Grafe, 2013). In contrast to the other plant species, *N. hemsleyana* and *K. hardwickii* interact mutualistically: The bats receive a parasite-free roost with beneficial microclimate, and in turn fertilize the plants with their nitrogen-rich faeces (Grafe *et al.*, 2011; Schöner, Schöner, Kerth & Grafe, 2013; Schöner *et al.*, 2016). Earlier studies already showed that *N. hemsleyana* shows traits that facilitate the mutualism including a typical shape that prevents the bats from falling into the digestive liquid or an echo-reflecting structure that is attractive for the bats (Lim *et al.*, 2015; Schöner, Schöner, Kerth & Grafe, 2013; C.R. Schöner *et al.*, 2015). However, it has never been investigated whether *K. hardwickii* also has traits that facilitate roosting in funnel-shaped plant structures and thus stabilize the interaction.

Compared to other *Nepenthes* species, the pitchers of *N. hemsleyana* are very sensitive to damage due to their low lignin content (Osunkoya, Daud & Wimmer, 2008). While *N. hemsleyana* pitchers usually last for around 90 days (C.R. Schöner *et al.*, 2015), we observed that pitchers, which we involuntarily had injured, completely wilted within only 7 days. The low lignin content (Osunkoya *et al.*, 2008) not only accelerates wilting it also results in crumpling of the *N. hemsleyana* pitchers so that they are no longer accessible for the bats. Because of this fragility and the fact that the pitchers are generally rare and thus hard to find for the bats (M.G. Schöner *et al.*, 2015), *K. hardwickii* should handle their roosts with care when roosting and moving inside. This is in agreement with our previous findings that the bats do not injure the soft tissue of *N. hemsleyana* pitchers and that the pitchers' longevity was not reduced when bats used them compared to unoccupied roosts (C.R. Schöner *et al.*, 2015).

We assumed that there should be a high selection pressure on *K. hardwickii* of having morphological structures, which avoid injuries of their host plants while moving and roosting inside. More specifically, we hypothesized that *K. hardwickii* has acquired pad-like structures on their extremities, which help them to cling to and move on the plants' surfaces. To test our hypothesis, we investigated *K. hardwickii*'s feet and thumbs as they are in contact with the plant surface when the bats roost and move inside pitchers and furled leaves. We compared the relative size of these morphological structures to that of closely related *Kerivoula* species (*Kerivoula intermedia* Hill & Francis, 1984; *Kerivoula pellucida* Waterhouse, 1845; Khan *et al.*, 2010; Hasan & Abdullah, 2011) that roost in wilted plant structures as well as to that of a not closely related species (*Myotis muricola* Gray, 1846) that – similarly to *K. hardwickii* – uses furled banana leaves as roosts (Francis & Barrett, 2008; Phillipps, 2016; Pottie, Lane, Kingston & Lee, 2005; own observation). Moreover, we determined *K. hardwickii*'s capability to cling to smooth surfaces and compared it with that of the aforementioned bat species. We predicted that the relative size of the relevant morphological structures and the capability to adhere to plant surfaces should be more similar in species with similar roosting habits than in closely related bat species. Finally, we also tested if there are intra-specific differences between *K. hardwickii* that roost in furled leaves and those that use the less ephemeral but highly fragile *N. hemsleyana* pitchers.

METHODS

Description of study site and time

Field work and experiments took place in the Belait district of Brunei Darussalam and the Mulu National Park of Sarawak/Malaysia from 14 August 2011 to 14 January 2012, from 20 June 2012 to 3 December 2012, from 14 April 2014 to 1 September 2014 and from 7 to 25 February 2016. During each of these field seasons we caught the focal bat species (*K. hardwickii*, *K. intermedia*, *K. pellucida*, *M. muricola*) by using harp traps at night (for details see Schöner, Schöner, Kerth & Grafe, 2013). Moreover, we searched for *K. hardwickii* roosting inside *Nepenthes* pitchers (*N. hemsleyana*, *N. bicalcarata*, *N. ampullaria*) and for *K. hardwickii* and *M. muricola* inside furled leaves of different species of ginger (e.g., *Alpinia ligulata* K.Schum., 1899; *Boesenbergia grandis* R.M.Sm, 1982), banana (*Musa muluensis* M.Hotta, 1967) and aroid plants. We determined sex, forearm, thumb, and toe length of all captured bats. Only adult non-pregnant or non-lactating individuals were used for experiments and further analysis. All individuals were marked with a sterile biopsy punch (Stiefel Laboratories, Offenbach Germany; diameter: 2 mm) at their wing membrane to recognise recaptures. Additionally, *K. hardwickii* were marked with transponders (ISO 11784/11785; Peddy-Mark, UK; see Kerth & König, 1999) for long-time identification. Bats that we kept for experiments were placed at room temperature in humid bags to prevent dehydration of the animals. We fed bats and released them within 12 hours after capture into their original habitat. All procedures performed with bats were in accordance with the ethical standards of the institution at which the studies were conducted adhering to the Animal Behaviour Society (Guidelines for the treatment of animals in behavioural research and teaching, 2012).

Determination of pad size

To find out which body parts are essential for the bats to move on and cling to plant structures, we placed 10 *K. hardwickii* in Falcon tubes (diameter: 3.0 cm, length: 11.5 cm) that have a similar diameter compared to *N. hemsleyana* pitchers the bats normally roost in (4.53 ± 0.85 cm; see Schöner, Schöner, Kerth & Grafe, 2013). Moreover, we investigated whether *K. hardwickii* has conspicuous features at their extremities, e.g., enlarged morphological structures that provide better adhesion.

We compared *K. hardwickii*'s extremities to that of the closely related *K. intermedia* and *K. pellucida* (Khan *et al.*, 2010; Hasan & Abdullah, 2011) that occur sympatrically with *K. hardwickii* and have a similar body size as indicated by overlapping forearm lengths (*K. hardwickii*: 28.5-35.1 mm, *K. intermedia*: 26.6-30.1 mm, *K. pellucida*: 26.3 – 33.3 mm; Francis & Barrett, 2008). Further, we compared these morphological data with those of the sympatric *M. muricola* that is similar sized (forearm length: 33.3-36.3 mm) and roosts in furled banana leaves (Pottie, Lane, Kingston & Y.-H. Lee, 2005) that are also used by *K. hardwickii* (Table 1). Of all four bat species, we photographed the body parts (thumb and foot structures) with which *K. hardwickii* adhered to the surface of the Falcon tubes. Thumbs and feet were photographed from above with a 90° angle between forearm and thumb or in a 90° angle to the camera (Fig. 1). To calculate the pad area, we used ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2014). With this software, we first marked the pictures of thumbs/ toes with a line whose exact length was known from the earlier measurements of the referring individual. This defined line was used as scale. Then the area of the adhesive structures at the thumbs/ feet (Fig. 1) were marked with the freehand or the polygon selection and ImageJ automatically analysed the size of the area.

Sliding experiment

To find out how effective the thumb and foot pads in the different species are, we conducted a sliding experiment with all four bat species (Table 1). For the experiment, we placed the bats again in Falcon tubes, which we horizontally fixed in a self-built rotation device (see supplement). To not change adhesion effects due to external humidity, we only used dry falcon tubes and bats with dry fur. Moreover, we only tested the bats when they rested calm in the tube (which was particularly difficult in *K. intermedia*; see table 1). Due to the smooth and hard surface of the tube and its lid the bats could not use their claws to interlock to the surface. The rotation device automatically stopped rotating at angles of 30°, 60°, and 90°. After 5 s the tube rotated to the next angle. We filmed each experiment (Sony HDR-CX560VE) to determine the angle at which bats started to slide.

Moreover, we compared the pad sizes and sliding angles of *K. hardwickii* individuals that roosted in pitchers to those individuals that roosted in furled leaves (note that, we only used individuals that we had found in their roosts and not in harp traps).

Data analysis

We captured 304 bats of the four focal species. Some of these bats were so agitated that the quality of the photographs and the video was insufficient for measuring their thumbs and feet. Students without background knowledge of the hypotheses analysed the photographs and videos. To correct for slight body size differences of the different bat species or sexes, we calculated an individual's "relative pad size" using the formula

$$relative\ pad\ size = \frac{\text{absolute pad size (mm}^2\text{)}}{\text{forearm length (mm)}} \times 1\,mm \text{ (to correct for the unit).}$$

We used Monte Carlo tests to test the null hypothesis that the affiliation to a certain species does not affect relative pad size or sliding angle of the bats during the experiment. We first calculated each species' mean relative pad size/sliding angle, which we then compared to the distribution of values expected under the null hypothesis. The null hypothesis distribution was obtained by permuting the relative pad sizes/sliding angles between species and then calculating the mean relative pad size/sliding angle per species. This procedure was repeated 10,000 times from which the null distribution of the mean relative pad size/sliding angle was obtained. The *P*-value was then calculated by comparing the mean relative pad size/sliding angle for the considered species to the null distribution. The same Monte Carlo test was applied to test whether there are intra-specific differences in pad size and sliding angle of *K. hardwickii* individuals roosting in pitchers and those roosting in furled leaves. Fisher's exact tests for count data were used to compare the different bat species regarding their abilities to avoid sliding.

RESULTS

Interspecific comparison of pad sizes and sliding angles

We found that the bats do not use their claws to stick to the surface of the falcon tubes but used their thumbs and feet (Fig. 1). The relative pad size of thumbs and feet were significantly larger in *K. hardwickii* than expected by random distributions of the relative pad sizes of all species. In all other tested species the relative thumb and foot pad size was smaller than expected (Fig. 2). This different morphology is also reflected in different capabilities of the focal bat species to stick to the Falcon tube. 82.6% of all tested *K. hardwickii* did not slide at all. This significantly differed from *K. intermedia* and *K. pellucida* where only a minority of individuals (28.6% and 37.5% respectively) could avoid sliding at angles between 0° and 90° (Fig. 3). Astonishingly, 91.7% of all *M. muricola* individuals slid. Consequently, *M. muricola* and *K. hardwickii* differed most in their abilities to avoid sliding in the Falcon tube (Fisher's exact test for count data to compare all species: $P < 0.001$; for post hoc analysis see Fig. 3) although both species roost in furled leaves. Finally, *K. hardwickii* slid at significantly steeper angles than the other three tested bat species (Fig. 4).

Intraspecific comparison of K. hardwickii *roosting in pitchers vs. furled leaves*

Individuals of *K. hardwickii* roosting in furled leaves had significantly smaller relative foot pad sizes ($N = 45$, median = 0.27 m^2, range = 0.12-0.54 m^2, $P < 0.001$) than those using pitchers ($N =$

91, median = 0.30 m^2, range = 0.13-0.68 m^2). In contrast, the relative thumb pad size of individuals roosting in pitchers (N = 72, median = 0.07 m^2, range = 0.02-0.13 m^2) was not smaller (P = 0.16) nor larger (P = 0.84) than those using furled leaves (N = 34, median = 0.07 m^2, range = 0.03-0.16 m^2). The difference in the relative foot pad size did not influence the bats' performance in the sliding experiment in which all individuals very well attached to the slippery surface regardless of where we had found them roosting (for both individuals roosting in furled leaves and those roosting in pitchers: median sliding angle = 61-90°, range = 0-90°, respectively, P = 0.53).

DISCUSSION

Adhesive morphological structures in the focal bat species

Our results show that in comparison with closely related species, *K. hardwickii* has enlarged morphological structures at the base of their thumbs and feet, which apparently act as pads and help the bats to adhere to their roosts' surface without using their claws. In the closely related species, *K. intermedia* and *K. pellucida,* the examined morphological structures were smaller relative to their body size, which could explain why these bats started sliding at shallower angles during the sliding experiment. The only other species that roosted in furled leaves, *M. muricola,* had both the smallest relative thumb pads and the worst ability to adhere to the smooth surface of the Falcon tube in the sliding experiment. Only one of 12 tested individuals stayed attached to the surface of the plastic tube at a 90° angle. Despite differences in foot pad size, we did not find significant differences in the adhesive capabilities of *K. hardwickii* individuals that roost in pitcher plants and those roosting in furled leaves. All of these bats strongly adhered to the smooth surface of the tube. The enlarged foot pads suggest that there is strong directional selective pressure on *K. hardwickii* populations roosting in pitchers to enlarge foot pad structures.

Surprisingly, *K. hardwickii* does not have pads that are as sophisticated as those of bats in the genera *Thyroptera* and *Myzopoda* (and also do not roost with their heads upwards). One likely explanation is that the interaction between *Thyroptera/ Myzopoda* and their host plants is much older than that between *K. hardwickii* and its plant interaction partners. Schliemann (1970) suggested a gradual and probably long-lasting evolutionary process that has led to the evolution of such highly developed adhesive pads in these Neotropical and Malagasy bats (Schliemann, 1971). In contrast, the interaction between *K. hardwickii* and the pitcher plants is probably very young. It has been suggested that divergence within the genus *Nepenthes* is very recent and Borneo's peat swamp forests, in which *N. hemsleyana* occurs, are relatively young (Meimberg & Heubl, 2006; Chin, Moran & Clarke, 2010; Clarke, 2006) (Muller 1965).

Kerivoula hardwickii*'s enlarged adhesive pads are beneficial to both bats and pitcher plants*

There are two basic mechanisms how to cling to and move on smooth surfaces: mechanical interlocking and bonding (Bhushan, 2014). Interlocking is realized by the bat *M. muricola*, which is able to roost and move inside furled banana leaves only by using their claws. Bonding usually requires enlarged pads, which have evolved, e.g., in the bat species *Thyroptera tricolor* Spix, 1823, and *Myzopoda aurita* Milne-Edwards & A. Grandidier, 1878. Both roost in developing furled leaves as well (Riskin & Fenton, 2001; Riskin & Racey, 2010).

Although less obvious, *K. hardwickii* has enlarged foot and thumb pads, which facilitate roosting in furled leaves. To roost in furled leaves is probably more widespread and older than roosting in *Nepenthes* pitchers, which is so far only known from Northern Borneo (Grafe *et al.*, 2011; Clarke, Moran & Lee, 2011). We assume that the bats evolved their adhesive pads to facilitate roosting in furled leaves. However, this probably was an important prerequisite for the successful establishment of the bats' mutualism with the fragile *N. hemsleyana*. Potentially, *N. hemsleyana* pitchers can be used for several months by the bats (Osunkoya *et al.*, 2008; C.R. Schöner *et al.*, 2015). However, we observed several times that pitchers with only small injuries started wilting and crumpling within days so that they were no longer available for the bats. This and the fact that the plants need 2.5 months on average to produce a new pitcher (C.R. Schöner *et al.*, 2015) should put a high pressure on *K. hardwickii* to avoid damaging the delicate pitchers of *N. hemsleyana* and thus to reduce the availability of their roosts.

The fact that individuals found in *Nepenthes* pitchers had larger relative foot pad sizes could be a hint that individuals roosting in pitchers are at an advantage when it comes to roosting in pitcher plants and that in areas with high pitcher plant densities natural selection will benefit bats with larger pad structures. Such microevolutionary adaptation helps organisms to deal with new environments even within relatively short time spans (Hendry & Kinnison, 2001). Future research will need to further clarify the evolutionary trajectories and selective pressures that have led to relatively larger pad structures in *K. hardwickii* than in other *Kerivoula*. Moreover, it will be necessary to investigate how exactly these pads function, i.e., whether it is van der Waals forces or wet adhesion, which could be achieved via sweat glands.

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All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted adhering to the Animal Behaviour Society (Guidelines for the treatment of animals in behavioural research and teaching 2012). The University Brunei Darussalam Research Committee (UBD/PNC2/2/RG105 &193), the Forestry Department Brunei Darussalam and the Forestry Department Sarawak (NCCD.907.4.4(JLD.10)-209, (JLD.12)-20 and NO. 173/2014) gave us permission to capture and handle the bats and to work in the field.

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Table 1. Number of individuals whose pad size was measured and those bats which were tested in the sliding experiment.

Fig. 1. Thumb (A-C) and foot pads (D-F) measured for morphological comparison. As can be seen when placed in a Falcon tube (A, D), *Kerivoula hardwickii* attach to surfaces with thumb (B) and foot (E) pads. To measure the size of these pads (C, F) we used the length of the thumb and toes (indicated by lines) and then calculated the pad sizes (indicated by polygones).

Fig. 2. Results of morphological measurements and sliding experiments. (A) Relative thumb and (B) relative foot pad sizes of different species (see text for details). (C) Interspecific comparison of the proportions of sliding and not sliding bats. Fisher's exact tests for count data were applied to test whether the proportion of *K. hardwickii* starting to slide was smaller than that of the other species (significance level: *: $P < 0.05$; **: $P < 0.01$). There was no difference between the proportions of sliding individuals between the other species (Ki vs. Kp: $P = 1$; Ki vs. Mm: $P = 0.13$; Kp vs. Mm: $P = 0.08$). All significances remained after a sequential Bonferroni correction. (D) Angles where bats of different species started to slide. Significant positive (↑) or negative (↓) differences of sliding levels from random distributions (Monte Carlo tests) are shown below each boxplot: significance level: ↑, ↓: $P < 0.05$; ↑↑, ↓↓: $P < 0.01$ (Abbreviations: Kh = *Kerivoula hardwickii*; Ki = *Kerivoula intermedia*; Kp = *Kerivoula pellucida*; Mm = *Myotis muricola*).

Species	Thumb pad			Foot pad			Sliding experiment		
	♂♂	♀♀	∑	♂♂	♀♀	∑	♂♂	♀♀	∑
K. hardwickii	45	61	106	59	77	136	39	53	92
K. intermedia	23	28	51	24	27	51	9	5	14
K. pellucida	15	10	25	12	11	23	4	4	8
M. muricola	3	6	9	3	6	9	3	6	9

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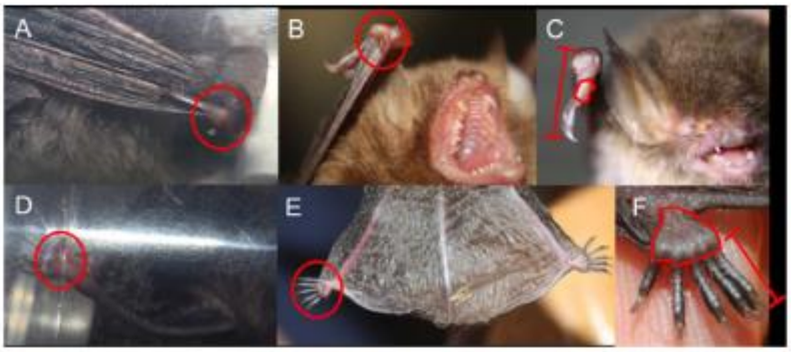
Table 1



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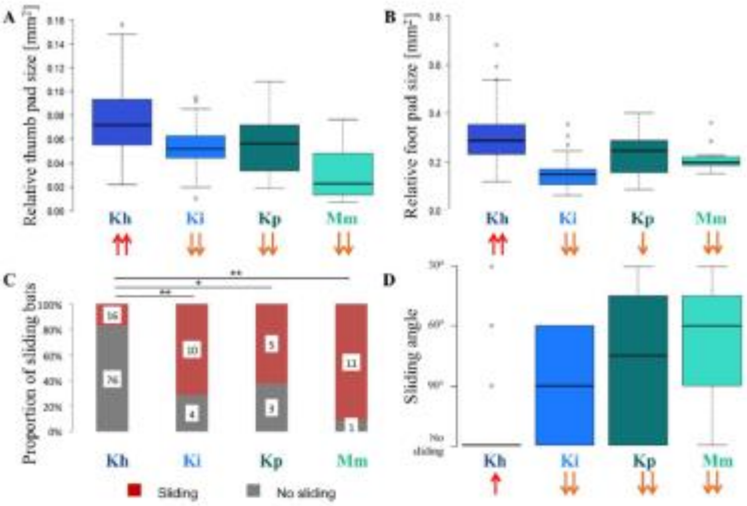

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Fig. 2

490x332mm (72 x 72 DPI)

4.2 Communicational traits that stabilize the mutualism

Manuscript 3

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Report

Current Biology

Bats Are Acoustically Attracted to Mutualistic Carnivorous Plants

Graphical Abstract

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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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.

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CellPress

Current Biology
Report

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

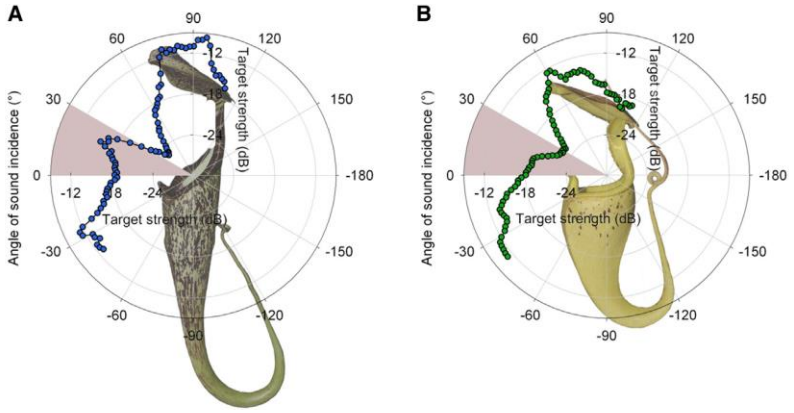


Figure 1. Echo Reflectance of *Nepenthes hemsleyana* and *Nepenthes rafflesiana* Pitchers for the Elevation Plane Given in Spectral Target Strength

(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°), 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° 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 ($13.0 \pm 1.5\ m^2$, mean ± SD) than for *N. rafflesiana* pitchers ($11.2 \pm 0.6\ m^2$; 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 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

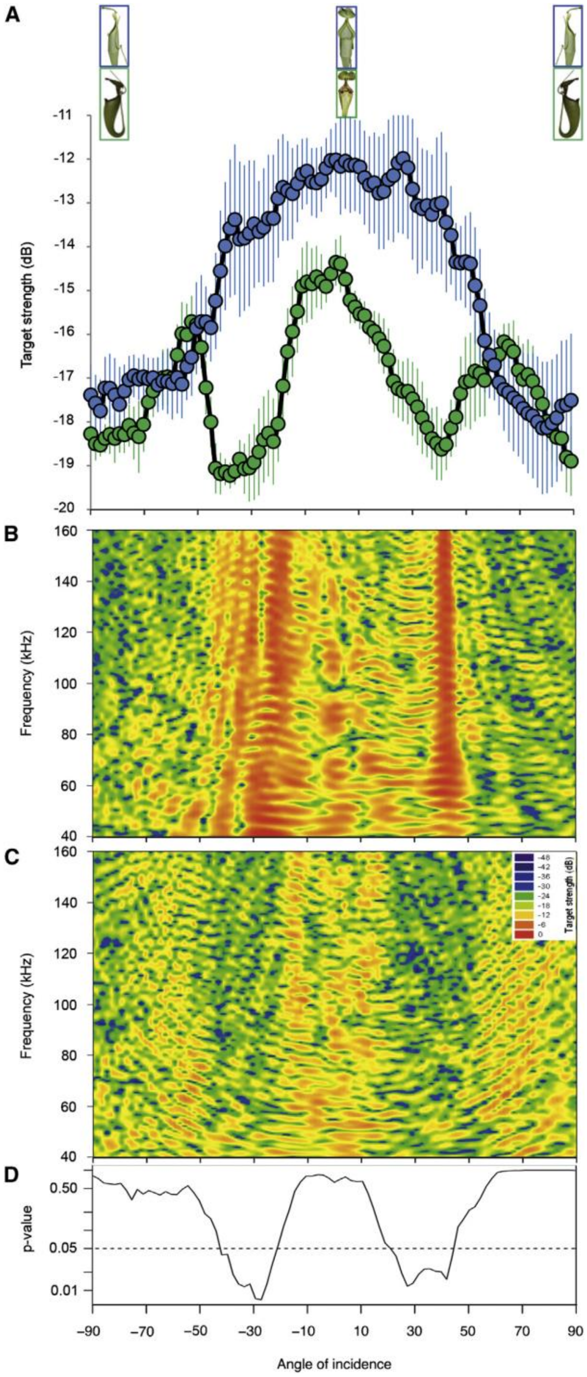


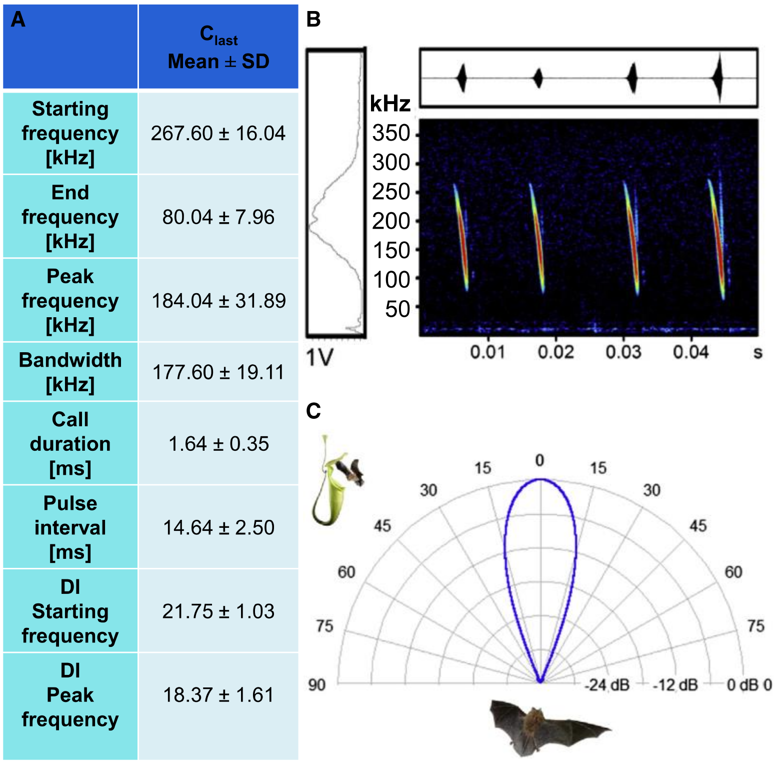
Figure 2. Echo Reflectance of *N. hemsleyana* and *N. rafflesiana* Pitchers in the Azimuth Plane

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).

(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).



A	C_{last} Mean ± SD
Starting frequency [kHz]	267.60 ± 16.04
End frequency [kHz]	80.04 ± 7.96
Peak frequency [kHz]	184.04 ± 31.89
Bandwidth [kHz]	177.60 ± 19.11
Call duration [ms]	1.64 ± 0.35
Pulse interval [ms]	14.64 ± 2.50
DI Starting frequency	21.75 ± 1.03
DI Peak frequency	18.37 ± 1.61

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

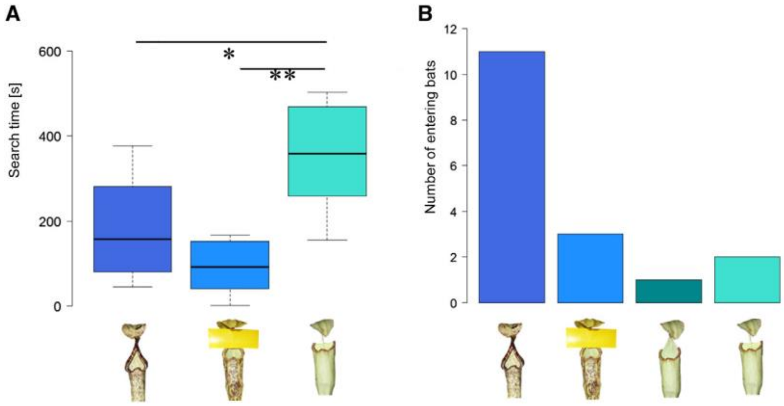


Figure 4. Behavioral Responses of *K. hardwickii* to Reflector Modifications

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 to160 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 permutated 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

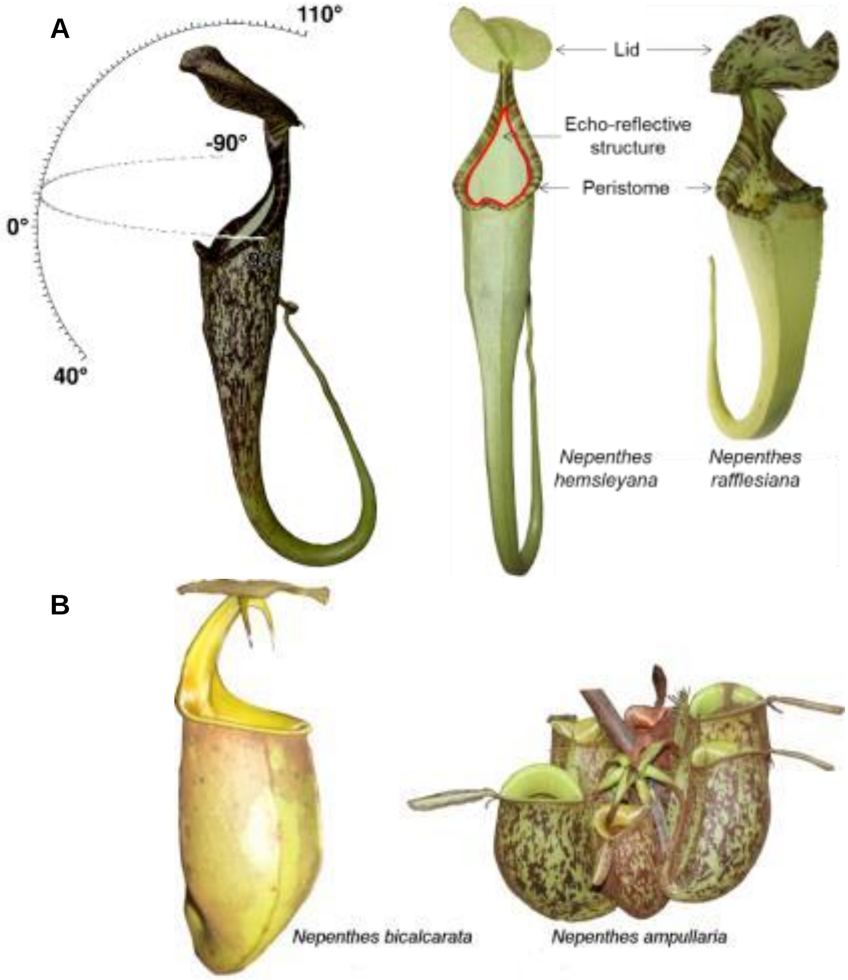


Figure S1, Related to Figure 1 and 2. ***Nepenthes hemsleyana*** **and other sympatric** ***Nepenthes*** **species.**

(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).

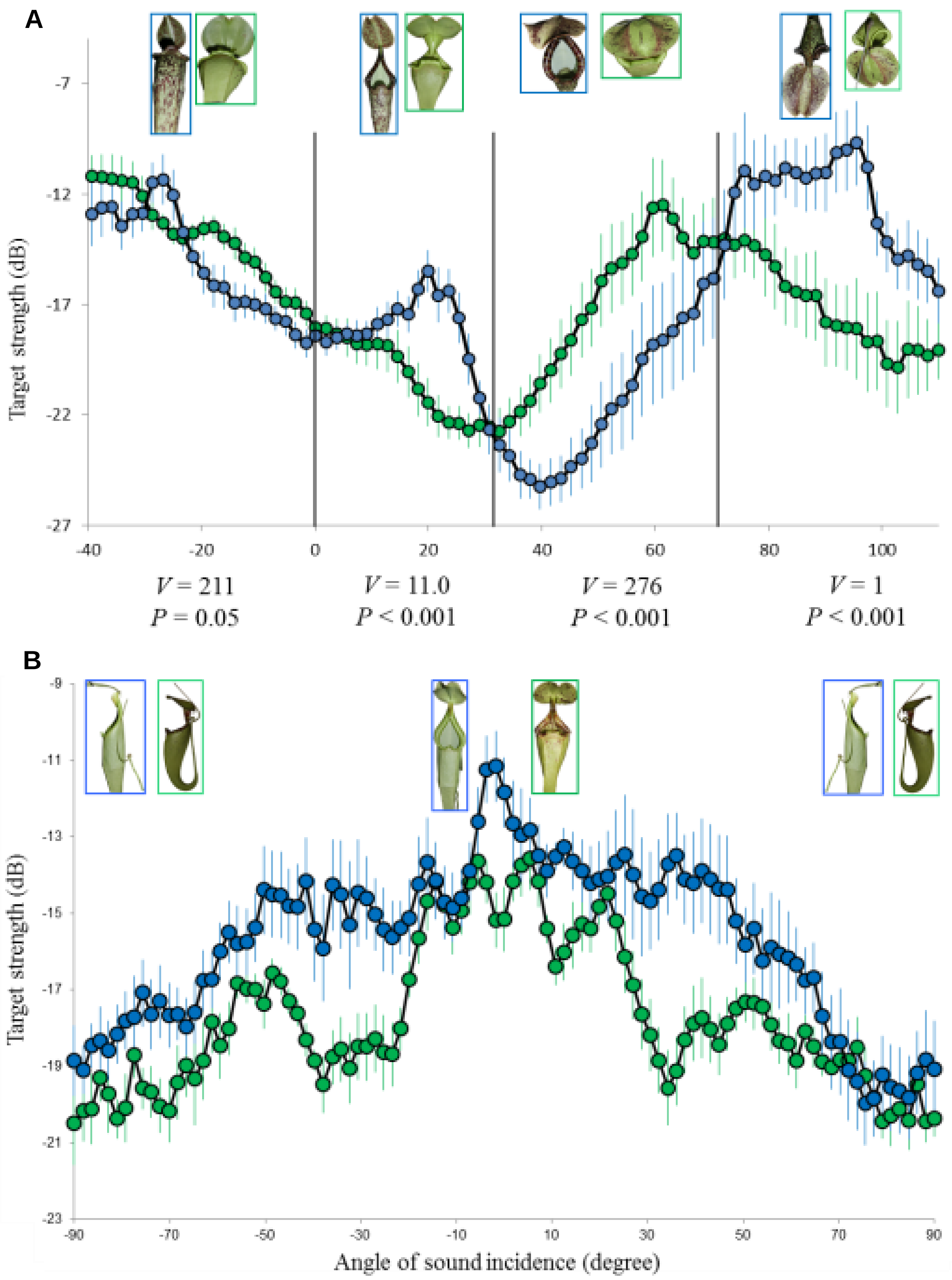


Figure S3, Related to Figure 1 and 2. Target strength of *N. hemsleyana* and *N. rafflesiana*.

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

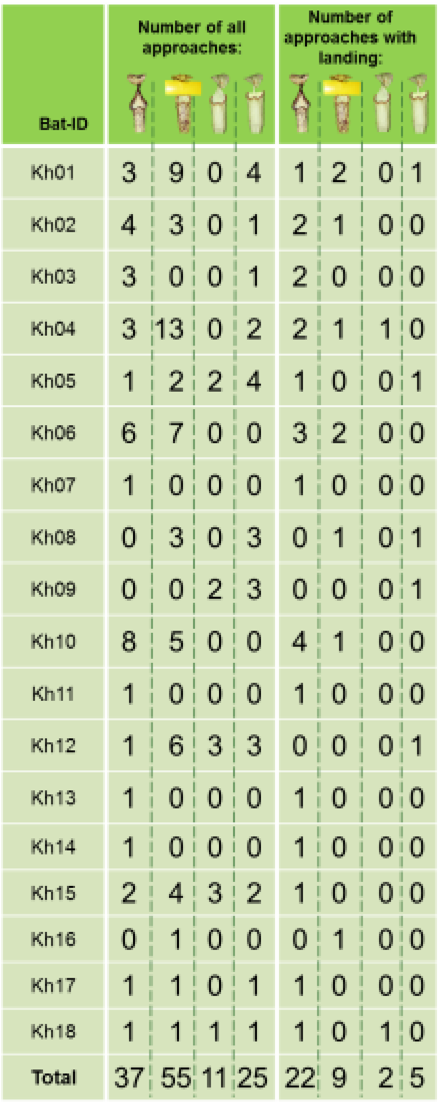
Table S1. Modifications of *Nepenthes hemsleyana* pitchers in three behavioral experiments and experimental set ups.

(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
unmodified	unmodified 11	unmodified 3	unmodified 2
enlarged	enlarged 3	1/3 removed 4	1/3 removed 3
completely removed	partly removed 1	1/2 removed 2	1/2 removed 2
	completely removed 2	completely removed 1	completely removed 1

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 $^1/_4$” 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°) and the azimuthal plane (±90°; 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:

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

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. Noiseinduced 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 ($DI = 20\log10(2\pi a/\lambda)$). 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 × 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 ± 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.

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Acoustic communication in plant–animal interactions

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Acoustic communication is widespread and well-studied in animals but has been neglected in other organisms such as plants. However, there is growing evidence for acoustic communication in plant–animal interactions. While knowledge about active acoustic signalling in plants (i.e. active sound production) is still in its infancy, research on passive acoustic signalling (i.e. reflection of animal sounds) revealed that bat-dependent plants have adapted to the bats' echolocation systems by providing acoustic reflectors to attract their animal partners. Understanding the proximate mechanisms and ultimate causes of acoustic communication will shed light on an underestimated dimension of information transfer between plants and animals.

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Introduction

Communication is widespread in nature and one of the most studied phenomena in biological sciences [1•,2]. In a broad sense communication is defined as the stimulation of a receiving individual's sensory system by trait values of a sender. This stimulation may lead to a change in the receiver's behaviour [2,3] and most often is beneficial for both organisms [4,5]. Acoustic communication occurs when organisms produce rapid vibrations that excite surrounding molecules and generate compression waves that travel away from the source in a fluid medium such as air or water [6]. If the propagating medium is solid, the communication is classified as vibrational. Acoustic and vibrational signals are mostly based on different underlying mechanisms [7,8]. However, in both cases the sender needs morphological traits and an encoding system to produce the vibrations and to transfer the information, while the receiver needs other morphological traits and a decoding system to access the informational content [4].

With acoustic communication it is possible to transfer variable, information-rich content [9]. Moreover, environmental barriers have less influence on the transmission of acoustic information over long distances compared to other forms of communication such as vision [10,11]. Limits to the detection range of information are set by attenuation, depending on the intensity and frequency of the produced sound and abiotic environmental factors such as temperature [12] and air humidity [13]. Generally, sound intensity is inversely proportional to the distance from the source (cube-root function) [6,12]. As a consequence, acoustic communication is difficult in noisy surroundings and sound production becomes costly there [14]. Attenuation and noise can lead to the selection of vocalizations that stand out from the environment [15,16] or even cause a shift to another sensory channel [17]. A further disadvantage is that acoustic signalling enables eavesdropping predators to locate the sender [18].

Intraspecific and interspecific acoustic communication has mainly been investigated in animals [1•,11,19] for which acoustic signals are shaped by sexual selection and ecological factors [19] to fulfil various functions such as attraction of mating partners [20], defence from predators [21] and coordination of social groups [22]. Outside the animal kingdom little is known and acoustic communication in protozoa or bacteria has rarely been investigated [23]. Recently, it was suggested that acoustic communication might be found in further organismal kingdoms, including plants [1•,24]. In contrast to animal–animal interactions, acoustic plant–animal communication is not mainly driven by sexual selection of mating partners but by selection of mutualism partners leading to a broader dimension of concepts such as sensory drive or exploitation of sensory bias [2]. Strong support for acoustic communication between animals and plants has been found in bat–plant interactions [25••,26••,27].

Do plants produce acoustic signals and do they react to sounds?

Recent studies investigated how chemical compounds, light or physical contact contribute to communication processes in plants. This can be seen in plants that transfer information about drought stress probably via root metabolites to unstressed neighbours, which react with stomatal closure [28]. Furthermore, plants perceive competitors for light via a decreased red to far-red light ratio, touches of surrounding leaves or the ethylene release of neighbouring plants resulting in cell elongations and vertical orientation of the leaves (hyponasty) [29–31]. It has also been shown that plants are able to recognize close relatives via root communication, opening up the possibility of kin selection in plants [32].

Acoustic traits have been neglected so far amongst others due to a lack of obvious sound producing or hearing organs. Thus, scientists repeatedly called for bio-acoustical research to examine the possibility of intraspecific and interspecific acoustic communication in plants [1•,24,33,34]. Plants are known to produce low and high frequency ultrasounds (10–300 kHz) [35,36], which might result from a rapid decrease of tensions in the plants' xylem after cavitation, for example, due to drought stress [37–38], from a bubble system in the xylem [36], from respiration and metabolic growth activity of the cambium [39] or from movements of organelles [1•]. Recent research demonstrates that plants are capable of producing sound in the absence of drought stress and cavitation processes. Young corn roots, for example, produce click-like sounds when submerged in water [40•]. Yet, proofs for communication purposes of such sounds are lacking so far.

Plants are also able to respond to sound waves, for example, by altering gene expression [41], phytohormone production [42], germination and growth [40•,43]. However, the underlying mechanisms are largely unknown. It is possible that complex mechanosensitive channels are responsible for the perception of acoustic signals [1•]. Weiming *et al.* recently developed an experimental platform to test the effects of sound on plants with more sophisticated experiments [44].

Not only the proximate mechanisms of sound production and responses to sound are unclear, but also the ultimate functions of plant acoustics are poorly understood. Using an experimental box Gagliano *et al.* tested whether plants are able to use other ways to recognize neighbouring plants than chemical signals, light and physical contact. As this was indeed the case the authors suggested this was an indication for acoustic communication between plants [24,45].

Do plants acoustically communicate with animals and how does it work?

To communicate with animal mutualists, parasites and herbivores [5], plants use various signals. The recent detection of floral electric fields that can be perceived by pollinators [46] demonstrates how cryptic such signals and cues can be for humans. The same may be true for acoustic communication with animals [2].

Well-supported examples of acoustic communication between plants and animals are found in bat-dependent plants, which mutualistically interact with echolocating bats. Finding plant partners echo-acoustically is challenging as plant echoes are highly diffuse, variable, and depend on the shape and position of each individual plant and its organs [47•,48,49]. Commonly, plants negatively influence acoustic communication of animals. Consequently, animals need to circumvent, overcome and adapt to them [12]. This is especially true in cluttered habitats where it is even more complicated for bats to detect and identify the mutualistic plants as surrounding vegetation could reflect ultrasound calls more intensely than the focal plants. Therefore, the bat-dependent plants evolved morphological structures that produce conspicuous echoes to catch the attention of bats (Figure 1).

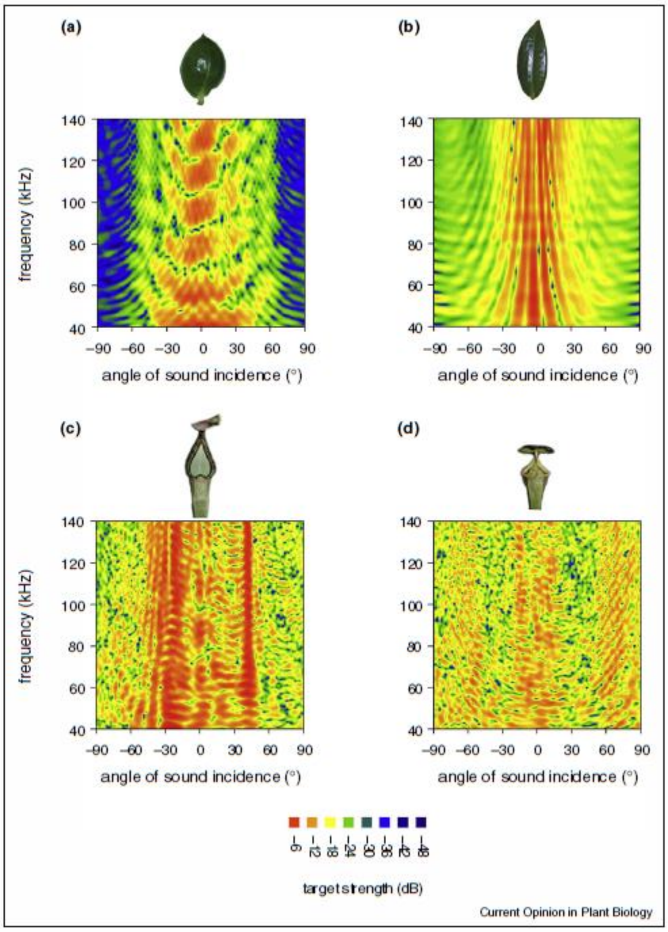
Several Neotropical bat-pollinated plants (e.g. *Mucuna holtonii* and *Marcgravia evenia*) evolved flowers with modified petals or leaves, which reflect the ultrasound calls of approaching bats with high intensity and in a broad range of angles [26••,27,50]. The flowers of some bat-pollinated cacti are surrounded by a hairy cephalium, which may enhance the echo reflection of the flower by attenuating background noise, for example, from the stem. Moreover, several bat-pollinated bell-shaped flowers (e.g. *Markea neurantha*) produce echoes of long duration with a complex spectral composition [51]. Experimental studies showed that these flower structures or leaves are essential for the animals to efficiently find their partner plants and exploit their nectar [26••,27].

Convergent structures can be found in the Paleotropics. In Borneo, a carnivorous pitcher plant (*Nepenthes hemsleyana*) is fertilized with nitrogen-rich faeces of a mutualistic bat species (*Kerivoula hardwickii*) that roosts inside the plants' pitchers [52]. A concave structure in the back wall of these plants' pitchers strongly reflects the ultrasound calls of the bats. This structure is missing in closely related *Nepenthes* species that do not interact with bats [25••].

Several bat species also use echolocation — often in combination with olfactory cues — to locate fruits [53–55]. Flagellichory, the exposed hanging of fruits on a long peduncle, seems to be a plant trait relevant for bats to echo-acoustically detect fruits. This is costly for the plants (e.g. *Gurania spinulosa*) as leafless, pendulous branches do not contribute to their photosynthetic yield. Nevertheless, these plants benefit from their animal partners (e.g. *Phyllostomus hastatus*) as bats are effective long-distance seed dispersers that do not harm the seeds [55]. The elongated fruits of *Piper* that stick out of the surrounding vegetation are another prime candidate for possible echo-acoustic adaptations in fruits [54]. However, these are all passive acoustic signals where plants reflect animal sounds to communicate with them.

Whether there is also active acoustic signalling in which plants produce sounds themselves to communicate with animals, still needs to be demonstrated (Figure 2). The last decades have revealed the astonishing capabilities of

Figure 1



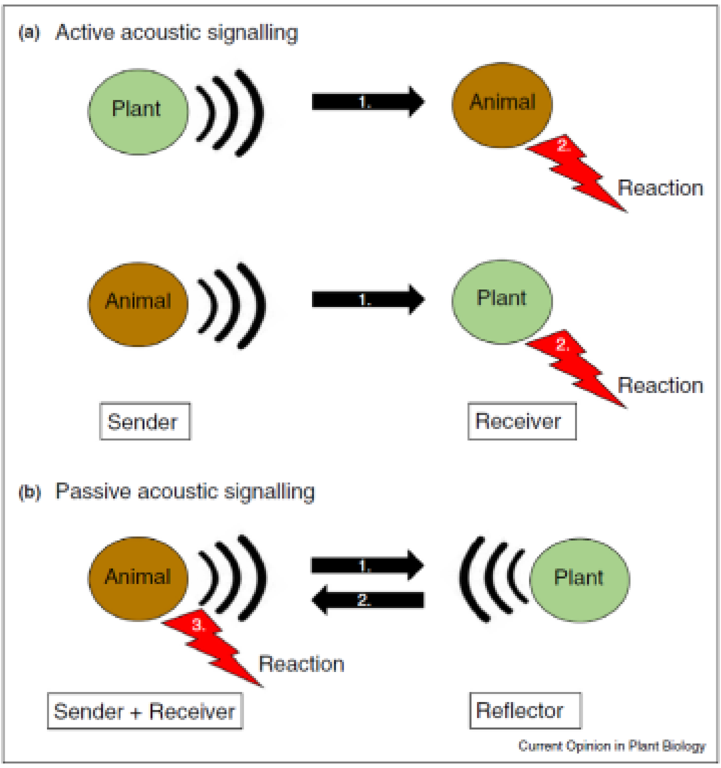
Passive acoustic signalling in two non-related plant species. The Neotropical vine *Marcgravia evenia* attracts bats, which pollinate its flowers. Exemplary spectral directional patterns of a dish-shaped leaf **(a)** and a foliage leaf **(b)** are shown. Dish-shaped leaves echoes' were of high intensity, multidirectional and had an invariant echo signature compared to foliage leaves [26**]. The Paleotropical carnivorous plant *Nepenthes hemsleyana* attracts bats that fertilize the plant with their faeces. Exemplary spectral directional patterns of the back wall of *N. hemsleyana* pitchers **(c)** show that this structure is a similar multidirectional echo-reflector as the dish-shaped leaves of *M. evenia*. Such structures are missing in other pitcher plant species such as *N. hemsleyana*'s closest relative *N. rafflesiana* **(d)**, which does not attract and host bats [25**].

plants to actively but non-acoustically communicate with animals in various ways, such as via volatile production to attract natural enemies of their herbivores [56]. Active communication might also be possible via sound; for example, sound waves produced by plants may not only have attractive [57] but also deterrent effects on potentially harmful herbivores.

How essential is acoustic communication with animals for plants and why have plants acquired acoustic traits?

Sophisticated acoustic signals have evolved for different purposes in different species: *Ma. evenia* has a patchy distribution pattern and grows in dense canopy vegetation. The inflorescences do not hang out on long peduncles and

Figure 2



Types of acoustic communication between animals and plants. **(a)** Active acoustic signalling indicates that either the animal or the plant emits sound, which leads to a reaction in the respective partner organism. **(b)** In passive acoustic signalling plants communicate with animals by reflecting sound produced by the partner animal, which will lead to a reaction in the animal partner.

thus need to stand out in the dense vegetation [58]. This is accomplished by the highly echo-reflective leaf close to the flowers, which helps the bats to efficiently find the flowers [26**].

In contrast, inflorescences of *Mu. holtonii* are easier to detect as they hang out of the canopy on long peduncles [27]. However, within the inflorescences single flowers are hard to locate and exploit as — the nectar is hidden and can only be released when the bats land on the flowers, press their snouts between two petals and thereby release an explosion mechanism. To coordinate these complex behaviours, the bats rely on the guiding properties of the echo-reflecting flower petal. Moreover, the spectral directional echo patterns of virgin and exploited flowers differ, which helps the bats to select virgin flowers that offer the highest amount of nectar [50]. The conspicuous echoes of many bell-shaped flowers help the bats to distinguish these flowers from other vegetation and may also guide bats to the nectar source [51].

In *N. hemsleyana* the echo-reflective back-wall structure of the pitchers seems to serve a mixture of different purposes. Similar to *Ma. evenia*, these pitcher plants grow in dense vegetation. The pitchers' echo reflector reduces the bats' search time for pitchers and guides them to the pitchers' entrance. Additionally, bats are able to discriminate *N. hemsleyana* pitchers from those of other pitcher plant species that are not suitable bat roosts, because the pitchers have a species-specific spectral signature [25**], which could be further supported by the waxy crystals on the surface of the echo reflector that are missing in most sympatric and closely related species [59].

All these plant species depend on their bat interaction partners relatively strongly but not exclusively. They occasionally interact with other partners (e.g. many bat-pollinated flowers are also visited by birds [60]) or use alternative resources (e.g. *N. hemsleyana* gains additional nutrients from arthropod prey [52,61]). Nonetheless, *N. hemsleyana* relies predominantly on the bats and assimilates nitrogen from their faeces faster than that from arthropods [62]. One reason for the high specialization might be that bats are reliable nitrogen providers that are faithful to their partner plants for years [61]. Bats are also reliable and effective pollinators having a large home range and an excellent spatial memory, which is especially beneficial for rare plants with a patchy distribution pattern [60].

Ultimately, acoustic attraction has several advantages for plants: First, it opens up alternative interaction possibilities and therefore enables species to occupy novel ecological niches. Second, competition for interaction partners, such as for pollinators [63], can be avoided. Third, acoustic attraction could be less costly than other attraction pathways that require, for example, the production of additional tissue for large flowers or chemical compounds for volatiles [40•,64]. This could be true for ***N. hemsleyana*** that occupies a new niche by interacting with bats and thus avoids competition for nutrients with other ***Nepenthes*** species [52,65–67]. By developing effective acoustic attraction mechanisms for the bats [25••] ***N. hemsleyana*** could reduce potentially more costly arthropod-attracting mechanisms, such as volatiles [68]. Future studies should assess costs of different attraction pathways and potential consequences for the plants' fitness.

Conclusions and future prospects

The evolution of intraspecific and interspecific acoustic communication in plants and their communication with animals still remains a puzzle [1•,2]. However, there is not only a lack of knowledge regarding the ultimate functions of active sound production and reception in plants but also regarding their proximate mechanisms [1•,33]. We still have limited knowledge on whether or how plant species react to animal sounds. There are further important questions. Which plants are capable of communicating acoustically? Which ecological parameters make them prone to this kind of communication? How did acoustic communication evolve and what was its origin? To solve these puzzles, well-designed experiments (e.g. playback/ensonification experiments) and screenings need to evaluate which acoustic signals are produced or perceived by plants and the ecological functions of these sounds [33,34].

The situation is better for passive acoustic signalling. For several bat–plant interactions, we have already gained detailed knowledge on how communication works, which parts of the plants are involved and why they are highly echo-reflective. Nevertheless, many ultimate questions remain. Is the echo-reflective structure an adaptation to the interaction with animals or an exaptation followed by successive secondary adaptation [69]? Currently, we only know of a few interactions that involve passive acoustic signalling. However, many more plants are likely to be involved in interactions with acoustic communication [25••,26••]. Around 250 genera of Neotropical plants are pollinated by echolocating bats [60]. Moreover, the fertilization of plants by bat faeces does not seem to be limited to ***N. hemsleyana*** and is also found in other plant species, including trees [70]. Thus, it is highly likely that many more plant species attract bats acoustically. Furthermore, it would be interesting if passive acoustic signalling could also be found in interactions with other animals that mainly or partly orientate acoustically (e.g. for frugivorous oilbirds it is unclear whether they use echolocation not only for orientation but also for foraging [71,72]). Various insects (herbivores, parasites and mutualists) might also acoustically interact with plants as they have excellent hearing capabilities. Moreover, several species are known to produce ultrasound (e.g. to avoid predation by bats) [73]. Yet, only few studies indicate the possibility of echolocation in insects [7,74]. Thus, research on echo-reflecting plant traits that attract mutualistic insects is missing so far. Finally, several mutualistic ant species colonize plants (e.g. ***Korthalsia robusta***), which they defend against herbivores and other ant species. If an intruder is detected, the ants start alarm signals by knocking with their heads and abdomens on the plant's stem, which creates a rustling sound that alerts colony members in the whole plant [75] and even colonies in neighbouring plants (M and C Schöner, personal observation). As the plants benefit from their ant guardians, selection should favour characteristics of the plant tissue that effectively transfer the sound.

Not only acoustical but also vibrational communication might be possible between several plant and animal species. It has been shown that vibrations specifically caused by herbivores, such as chewing and notably not by other causes, such as wind, induce chemical defence mechanisms in plants [76]. Comparable to volatile signals that attract predators of herbivores [56], plants could have been selected to amplify vibrational signals of herbivores to alert protective symbiotic species such as ants [77] after herbivore infestation. Various organisms also communicate in competitive and mating contexts through plant-borne vibrations as can be seen in green lacewings [78–80]. As the green lacewings also predate on herbivores from these plants it is likely that they select host plants that transfer their songs best. In contrast, plants should evolve traits that inhibit communication of herbivores (e.g. treehoppers).

The current challenge is to discover interactions where plants communicate with other plants or animals acoustically or vibrationally. Traditionally, plants have not been considered to behave or actively communicate, which is one reason why acoustic recordings of plants are still largely missing. In the case of ultrasound communication a further challenge is to discover interactions that cannot be heard by humans [25••,26••,39]. Consequently, echo-acoustic structures are difficult to detect and elaborate

analyses and experiments will need to reveal whether certain structures produce loud and conspicuous sounds or echoes, if and how animals will react to them, and if these sounds or echoes have common patterns [25••,26••]. Likewise, more knowledge is necessary about the underlying processes on the cellular level (but see the signalling model of Mishra *et al.* [81]). Finally, a promising area of research is the investigation of the question whether and to what degree, plants can facilitate or even use substrate-borne vibrational signals for communication purposes. Overall, discovering new ways of acoustic and vibrational communication in plants will open up a new world and will essentially increase our knowledge about how organisms interact with each other.

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Manuscript 5

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Bats attend to plant structures to identify roosting sites

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More than half of the extant bat species rely on plants as roosts. Nevertheless, it is largely unknown how bats find these roosts and whether they use characteristic plant structures for their identification. The bat *Kerivoula hardwickii* regularly roosts in damaged pitchers of the carnivorous pitcher plant *Nepenthes bicalcarata*. These pitchers are characterized by two sharp, long thorns directly above the pitchers' opening. In two behavioural experiments we tested, if 1) the length of the thorns or 2) the distance between thorns and pitcher opening has an attractive or deterrent effect on *K. hardwickii*. The bats preferred pitchers with longer thorns while the distance between thorns and pitcher opening did not influence them. This shows that the bats are not deterred by the thorns. It also suggests that they do not exploit the pitchers' thorns as a protection against being preyed on while roosting. In this case the bats should have chosen pitchers with thorns close to the pitcher's opening that provide the most effective protection. Instead, *K. hardwickii* seem to use the thorns as identification cues to find suitable roost sites. Generally, our study suggests that bats even attend to plant structures that do not provide them with a direct benefit.

Key words: roost finding, bat-plant interaction, *Kerivoula*, *Nepenthes*, pitcher plant

INTRODUCTION

Many animal species including bats rely on shelters to hide from predators (Manser and Bell, 2004), to minimize effects of adverse climatic conditions (Schwarzkopf and Alford, 1996; Shimmin *et al.*, 2002) and to engage in social interactions and information transfer (Wilkinson, 1992; Kerth *et al*., 2003; Kerth, 2008). To ensure optimal roosting conditions in a changing environment and due to changing physiological demands, many bats are forced to regularly switch and select new roosts (Kerth *et al*., 2001; Bartonička and Řehák, 2007). This is especially true when roosts are ephemeral as it is often the case in plant structures (Chaverri *et al*., 2010) that are used by more than half of the extant bat species (Kunz and Lumsden, 2005). Despite the importance and fitness relevance of roosts (Chaverri and Kunz, 2011), how bats find new roosts is poorly understood (Ruczyński *et al*., 2007). Most bat species are group living (Kerth, 2008) and it has been shown that individuals of some of these species use vocalizations of their conspecifics to find new roosts (Ruczyński *et al*., 2007; Chaverri *et al*., 2010; Schöner *et al*., 2010; Chaverri and Gillam, 2013). However, how individual bats initially find new roosts and which roost attributes are relevant for roost location and identification is barely understood. This is especially true for solitary bats: they cannot rely on the help of their conspecifics resulting in increased selective pressure to find and identify new roosts when the former roosts are no longer suitable.

One possible explanation for how bats find and identify their roosts in plant structures is that they cue in on such characteristic structures. Many plant species intentionally advertise their presence with conspicuous signals and structures to attract beneficial animal species (Hossaert-McKey *et al*., 2010) including bats (von Helversen and von Helversen, 1999; Simon *et al*., 2011) or to deter harmful ones (Huang and Renwick, 1994; Schaefer *et al.*, 2004). However, such traits can also be exploited by animals for their own advantage, e.g., when plants produce volatiles for certain pollinators but instead attract herbivores (Andrews *et al*., 2007; Cha *et al*., 2011). In bats it is unknown so far whether they attend to typical plant structures to find and identify their roost plants.

Here we investigate the role played by the prominent, sharp, thorn-like structures of the carnivorous pitcher plant *Nepenthes bicalcarata* for the woolly bat *Kerivoula hardwickii* that uses *N. bicalcarata*'s pitchers as roosting sites (Schöner *et al.*, 2013, 2015*a*). These thorns are a unique characteristic of *N. bicalcarata* pitchers (Clarke, 2006). In fact, thorns are missing in all other *Nepenthes* species including the sympatric *N. mirabilis*, *N. gracilis*, *N. rafflesiana*, the rarely used *N. ampullaria* or the bats' preferred pitcher plant roost, *N. hemsleyana* (Schöner *et al.*, 2013, 2015*a*). *Nepenthes bicalcarata*'s thorns are positioned at the lid of the pitcher directly pointing towards its opening (Fig. 1). They contain nectar glands that serve as a food source for symbiotic ants (Merbach *et al.*, 1999). It has also been suggested that the thorns discourage mammals, e.g., western tarsiers and other small mammals from damaging the pitchers (Merbach *et al.*, 1999; Phillipps and Phillipps, 2016). As *N. bicalcarata* pitchers usually are full of digestive fluid, *K. hardwickii* can only roost in damaged pitchers in which the fluid has drained out through small holes. To make them suitable roosts, it is possible that the bats bite these holes into the pitchers (Schöner *et al.*, 2013). Damaged and without digestive fluid, *N. bicalcarata* cannot take up nutrients from bat faeces as done by *N. hemsleyana* (Grafe *et al.*, 2011; Schöner *et al.*, 2013, 2015*a*). *Kerivoula hardwickii* use both pitcher plants as roosts but prefer roosting in *N. hemsleyana* probably due to better microclimatic conditions and reduced parasite infestation risk. In contrast to the bulky *N. bicalcarata* pitchers, *N. hemsleyana* also has a shape that perfectly fits to the bats leaving just enough space for a single *K. hardwickii* individual or a mother with pup (Schöner *et al.*, 2013; Lim *et al.*, 2015). Nevertheless, the bats regularly use *N. bicalcarata* pitchers as *N. hemsleyana* is rare and the bats' range would otherwise be strongly limited (Schöner *et al.*, 2013).

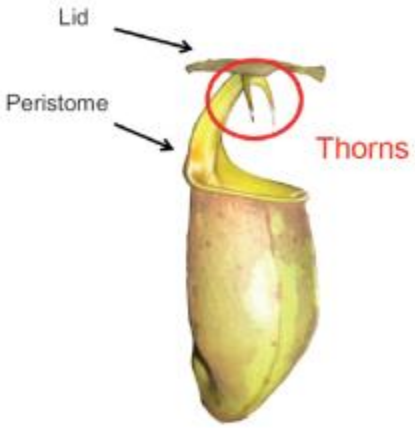


FIG. 1. Morphology of a *N. bicalcarata* pitcher: the most typical and unique morphological characteristic of *N. bicalcarata* pitchers are two thorns, which derive from the pitcher's lid. These thorns are located directly above the pitcher's opening and contain large extra-floral nectaries

Several reactions of *K. hardwickii* towards the thorns are possible: If the bats indeed act as parasites and damage the pitchers, *N. bicalcarata* should deter them from using their pitchers. The sharp and potentially harmful thorns could then fulfil this function especially as the bats' wing membranes are highly vulnerable. Hence, *K. hardwickii* should avoid pitchers with such thorns if they also can choose a thorn-free pitcher. Alternatively, the bats could exploit the protective function of the thorns to be safe from predators, such as snakes or other mammals (Phillipps and Phillipps, 2016), while roosting in these pitchers. We regularly find *K. hardwickii* with severe injuries suggesting that predation takes place (personal observation). It has been reported that western tarsiers feed on both bats and the prey of pitcher plants. Noteworthy, the tarsiers had more difficulties to exploit the thorny *N. bicalcarata* than *N. rafflesiana* pitchers (see Phillipps and Phillipps, 2016). If the bats use the thorns as protection, they should prefer pitchers with thorns close to the pitchers' opening so that predators, such as the western tarsier, have limited access to the pitchers. However, as such thorns are unique for *N. bicalcarata* we also hypothesized that the bats use the thorns for roost identification. If so, the mere presence of the thorns should be important and not their distance to the peristome. Thus, one major aim of this study was to find out whether the bats make use of these characteristic roost attributes to efficiently find new roosts and whether they exploit the plant's traits for their own purpose.

Materials and Methods

From 25th June to 24th November, 2012 and from 5th April to 10th September, 2014 we caught *K. hardwickii* roosting in *N. bicalcarata* pitchers in peat swamp and heath forests of Brunei Darussalam's Belait district. We marked the bats with PIT-tags (ISO 11784/11785; Peddy-Mark, UK; see Kerth and König, 1999) to ensure individual identification. 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 (2012).

To test the bats' reaction to *N. bicalcarata* thorns, we conducted two different choice experiments during which bats could choose between one unmodified and up to three modified *N. bicalcarata* pitchers. In a flight arena (Eureka! Breezeway — Screen House, Canada; ground area 3.5 × 3.5 m, 2.5 m central height) we fixed the experimental pitchers on sticks at a height of 1.5 m (a common height in which bats use pitchers — Schöner *et al.*, 2013) and with a distance of 0.5 m next to each other. We kept 28 adult bats (11 ♂♂, 17 ♀♀ none of which were pregnant or lactating) for maximally 12 hours and fed them during this time before we started the experiments in the morning hours (06:30–10:30). Each bat was tested individually and their approaches to pitchers were recorded with a digital camcorder (Sony HDR-CX560VE, Japan; because of camera problems one experiment had to be analysed during its performance). Experiments were finished as soon as the bats entered a pitcher.

To avoid pseudo-replication, each bat was tested once, all pitchers were randomly chosen regarding the modifications and their order was randomly arranged for each experiment. To avoid removing too many potential roost pitchers from the bats' habitat, we collected eight pitchers per treatment and randomly chose one of them for an experiment. Damaged pitchers were constantly replaced between trials.

In the first experiment ('thorn length experiment' — see Supplementary Video 1), we tested whether the length of *N. bicalcarata*'s thorns positively or negatively influenced *K. hardwickii*'s roost choice. Thirteen bats could choose between four pitchers: i) one with unmodified thorns (thorn lengths: 1.0–1.5 cm), ii) one with reduced thorns (0.5 cm), iii) one with thorns fully cut, and iv) one with thorns artificially extended with wooden tooth picks (2.0–2.5 cm). In a second experiment ('thorn distance experiment' — see Supplementary Video 2), we tested whether the potential attractive or deterrent effect of the thorns depends on the distance between thorns and pitcher opening. Fifteen bats could choose between two pitchers: a pitcher with enhanced distance between the basis of the thorns and peristome (distance, $\bar{x}$ ± SD = 8.54 ± 0.58 cm) and an unmodified one (distance = 1.62 ± 1.26 cm).

Three bats made no final roost choice within the maximal time span of 20 min and thus were excluded from the analysis of the bats' final roost choice. Persons without knowledge about the hypotheses analysed the videos regarding approaches (i.e., a bat hovered head first within a distance of about 10 cm towards the pitcher) with and without bats landing on the pitchers, and the bats' final roost choice (i.e., entering the pitcher). For statistical analyses we tested the null hypothesis that the treatments did not affect the number of approaches, using permutation tests. We first calculated the mean number of approaches per treatment, which we then compared to the distribution of values expected under the null hypothesis. To obtain the null hypothesis distribution, we randomly distributed the number of approaches of each animal to the different treatments 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.

RESULTS

In the thorn length experiment, the 13 tested *K. hardwickii* individuals approached the pitchers 37 times without landing on them and 18 times with landing (Fig. 2A). Bats did not show a significant preference for a certain pitcher type during approaches without landing (Fig. 3). In contrast, more approaches (17 out of 18; 94.4%) than expected by random distributions during which bats landed on a pitcher were directed either towards the unmodified pitcher (n = 8, P = 0.04) or the one with the extended thorns (n = 9, P = 0.01). Only one bat once landed on the pitcher with reduced thorns (P = 0.01) and never on the pitcher with fully cut thorns ($P < 0.001$). Similarly, the bats' final roost selection significantly deviated from random distributions (P = 0.003): Bats most often entered unmodified pitchers (n = 6) and those with elongated thorns (n = 6) while they never entered pitchers with fully cut thorns and only once entered a pitcher with reduced thorns.

In the thorn distance experiment, 15 tested bats approached the pitchers 73 times without landing and 19 times with landing on one of the two provided pitchers (Fig. 2B). Approaches with and without landing were directed to both pitcher types without any significant preference for one of them (Fig. 3). Interestingly, regarding the final pitcher choice the bats also did not show a significant preference for one of the pitcher types (unmodified: n = 4, extended gap: n = 9; P = 0.22).

DISCUSSION

There are several theories concerning the function of *N. bicalcarata*'s thorns. These thorns have been shown to be nectaries on which symbiotic ants feed (Merbach *et al.*, 1999). It has also been suggested that these nectaries might serve to attract arthropod prey (Clarke, 1993). Another early assumption was that the thorns could protect the plants from animals that steal the pitchers' prey (see Dodd, 1982; Clarke, 1993; Merbach *et al.*, 1999; Phillipps and Phillipps, 2016). However, whether the thorns have any effect on the bats roosting in the pitchers has never been studied.

Astonishingly, in our experiments the bats did not avoid pitchers with sharp thorns, which could easily injure the bats' delicate wing membranes. In contrast, our results clearly show that the bats preferred *N. bicalcarata* pitchers with intact or even enlarged thorns and avoided pitchers with removed thorns. The fact that *K. hardwickii* tend to approach *N. bicalcarata* pitchers more laterally compared to the more frontal approaches towards *N. hemsleyana* pitchers, its alternative roost, could be connected to

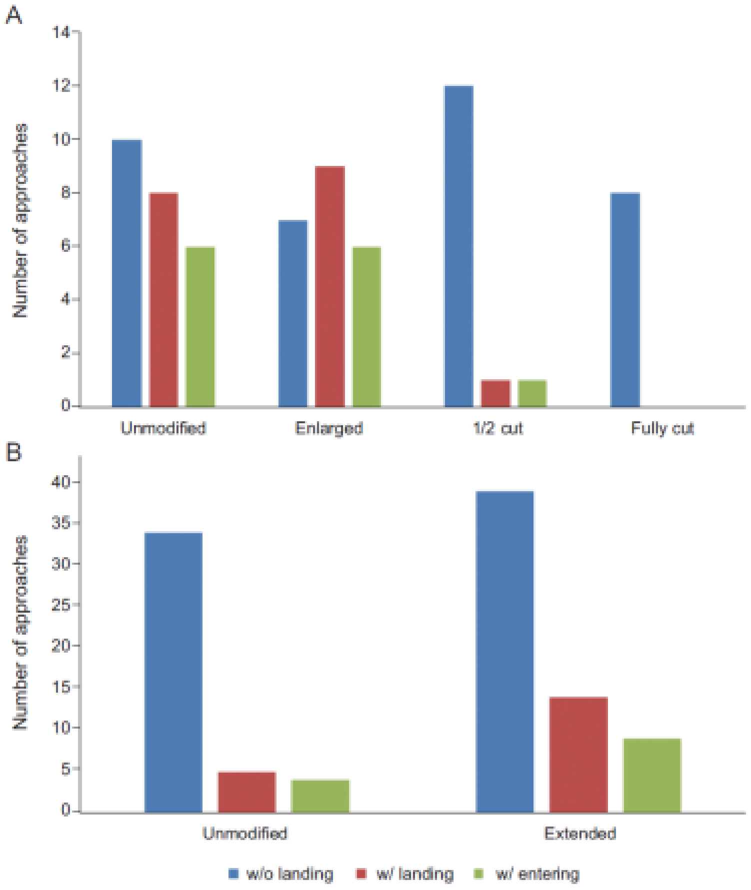
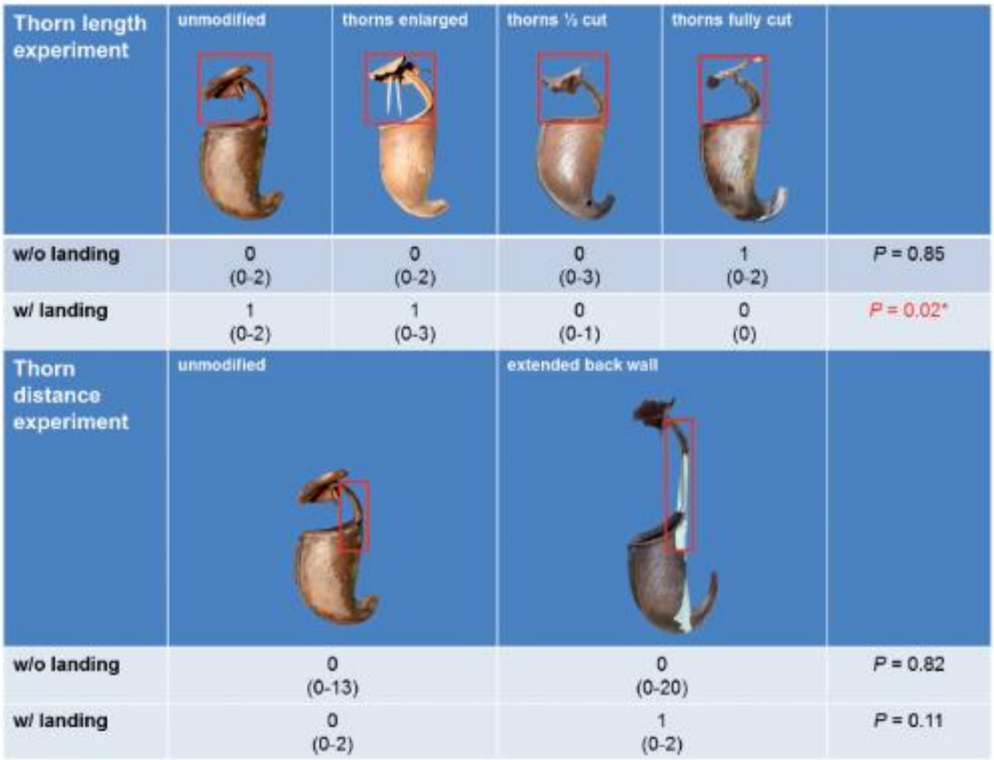


FIG. 2. Total number of bat approaches to different pitcher treatments. Approaches of all individuals grouped into three categories: approaches 1) without (w/o) landing on the pitcher, 2) with (w/) landing but without entering, and 3) with (w/) entering the pitcher. In the thorn length (A) and in the thorn distance (B) experiment all individuals' approaches to one pitcher were summed up

the pitchers' different morphology and a strategy to minimize the risk of injuries (personal observation — Fig. 4). The bats themselves were neither negatively affected by *N. bicalcarata*'s thorns nor are these thorns the only risk of injury in *K. hardwickii*'s habitat: the dense vegetation of peat swamps and heath forests consists of many plants with prickles and thorns, e.g., pandan (Anderson, 1963). Interestingly, less than 1% of all *K. hardwickii* individuals that we captured had large holes or other injuries in their delicate wing membranes. Bats of the genus *Kerivoula* use outstandingly high frequency echolocation calls, which provide a high resolution of the environment (Kingston *et al*., 1999; Schöner *et al*., 2015*b*). This should help the bats to avoid injuries from both the vegetation and *N. bicalcarata*'s thorns.

The bats could also exploit the thorns to protect themselves against predators while roosting in the pitchers. Usually the thorns are close to the pitcher's entrance (leaving a gap of 1.62 ± 1.26 cm). We often experienced how difficult and painful it is to remove bats from *N. bicalcarata* pitchers because the thorns pricked our skin. The same would be true for potential predators such as snakes, tarsiers, or monkeys. To effectively exploit the thorns' protective function, the bats should choose pitchers whose thorns are close to the orifice and prevent predators from



Thorn length experiment	unmodified	thorns enlarged	thorns ½ cut	thorns fully cut	
w/o landing	0 (0-2)	0 (0-2)	0 (0-3)	1 (0-2)	*P* = 0.85
w/ landing	1 (0-2)	1 (0-3)	0 (0-1)	0 (0)	*P* = 0.02*

Thorn distance experiment	unmodified	extended back wall	
w/o landing	0 (0-13)	0 (0-20)	*P* = 0.82
w/ landing	0 (0-2)	1 (0-2)	*P* = 0.11

FIG. 3. Pitcher modifications and median number of bat approaches: approaches were categorised as those with (w/) and without (w/o) subsequent landing on the pitchers. Numbers indicate the median approach number (and range) per bat to each of these modified or unmodified pitcher treatments

gaining access to the pitchers' interior. However, our thorn distance experiment showed that the gap between thorns and the pitchers' orifice had no influence on the bats' decision. Thus, in our experiments neither *N. bicalcarata*'s thorns deterred the bats from entering the pitchers nor did they provide evidence for the hypothesis that the bats exploit the thorns' potential protective function for their own safety.

Instead, our results are in line with the hypothesis that the bats use the unique thorns (Clarke, 1993; Merbach *et al*., 1999) to identify *N. bicalcarata* pitchers. This could help the bats to distinguish the pitchers from less suitable hybrids or other sympatric pitcher plant species. Although pitchers of those species can be similar in size and shape, they are never or only rarely used by the bats (Schöner *et al*., 2013). *Nepenthes ampullaria*, for example, are only used by the bats when no other pitcher plants are available (Schöner *et al*., 2013) probably because of the pitchers' missing lid, which exposes the bats to unfavourable weather and predators. The bats do not use *N. rafflesiana* pitchers presumably because they usually occur at locations with low canopy cover that are avoided by the bats (Schöner *et al*., 2013).

The above-mentioned lateral approaches should not only reduce the bats' risk of injuries, they might also be a strategy to better perceive the thorns. During frontal approaches the bats' echolocation calls will mainly be reflected from other pitcher parts, such as the lid, which partly covers the thorns from the front. Moreover, it is likely that reflections from the pitcher's lid mask the reflections of the thorns. During lateral approaches the bats should receive a clearer reflection of these structures.

Parasites and predators often identify and locate hosts by attending to characteristic traits and signals

FIG. 4. *Kerivoula hardwickii* approaching *Nepenthes* pitchers: lateral approach to a *N. bicalcarata* pitcher (A), frontal approach to a *N. hemsleyana* pitcher (B)

the host evolved for completely different reasons. Mating calls of male frogs, for example, attract frog-eating bats and frog-biting midges (Tuttle and Ryan, 1981; Bernal *et al*., 2007; Grafe *et al*., 2008). In the case of *N. bicalcarata* and the woolly bats, further experiments are needed to clarify the mechanisms of how the bats detect and identify these pitchers and if the thorns produce a species-specific echo pattern (see, e.g., Schöner *et al*., 2015*b*, 2016).

Several bat species seem to be highly specialized on certain plant species with characteristic traits and reject roost types that lack these traits (Chaverri and Kunz, 2011; Schöner *et al*., 2015*b*). This is also important for conservation issues as various bat species strongly rely on their plant partners (Kunz and Lumsden, 2005; Chaverri and Kunz, 2011). The risk of extinction is especially high in those species that are specialized on their roost, which could be ascribed to morphological adaptations of the bats (Sagot and Chaverri, 2015). Here we emphasize that the use of very specific identification cues could intensify the degree of roost specialization in many bat species. On a broader scale, our study suggests that bats react sensitively to certain plant structures even when they do not provide them with a direct benefit. To improve our understanding of bat-plant interactions, more knowledge about the effect of plant structures on bats is necessary.

SUPPLEMENTARY INFORMATION

Contents: Supplementary Video 1. Thorn size experiment in the flight arena: one unmodified and three modified *N. bicalcarata* pitchers with different thorn sizes were offered to individuals of *K. hardwickii*; Supplementary Video 2. Thorn distance experiment: the bats could choose between one unmodified *N. bicalcarata* pitcher and one pitcher with enhanced distance between the thorns and the pitcher's orifice. Supplementary Information is available exclusively on BioOne.

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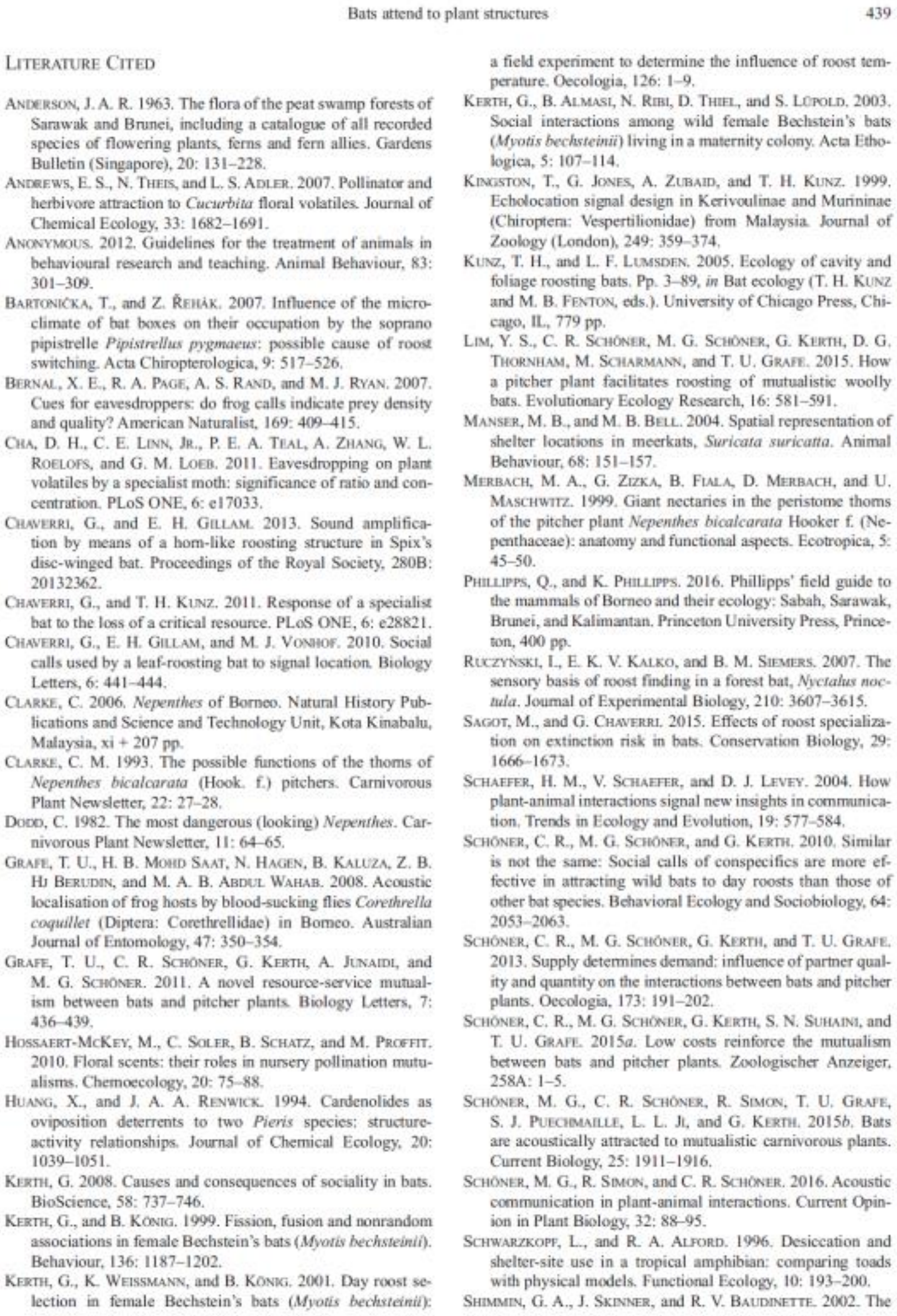

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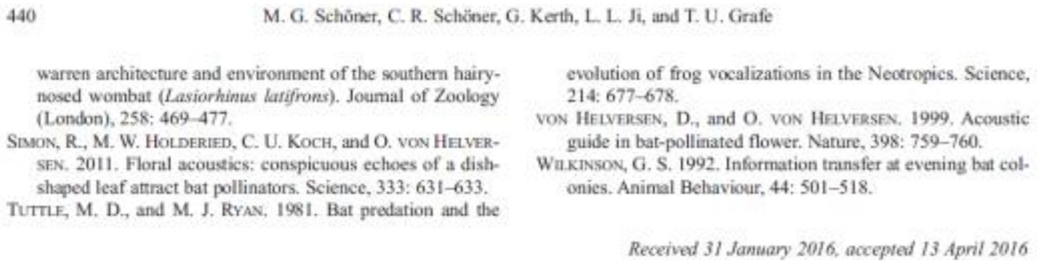

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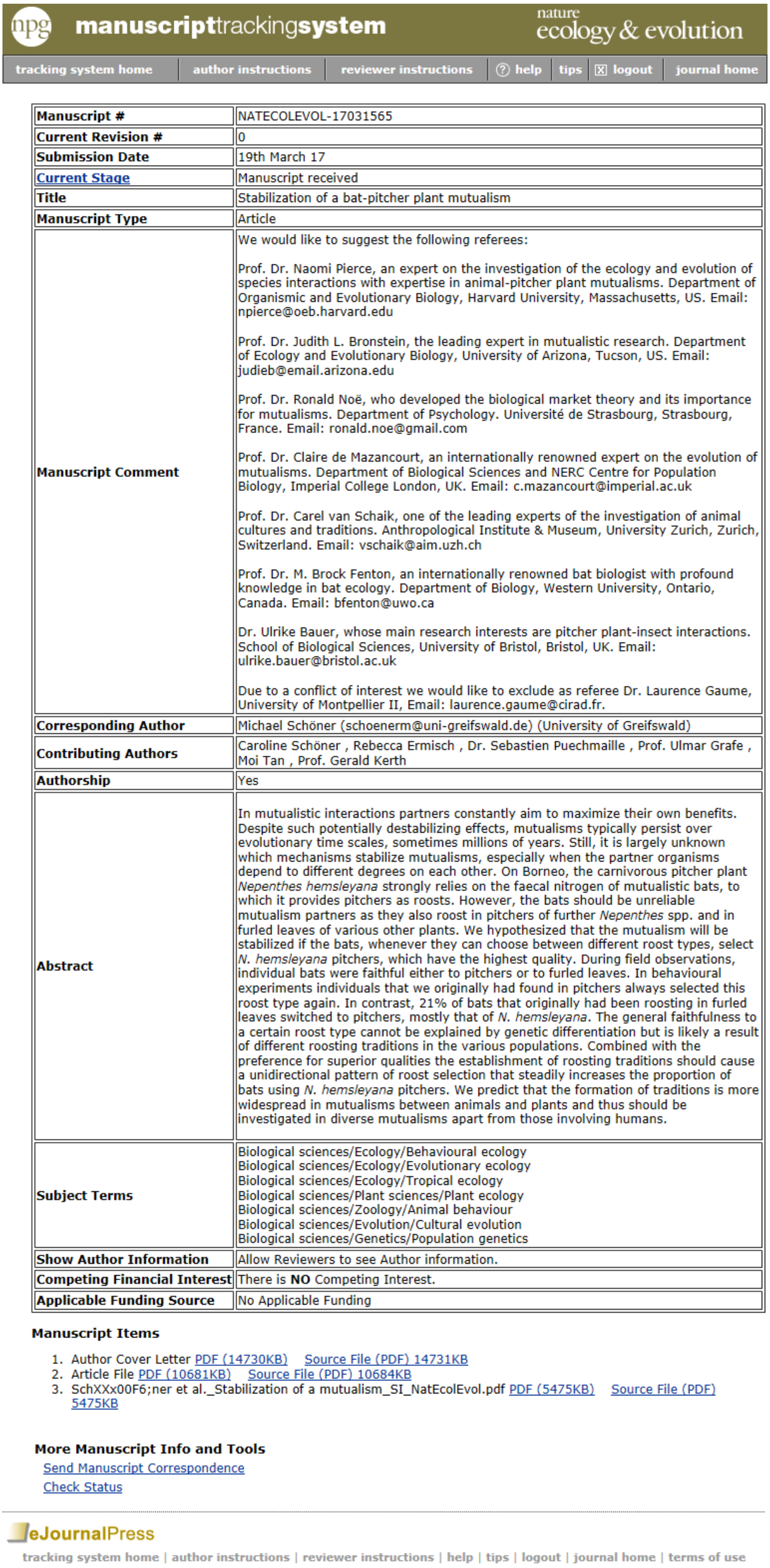
4.3 Behavioural traits that stabilize the mutualism

Manuscript 6

Schöner, M.G., Schöner, C.R., Ermisch, R., Puechmaille, S.J., Tan, M.C., Grafe, T.U. & Kerth, G. (*submitted*): Stabilization of a bat-plant mutualism.



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Manuscript Comment	We would like to suggest the following referees: Prof. Dr. Naomi Pierce, an expert on the investigation of the ecology and evolution of species interactions with expertise in animal-pitcher plant mutualisms. Department of Organismic and Evolutionary Biology, Harvard University, Massachusetts, US. Email: npierce@oeb.harvard.edu Prof. Dr. Judith L. Bronstein, the leading expert in mutualistic research. Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, US. Email: judieb@email.arizona.edu Prof. Dr. Ronald Noë, who developed the biological market theory and its importance for mutualisms. Department of Psychology. Université de Strasbourg, Strasbourg, France. Email: ronald.noe@gmail.com Prof. Dr. Claire de Mazancourt, an internationally renowned expert on the evolution of mutualisms. Department of Biological Sciences and NERC Centre for Population Biology, Imperial College London, UK. Email: c.mazancourt@imperial.ac.uk Prof. Dr. Carel van Schaik, one of the leading experts of the investigation of animal cultures and traditions. Anthropological Institute & Museum, University Zurich, Zurich, Switzerland. Email: vschaik@aim.uzh.ch Prof. Dr. M. Brock Fenton, an internationally renowned bat biologist with profound knowledge in bat ecology. Department of Biology, Western University, Ontario, Canada. Email: bfenton@uwo.ca Dr. Ulrike Bauer, whose main research interests are pitcher plant-insect interactions. School of Biological Sciences, University of Bristol, Bristol, UK. Email: ulrike.bauer@bristol.ac.uk Due to a conflict of interest we would like to exclude as referee Dr. Laurence Gaume, University of Montpellier II, Email: laurence.gaume@cirad.fr.
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Authorship	Yes
Abstract	In mutualistic interactions partners constantly aim to maximize their own benefits. Despite such potentially destabilizing effects, mutualisms typically persist over evolutionary time scales, sometimes millions of years. Still, it is largely unknown which mechanisms stabilize mutualisms, especially when the partner organisms depend to different degrees on each other. On Borneo, the carnivorous pitcher plant *Nepenthes hemsleyana* strongly relies on the faecal nitrogen of mutualistic bats, to which it provides pitchers as roosts. However, the bats should be unreliable mutualism partners as they also roost in pitchers of further *Nepenthes* spp. and in furled leaves of various other plants. We hypothesized that the mutualism will be stabilized if the bats, whenever they can choose between different roost types, select *N. hemsleyana* pitchers, which have the highest quality. During field observations, individual bats were faithful either to pitchers or to furled leaves. In behavioural experiments individuals that we originally had found in pitchers always selected this roost type again. In contrast, 21% of bats that originally had been roosting in furled leaves switched to pitchers, mostly that of *N. hemsleyana*. The general faithfulness to a certain roost type cannot be explained by genetic differentiation but is likely a result of different roosting traditions in the various populations. Combined with the preference for superior qualities the establishment of roosting traditions should cause a unidirectional pattern of roost selection that steadily increases the proportion of bats using *N. hemsleyana* pitchers. We predict that the formation of traditions is more widespread in mutualisms between animals and plants and thus should be investigated in diverse mutualisms apart from those involving humans.
Subject Terms	Biological sciences/Ecology/Behavioural ecology Biological sciences/Ecology/Evolutionary ecology Biological sciences/Ecology/Tropical ecology Biological sciences/Plant sciences/Plant ecology Biological sciences/Zoology/Animal behaviour Biological sciences/Evolution/Cultural evolution Biological sciences/Genetics/Population genetics
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Stabilization of a bat-pitcher plant mutualism

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Author contribution: CRS, MGS, TUG and GK developed the concept of the study. CRS, MGS and RE performed the experiments. CRS, MGS and SJP conducted the genetic analysis. MGS and CRS wrote the first draft of the manuscript. All authors contributed substantially to revisions.

Running Title: Stabilization of a mutualism

Keywords: mutualism, animal-plant interaction, population genetics, behaviour, tradition, social learning, *Nepenthes*, *Kerivoula*, bat, pitcher plant

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In mutualistic interactions partners constantly aim to maximize their own benefits. Despite such potentially destabilizing effects, mutualisms typically persist over evolutionary time scales, sometimes millions of years. Still, it is largely unknown which mechanisms stabilize mutualisms, especially when the partner organisms depend to different degrees on each other. On Borneo, the carnivorous pitcher plant *Nepenthes hemsleyana* strongly relies on the faecal nitrogen of mutualistic bats, to which it provides pitchers as roosts. However, the bats should be unreliable mutualism partners as they also roost in pitchers of further *Nepenthes* spp. and in furled leaves of various other plants. We hypothesized that the mutualism will be stabilized if the bats, whenever they can choose between different roost types, select *N. hemsleyana* pitchers, which have the highest quality. During field observations, individual bats were faithful either to pitchers or to furled leaves. In behavioural experiments individuals that we originally had found in pitchers always selected this roost type again. In contrast, 21% of bats that originally had been roosting in furled leaves switched to pitchers, mostly that of *N. hemsleyana.* The general faithfulness to a certain roost type cannot be explained by genetic differentiation but is likely a result of different roosting traditions in the various populations. Combined with the preference for superior qualities the establishment of roosting traditions should cause a unidirectional pattern of roost selection that steadily increases the proportion of bats using *N. hemsleyana* pitchers. We predict that the formation of traditions is more widespread in mutualisms between animals and plants and thus should be investigated in diverse mutualisms apart from those involving humans.

Despite their importance and ubiquity, “the evolution and maintenance of mutualisms remains a largely unsolved puzzle”[1]. Clear evidence exists that mutualisms evolved and disappeared repeatedly and that the transition from autonomy to mutualism and vice versa is often fluid[2–4]. Empirical research on the stabilization of mutualisms is generally rare, has mostly focused on obligate mutualisms (e.g., between figs and fig wasps[1]) and partly contradicts theoretical models (see, e.g.,[5]). It is generally assumed that the quality of the partners is crucial for the stabilization of facultative mutualisms[6–8]. However, recent studies also show that this is not necessarily the case, e.g., because alternative behavioural patterns such as punishment of cheaters stabilize the interaction[9].

Here, we empirically investigated how the mutualism between the woolly bat *Kerivoula hardwickii* and the carnivorous pitcher plant *Nepenthes hemsleyana* is stabilized. In this mutualistic system the bats fertilize the pitcher plants with faeces while using the plants’ pitcher-shaped trapping organs as high quality roosts[10–12]. As arthropod capture is insufficient for the plants’ nutrient demand, *N. hemsleyana* strongly relies on its bat partners[13]. In contrast, *K. hardwickii* depends less on its mutualism partner, as they have been reported to also roost in plants that do not profit from the bats’ presence such as in dead pitchers of *Nepenthes bicalcarata* and *Nepenthes ampullaria* and additionally in furled leaves of the families Araceae, Musaceae and Zingiberaceae[11,14,15].

In this study, we investigated whether the bats show behavioural traits that stabilize their unique mutualistic relationship with the pitcher plant *N. hemsleyana*. We hypothesized that the roost choice of *K. hardwickii* individuals is not arbitrary but that the bats prefer the roost with the highest quality when several roost types are available (c.f.[6–8]). If this is the case, and the majority of bats choose pitchers of their mutualism partner *N. hemsleyana* whenever they can select between different potential roosts, this unique bat-pitcher plant mutualism would be stabilized. *Nepenthes hemsleyana* has acquired traits that are highly attractive for the bats including a typical shape that perfectly fits to the bats' body size[10,12] or a more stable microclimate than in other *Nepenthes* species[11]). Moreover, *N. hemsleyana* pitchers are available on more successive days than furled leaves[16] (personal observation). Most important, due to an effective echo-reflecting structure, *N. hemsleyana* can easily be detected and identified by *K. hardwickii* in the dense vegetation where they occur[17,18]. Because of the possibility for experimental manipulations this bat-pitcher plant interaction is a candidate system to reveal how mutualisms potentially are stabilized. Such stabilizing mechanisms, such as the evolution of traditions, may be similarly found in other animal-plant mutualisms, but so far are largely undocumented.

Results

Which roosts do the bats select under natural conditions?

Using radio-telemetry and passive integrated transponder (PIT) tags to individually mark bats we monitored *K. hardwickii* individuals roosting in *Nepenthes* pitchers (n = 174 bats) or furled leaves (n = 152 bats) in 10 different study sites in Brunei Darussalam and Sarawak/Malaysia for 30 ± 18 (mean ± s.d.) days per site (Supplementary Table S1). In two out of 10 study sites only furled leaves were present. Individuals living in the remaining eight study sites additionally could use pitchers of different *Nepenthes* species. In these sites, roost selection generally seemed not to be influenced by abundance of the available roost types/species as *N. hemsleyana* was disproportionately used by the bats. In study site “Long Iman”, e.g., *N. hemsleyana* pitchers only made up 5% of all available roosts. The bats clearly preferred these pitchers and occupied all of them every day. In contrast, 38% of the available potential roosts were furled leaves, in which we never found bats in that study site. This suggests that the individual bats do not select roosts in relation to their relative abundance in the wild.

In altogether seven study sites, including the already mentioned site “Long Iman”, where pitchers and furled leaves co-occurred, individual bats only used pitchers (six sites) or furled leaves (one site; Supplementary Table 1). This was also the case at the study site „Airport“, the only site where furled leaves and pitchers (of *N. hemsleyana* and *N. bicalcarata*) were both used as roost. All 43 individual bats marked and followed in this site (over 3.70 ± 3.20 days on average, range: 1 – 14 days) were faithful to their respective roost types and we never observed them switching between pitchers (used by seven individuals) and furled leaves (used by 36 individuals).

Which roosts do the bats select under controlled conditions?

In a series of behavioural experiments, we investigated whether the bats are faithful to one roost type (pitcher versus furled leaf) or even plant species or whether they have a general preference for *N. hemsleyana.* In a flight arena, we offered different potential plant roosts to the bats. Each roost type was offered once and all roosts were randomly arranged for each trial (Figure 1). The number of approaches to a roost and the final roost selection were taken as an indication for roost preference. We also scored whether the bats’ roost choice was independent of the roost type in which the individuals had been found in the wild.

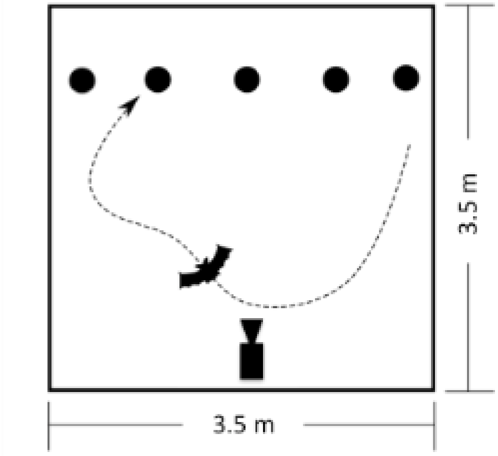

Figure 1. Experimental set-up of the behavioural experiments. Bats were offered three to five potential roost types in a randomized linear array (full circles) depending on the experiment.

First, we tested whether the bats prefer pitchers of certain *Nepenthes* species to others. Forty-one bats from areas where we had found *K. hardwickii* to only use pitchers (*N. hemsleyana* and/or *N. bicalcarata*) could choose between one *N. hemsleyana*, one *N. bicalcarata*, one *N. ampullaria* pitcher, one *Nepenthes rafflesiana* pitcher (which is not used by the bats), and a plastic tube that was similar in dimensions as the pitchers (width: 4.5 cm, length: 18.5 cm). The experiments showed that bats, which we had found roosting in *N. hemsleyana* or *N. bicalcarata* pitchers, tended to be faithful to pitcher plant species in which they had originally been found in the wild (Fisher's exact tests for count data: *P* = 0.02; the result did not stay significant after a sequential Bonferroni correction; Table 1; Figure 2a; Supplementary results).

Table 1. Post hoc test results (Fisher's exact tests for count data) of the behavioural experiments. We investigated if bats prefer a certain roost type/species depending on where they had been found in the wild. Symbols indicate the roost plants that could be chosen by the bats. a) (red part of the matrix): Post hoc tests for bats found roosting in *N. hemsleyana* pitchers vs. bats found roosting in *N. bicalcarata* pitchers (global Fisher's exact tests for count data: $P = 0.04$). b) (blue part of the matrix): Post hoc tests for bats found roosting in pitchers vs. bats found roosting in furled leaves (global Fisher's exact tests for count data: $P < 0.001$). Red values indicate significance after sequential Bonferroni correction (Abbreviation: Nh = *N. hemsleyana,* Nb = *N. bicalcarata,* Na = *N. ampullaria,* Nr = *N. rafflesiana,* Pt = Plastic tube, Fl = Furled leaf).

	Nh	Nb	Na	Nr	Pt	a)
Nh		0.02	0.11	0.28	0.31	Nh
Nb	1.00		1.00	0.62	1.00	Nb
Na	0.27	0.23		1.00	1.00	Na
Pt	0.33	0.29	1.00		1.00	Nr
Fl	<0.001	<0.001	0.09	1.00		Pt
b)	Nh	Nb	Na	Pt	Fl	

In another experiment, we tested 14 bats that we had found in furled leaves of *Alpinia ligulata* (n = 5), *Boesenbergia grandis* (n = 3), and *Musa muluensis* (n = 6) if they prefer one of these species when all three are offered simultaneously (one leaf per plant species). Except of one bat that did not choose in the end, all tested bats approached (Supplementary results) and entered furled leaves of the different species regardless of the species where we had found them roosting originally (Fisher's exact tests for count data: $P = 0.76$; Figure 2b).

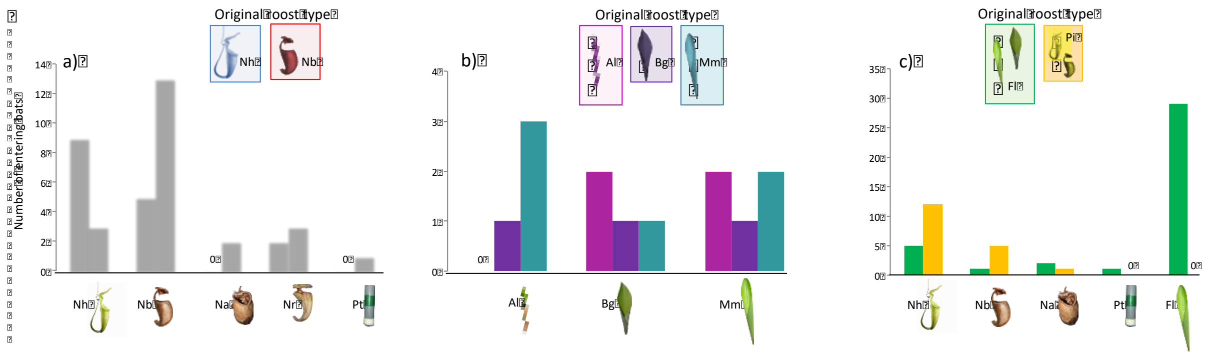

Figure 2. Roost preferences of *Kerivoula hardwickii* that used different roosts in the wild (= original roost type). a) Bats found in *Nepenthes hemsleyana* (Nh) or *Nepenthes bicalcarata* (Nb) could choose between pitchers of different *Nepenthes* species (*N. hemsleyana*, *N. bicalcarata*, *N. ampullaria* (Na), *N. rafflesiana* (Nr)) and a plastic tube (Pt)). b) Bats found in furled leaves of *Alpinia ligulata* (Al), *Boesenbergia grandis* (Bg) or *Musa muluensis* (Mm) could choose between furled leaves of these three species. c) Bats found in furled leaves (Fl) or pitchers (Pi) could choose between different roost types (*N. hemsleyana*, *N. bicalcarata*, *N. ampullaria*, one furled leaf and the plastic tube).

Finally, we investigated whether the bats generally prefer pitchers to furled leaves or vice versa. We conducted another behavioural experiment where *K. hardwickii* individuals (47 captured in furled leaves, 21 in pitcher plants) could choose between one furled leaf (*A. ligulata*, *B. grandis*, *M. muluensis*, depending on the plant species in which we had found a bat; for bats found in pitchers we used furled leaves of the species that we found within a distance of 20 m), one *N. hemsleyana*, one *N. bicalcarata*, one *N. ampullaria* pitcher and a plastic tube. We provided several pitcher plant species but only one furled leaf as the earlier experiments (see above) had shown that the bats randomly selected furled leaves of different plant species but tended to discriminate between pitchers of different *Nepenthes* species.

We found that bats from pitchers (individuals from all *Nepenthes* species pooled) chose *N. hemsleyana* and *N. bicalcarata* pitchers significantly more often than bats that originated from furled leaves (all species pooled; Table 1; Figure 2c; Supplementary Table S1). However, eight (21%) of the bats roosting in furled leaves chose pitchers during the experiment (five of these bats chose *N. hemsleyana* pitchers, one

N. bicalcarata and two *N. ampullaria* pitchers). In contrast, not a single bat switched from pitchers to furled leaves.

Is roost selection related to genetic differentiation?

An explanation why the bats have different roosting habits could be that they belong to different cryptic species. In fact, it has been suggested, that *K. hardwickii* is comprised of up to five different species[19,20]. Our population genetic analyses showed low genetic differentiation between the sampled *K. hardwickii* individuals from the different study sites (pairwise F_{ST}-values: mean = 0.03 ± 0.02; range: 0.001 to 0.09; Figure 3; Supplementary Table S2), which was clearly connected to distance and not to the roost type. This indicates that all individuals do belong to the same species and that it is not cryptic species, which roost in different roost types (pitchers versus furled leaves).

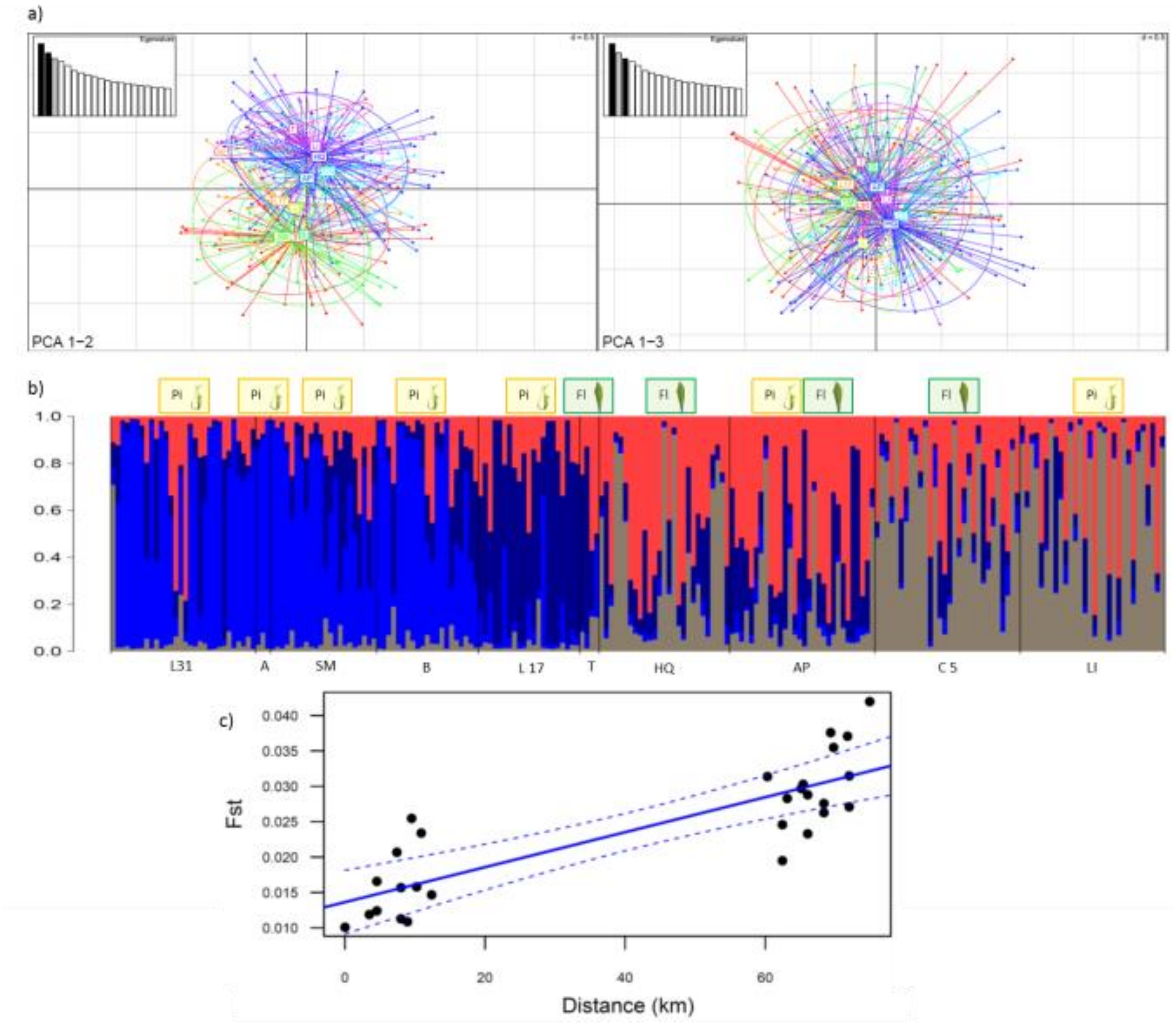

Figure 3. Population differentiation based on the microsatellite data. a) Principal component analysis (PCA) showing the axes 1-2 (axis 1 explaining 3.90 % of the variance, axis 2 explaining 3.40 %) and 1-3 (axis 3 explaining 3.09 %) for the population structure of individuals. For each graph, the inset represents the eigenvalues of the two axes. Individuals from each of 10 study sites are represented in different colours. b) Results of the STRUCTURE analysis are shown for the number of populations with the BestK and the estimators MedMeaK, MaxMeaK, MedMedK and MaxMedK. c) Relationship between genetic distance (F_{ST}) and geographic distance for eight study sites (only populations with more than five individuals were used; the majority of these study sites were connected by habitats in which *K. hardwickii typically occur.* Mantel test: r = 0.84*,* *P* = 0.0007). The solid line represents linear regression, dashed lines the 95 % confidence interval.

However, within the study site “Airport” pairs of bats that were both roosting in pitchers were significantly more closely related than expected by chance (Triadic Likelihood Relatedness Estimate, TrioML = 0.17 ± 0.21; permutation test: *P* = 0.004) while the relatedness of pairs with one bat roosting in pitchers and one roosting in furled leaves was significantly lower than expected by random distributions (TrioML = 0.03 ± 0.05; *P* = 0.01). Relatedness of pairs where both bats preferred furled leaves did not differ from random distributions (TrioML = 0.05 ± 0.10; *P* = 0.37).

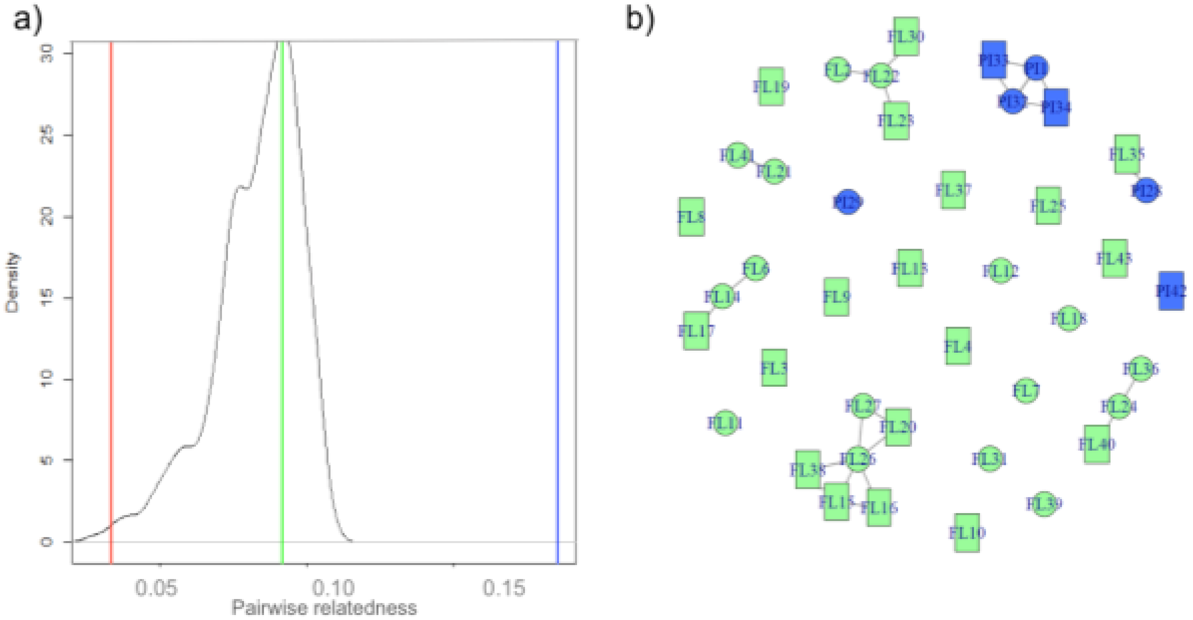

Figure 4. Pairwise relatedness of *K. hardwickii* at the study site “Airport”. a) The graph represents mean relatedness values (TrioML, 10,000 permutations) of randomly selected individuals (null hypothesis distribution). Coloured lines show the mean observed relatedness for bat pairs roosting in pitchers (blue), roosting in furled leaves (green), and those pairs with one bat roosting in pitchers and the other in furled leaves (red). b) Social network of bats based on pairwise relatedness (TrioML). Only potential parent offspring pairs and full sibling pairs are linked (r>0.44). Circles represent the females, rectangles the males, blue colour indicates bats roosting in pitchers, green those in furled leaves.

Discussion

Why should the bats prefer *N. hemsleyana* pitchers?

Our results showed that whenever *Nepenthes* and furled leaves were present at a given site the bats exclusively or, in one site, additionally used pitchers. All bats that we originally had found in pitchers chose pitchers again when they could select between different roost types during our behavioural experiments. Additionally, 21% of bats that originally had been found in furled leaves switched to pitchers during the behavioural experiments. According to the biological market model, a species should provide resources of higher quality to its mutualism partner in order to outcompete alternative resources[6,7]. This is the case in *N. hemsleyana* whose pitchers offer optimal roosting conditions for bats. Also for bats, quality (e.g. perceptibility or absence of parasites) is one of the most important aspects to choose or to reject roosts[11,21], which explains why the bats probably preferred roosts provided by *N. hemsleyana*. This preference for pitchers provides a unidirectional mechanism that should steadily increase the number of bats using *N. hemsleyana* in areas where both pitchers and furled leaves are available. However, contrary to our initial hypotheses that the bats should generally prefer their mutualism partner *N. hemsleyana,* the majority of bats (79%) that originally had been roosting in furled leaves stayed faithful with this roost type.

How does the bats' faithfulness to different roost plants affect the stabilization of the mutualism with *Nepenthes hemsleyana*?

The mutualism between bats and pitcher plants is not symmetric: *N. hemsleyana* is specialized and strongly dependents on the bats. In contrast, the bats cannot solely rely on *N. hemsleyana* because of the plants' patchiness and restricted distribution range[22]*.* Asymmetries are common in many mutualistic interactions and have been shown to act as a stabilizing mechanism. While two species that are highly specialised on each other influence each other negatively when one of them is faced with adverse biotic or abiotic influences, such negative influences are buffered in interactions between a specialist and its less sensitive generalistic partner (Bascompte et al. 2006)[23]. By being faithful to *Nepenthes* and to the more widespread plants with furled leaves, *K. hardwickii* can extend its range[11] and, as our results show, the gene flow between the bat populations is maintained. Thus, the mutualism with *N. hemsleyana* could be stabilized due to the aforementioned buffering effects[23]. Finally, although the bats do not rely on *N. hemsleyana*, they clearly prefer the pitchers of their mutualism partner in areas where they can select.

How can the bats' faithfulness to different roost plants be explained?

The mere abundance of the different roost types seems not to explain the bats' different roosting behaviour as most bats were faithful to either pitchers or furled leaves even when both roost types were available in the wild or in the flight arena. Moreover, genetic differences among populations were not related to the roost use of the bats. However, on an intra-population level, individuals from study site "Airport" that shared the same roost preferences were more closely related than individuals with another roost preference. This indicates that roost preferences are transmitted between closely related individuals probably due to imprinting or social learning including the formation of traditions.

Konrad Lorenz defined imprinting as an individual's behavioural response to a certain stimulus (e.g., in our study system possibly the species-specific shape or smell of a roost) to which it had been exposed during a sensitive period in early life[24]. Several animal species are imprinted to their habitats or nesting sites[25–28]. However, if imprinting would account for *K. hardwickii*'s roosting habits, the bats' roost selection should be highly specific and stable during an individual's life[29], which is not the case. Neither bats roosting in furled leaves nor those roosting in pitchers were completely fixed to a certain species. Especially in the case of pitchers, the traits (e.g., shape, smell, etc.) of the different *Nepenthes* species used by the bats are so diverse[30] that a general imprinting to the roost type "pitcher" seems unlikely.

In contrast to imprinting, social learning allows for more flexible behaviours[31]. General advantages of learning from experienced individuals can be seen in abridged learning processes compared to individual learning as juveniles can easily reproduce the behaviour of their conspecifics or learn to focus on particular cues[32]. As *K. hardwickii* is a solitary roosting bat[10], horizontal social learning from conspecifics of the same generation can probably be neglected. Vertical social learning, in contrast, is facilitated because the juveniles stay for relatively long times with their mothers (at least 77 days; own observation). The bats' faithfulness to a certain roost type could thus be the result of maternal social transmission that leads to different regional roosting traditions. Populations that socially transmit the preference for pitchers are reliable mutualism partners for *N. hemsleyana*.

Conclusion

In mutualistic research the high relevance of partner quality for the stability and maintenance of mutualisms has been broadly discussed for example with respect to cheating[9]. In contrast, the importance of social transmission for mutualistic interactions has mostly been considered as unique for humans and their domesticated plants and animals[33]. Apart from that only few studies indicate that socially transmitted behaviours, e.g., in bumblebees, could affect mutualisms[34]. Our study indicates that social transmission in combination with a general preference for high quality roosts could be one factor to stabilize a facultative mutualism and potentially could lead to an obligate interaction. This phenomenon is probably more widespread and should be investigated in diverse mutualisms apart from those involving humans.

Methods

Study periods and study sites

Bats were caught in harp traps or in their roosts (*Nepenthes* pitchers and furled leaves of the plant families Zingiberaceae, Musaceae, Araceae) in the Belait district of Brunei Darussalam and in the Mulu National Park in Sarawak/Malaysia during four field seasons (from 14 June to 30 July 2009, from 14 August 2011 to 14 January 2012, from 20 June to 3 December 2012 and from 14 April to 1 September 2014; see Supplementary Table 1). All adult males and non-reproductive females were marked with PIT-tags (ISO 11784/11785; Peddy-Mark, UK) for individual identification[11]. Capturing and handling of the bats was conducted with permission of the University Brunei Darussalam Research Committee (UBD/PNC2/2/RG105 &193) and the Sarawak Forestry Department (NCCD.907 4.4(Jld.10)-209) adhering to the Animal Behaviour Society[35].

Kerivoula hardwickii's roost choice

Field observations

In each study site we monitored the occurring *K. hardwickii* individuals for 30.0 ± 18.3 days (mean ± s.d.) by daily checking all potential roosts (furled leaves and *Nepenthes* pitchers below a height of 2.5 m) and additionally by catching individuals with harp traps. We radio-tracked on average 5.5 ± 3.8 (range: 0 – 12) individuals per site. Parts of the radio-tracking data have already been published[10,11]. Additionally, individuals could easily be identified from outside the roost with a handheld PIT-tag reader (LID-575 Midrange Reader, Trovan, UK). Of special interest to us was study site “Airport” where bats not only use pitchers of the species *N. hemsleyana* and *N.*

bicalcarata but also furled leaves (*Musa muluensis, Zingiber kelabitianum*, *Plagiostachys albiflora*, *Plagiostachys strobilifera*) as roost. In this study site we radio-tracked three *K. hardwickii* individuals (two males, one female) from furled leaves and one male individual from a pitcher for an overall mean of 8.50 ± 2.87 days.

Experimental set-up

We conducted several behavioural experiments where bats could choose between different potential roosts. All experiments were filmed (Sony HDR-CX560VE) in a flight arena (length and width 3.5 m, height 2.5 m, Figure 1). Bats were fed and released within 24 hours of capture into their original habitat. We excluded pregnant and lactating females as well as juveniles.

By performing a suite of experiments we wanted to determine how the bats react to different roost types (pitchers or furled leaves) or plant species. In a first experiment we aimed to find out if bats that use pitchers in the wild have a preference for the species in which we had found them, if they prefer pitchers of certain *Nepenthes* species or if they randomly choose between different pitcher plant species. We simultaneously offered one *N. hemsleyana*, one *N. bicalcarata*, and one *N. ampullaria* pitcher, and additionally a pitcher of *Nepenthes rafflesiana,* which is not used by the bats, as well as a plastic tube. We tested 41 bats (12 males, 29 females) from areas where *K. hardwickii* only used pitchers as roosts, although furled leaves were available (Table S1). Sixteen individuals derived from an area where the bats exclusively use *N. hemsleyana* pitchers, the other bats were captured at study sites where the bats exclusively roost in *N. bicalcarata* pitchers or where they use both pitcher plant species (see Table S1 for roost availabilities per plant species and site). In the latter case, we only tested individuals that exclusively roosted in *N. bicalcarata* pitchers during a radio-tracking period of 5-13 days (9.82 ± 2.64 days; for details see[11]).

Similarly, in a second experiment we tested 14 bats (10 males, 4 females) that had roosted in furled leaves. For the experiment we simultaneously offered a total of three furled leaves, one of each species: *Alpinia ligulata*, *Boesenbergia grandis*, and *M. muluensis*.

Finally, we tested how the bats react to different roost types (pitchers versus furled leaves). We offered one *N. hemsleyana*, one *N. bicalcarata*, and one *N. ampullaria* pitcher, one furled leaf (*A. ligulata*, *B. grandis* or *M. muluensis*; in the case of bats roosting in furled leaves we used the species where we had found the bat roosting in; in the case of bats found in pitchers we used the plant species that occurred within a distance of 20 m from the roost) as well as the plastic tube as roost. We tested 68 bats (56 males, 12 females) of which 47 were found roosting in furled leaves, 21 in pitcher plants.

In all experiments, pitchers and furled leaves were randomly arranged within the flight arena (distance to each other = 0.5 m; height = 1.5 m). To prevent the plants from excessive damage by cutting pitchers and leaves, the same experimental leaf/pitcher was offered to up to three bats. Each bat was tested only once per experiment. We defined an approach as hovering flight in front of an object within a distance of 10 cm. Three bats in the first, one bat in the second and eleven bats in last experiment did not choose a roost within the maximum time span of 30 min per trial and thus were excluded from the analyses of the bats' final roost selection.

Statistical data analysis

We compared the distribution of observed approaches to permutated datasets in which observed approach numbers were randomly allocated to the three/five provided roost types following the approach used in[17]. For the permutation tests, we tested the null hypothesis that the roost type did not affect the number of approaches. We first calculated the mean number of approaches for each roost type, which we then compared to the distribution of values expected under the null hypothesis. The null hypothesis distribution was obtained by permuting the number of approaches between roost types for each tested animal and then calculating the mean number of approaches per roost type. This procedure was repeated 10,000 times from which the null distribution of the mean number of approaches was obtained. The *P*-value was then calculated by comparing the mean number of approaches for the considered roost type to the null distribution.

Genetic analysis

Sample collection and DNA extractions

We took samples with a sterile biopsy punch (Stiefel Laboratories; diameter: 2 mm) of 317 bats from 10 locations (six in Brunei Darussalam, four in Sarawak). Samples were stored in 90 % ethanol or dried with silica gel until DNA extraction (Silica Gel Orange, Carl Roth GmbH). DNA was extracted from wing biopsy punches using a modified ammonium acetate extraction protocol[36], eluted in Low TE and stored at -20 °C. We used DNA samples at final concentrations of at least 2 ng μl^{-1} (quantified from extracted samples on a NanoDrop ND-1000 Spectrophotometer, Thermo Fisher Scientific).

Microsatellite development

We sent genomic DNA to the Max-Planck-Institute for Evolutionary Biology in Plön where a microsatellite library was created using high-throughput shotgun 454-sequencing. Using the programme MISA (Microsatellite Identification Tool; http://pgrc.ipk-gatersleben.de/misa/misa.html) 66,289 potential microsatellite sequences were found from which we developed 40 unlabelled primer pairs using the programmes Nucleic Acid Sequence Massager (http://www.attotron.com/cybertory/analysis/seqMassager.htm) for cleaning the sequences and Primer 3 v. 4.0.0 (http://sourceforge.net/projects/primer3/)[37,38] to design the primers. We tested these primer pairs for amplification and polymorphism using pooled DNA from two individuals. All primer pairs were tested with a range of four different annealing temperatures (56–62° C; ABI 3130xl Genetic Analyser, Applied Biosystems).

Microsatellite amplification and data analysis

Two multiplex reactions (MP1/MP2) were conducted for each individual in 8 µl (MP1) and 5 µl (MP2) reaction volumes, each consisting of 1.0 µl DNA, 4.0 µl (MP1) and 2.5 µl (MP2) Multiplex PCR Master Mix (Qiagen) and primer concentrations as indicated in Table S3. The following amplification conditions were used: 95° C for 15 min; 32 cycles of 94° C for 30 s, 60° C for 90 s, 72° C for 60 s; 60° C for 30 min. All PCR products were run on an ABI 3130xl Genetic Analyser (Applied Biosystems) and sized with an internal lane standard (GeneScan™ 500 LIZ™ dye Size Standard, Thermo Fisher) and the software GENEMAPPER v. 5 (Applied Biosystems).

To check for genotyping consistency, 23.0 % of samples were amplified and genotyped twice. We could not detect departures from Hardy-Weinberg and linkage equilibrium at the site level after Bonferroni correction using Genepop v. 4.1.4 (except for individuals of study site “Labi 31” where we had 33 significant linkages between markers probably due to inbreeding). We also found no evidence for the presence of null alleles, large allelic drop-out or possible scoring errors across populations within our dataset (tested with MICRO-CHECKER v. 2.2.3)[39].

To investigate if there is a correlation between the populations’ pairwise genetic distance and pairwise geographic distance matrices, we conducted a Mantel test (99,999 permutations) with the R package ecodist[40]. We calculated F_{ST} with Geno-Dive v.2. Ob27 to measure pairwise population differentiation. With STRUCTURE v. 2.3.4[41,42] we investigated the population structure using a burn-in length of 20,000 and a run length of 200,000 without prior population information. The admixture model and the correlated allele frequencies between population options were selected. After an initial test we chose the burn-in and run length by looking at the convergence of the values of summary statistics and consistency between runs. All other parameters were left as by default. We undertook ten independent runs for K-values ranging from one to ten, which reflects the minimum and maximum number of populations suspected. The number of populations was inferred from the corrected posterior probability and four new estimators that have been shown to outperform other estimators, namely MedMeaK, MaxMeaK, MedMedK and MaxMedK[43]. Additionally, we conducted a Principal Component Analysis (based on individual allelic frequencies) using the adegenet v. 1.3-9[44] and ade4 v. 1.4-14[45] packages in R.

For all bats of the study site “Airport” (which is the only study site where bats are roosting in pitcher plants and in furled leaves) we calculated pairwise relatedness (triadic likelihood relatedness estimate (TrioML)[46] with Coancestry v. 1.0.1.5[47]. With permutations we tested the null hypothesis that the pairwise relatedness of bats did not differ in relation to the preferred roost type (pitchers, furled leaves). Therefore, we randomly selected (1,000 times) seven individuals roosting in furled leaves and combined them with the seven individuals roosting in pitcher plants. We compared the mean pairwise relatedness of bat pairs roosting in pitchers, of bat pairs roosting in furled leaves, and of bat pairs with differing roost preference (one in pitchers, one in furled leaves) to the distribution of values expected under the null hypothesis. The null hypothesis distribution was obtained by randomly assigning roost preferences and then calculating mean difference for pairs roosting in furled leaves, in pitchers, or in both. This procedure was repeated 10,000 times. The *P*-values were then calculated by comparing the observed mean values of relatedness to the null distributions. To visualize the observed pairwise relatedness (TrioML) between the individuals at the study site “Airport”, we constructed an unweighted and undirected network of the bats using the R package igraph v. 0.7.1[48]. To focus on very closely related pairs of bats (parent-offspring or full-sibling pairs), we kept only links with TrioML relatedness > 0.44.

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Stabilization of a bat-pitcher plant mutualism

Supplement

Supplementary Results

Behavioural experiments

When the bats could select between pitchers of different *Nepenthes* species, bats originally roosting in *N. hemsleyana* pitchers also approached *N. hemsleyana* pitchers more often than expected by chance while they approached *N. rafflesiana* significantly less often than expected by chance (Figure S1d). In contrast, bats from *N. bicalcarata* pitchers randomly approached all potential roost species (Figure S1e). When bats could choose between pitchers of different species and a furled leaf, bats from furled leaves approached more often to furled leaves and visited *N. bicalcarata* pitchers less frequently (Figure S1f) while bats from pitchers significantly more often approached *N. hemsleyana* pitchers but less often furled leaves and the plastic tube (Figure S1e).

To correct for the higher proportion of pitchers compared to the single furled leaf (3 : 1), we divided the number of bats that selected pitchers in the flight arena by three (assuming equal preference for all pitchers). Still, bats clearly preferred their original roost type to the unfamiliar one: Only 2.67 of the 8.67 bats that chose pitchers derived from furled leaves while none of the 29 bats that chose furled leaves derived from pitchers (Fisher's exact test for count data: $P < 0.0001$).

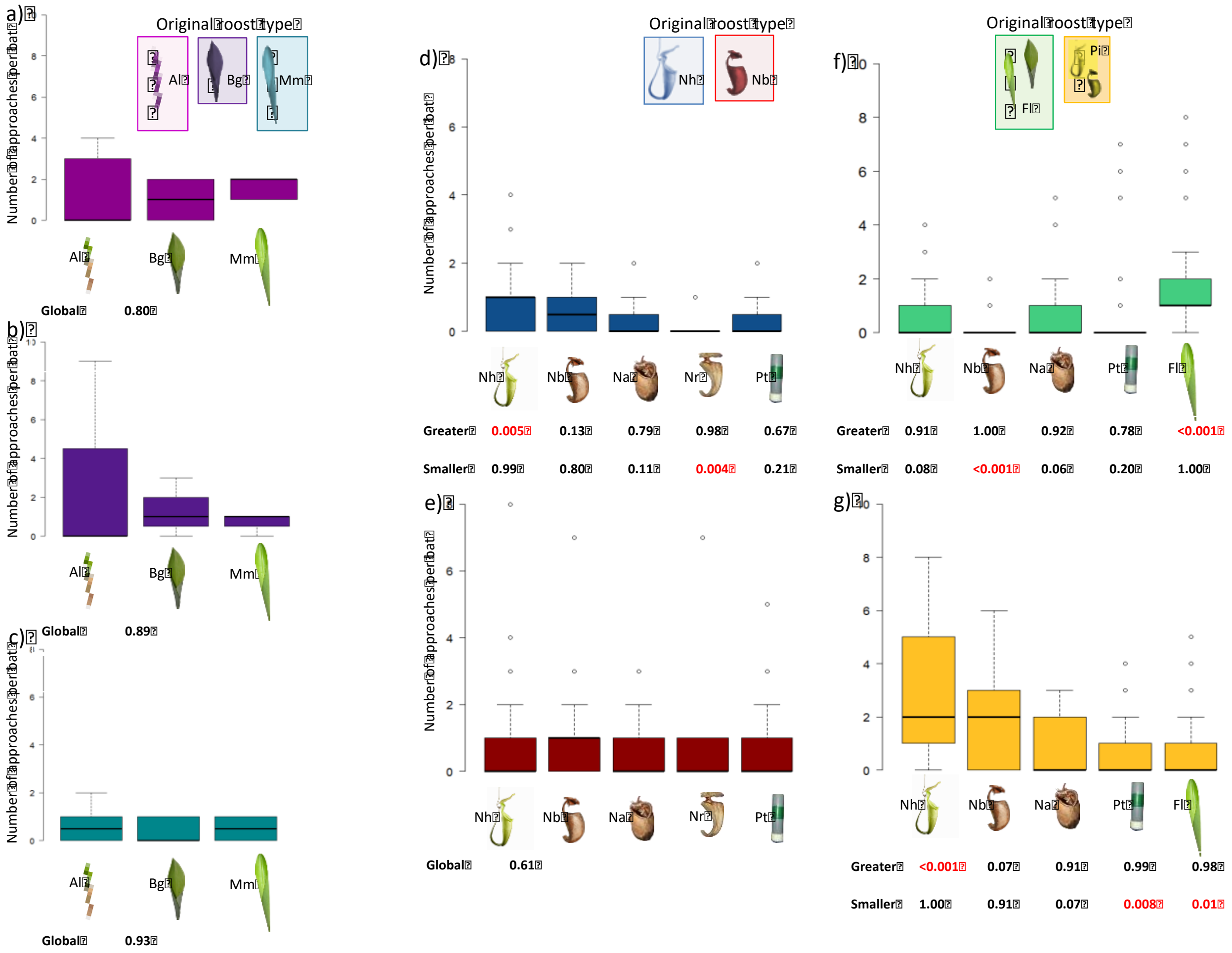
Genetic analysis of the different populations

Between study sites, pairwise F_{ST}-values (mean = 0.03 ± 0.02; range: 0.01 to 0.09; Table S2) showed low population differentiation and differentiation seemed to be independent of the bats' roost preference. Rather than bat roost preference, population structure was linked to geography. We identified three clusters within our ten sampling locations/populations: Cluster 1) "Labi 31", "Andulau", "Saw Mill", "Badas" (Brunei); Cluster 2) "Labi 17", "Teraja" (Brunei); Cluster 3) "Camp 5", "Airport", "Headquarter", "Long Iman" (Sarawak/Malaysia; Figure 3b). Clusters 2) and 3) comprise both bats roosting in pitchers and bats roosting in furled leaves. Similarly, a PCA analysis showed no clear differentiation between bats roosting in pitchers and those in furled leaves (Figure 3a). However, there was a significant relationship between geographic and genetic distance (F_{ST}) regarding the 10 sampling sites ($r = 0.29$, $P = 0.009$; Figure 3c).

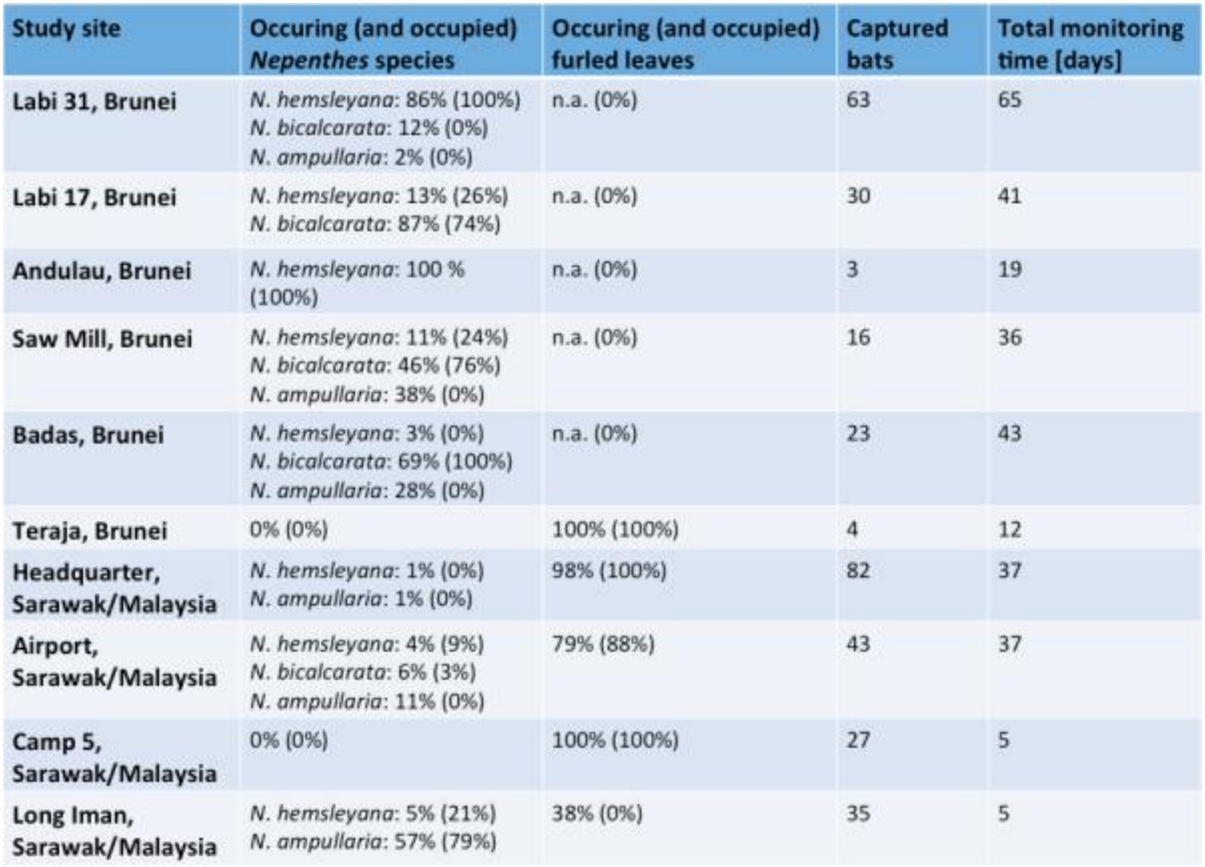
Roost choice and its effect on the relatedness of the bats

Although in six of the 10 study sites the monitored bats used different roost species, they never used both, pitchers and furled leaves, except of the study site "Airport". Here we monitored 42 bats for a mean time period of 3.76 ± 3.24 days. On an individual level, however, the bats did not switch but either used pitchers (seven bats roosted in 12 *N. hemsleyana* pitchers, two of them additionally in three *N. bicalcarata* pitchers) or furled leaves (35 bats roosted in 136 *M. muluensis* plants, which provided 79.40 % of all furled leaves, one of these bats switched between furled leaves of *M. muluensis*, *Z. kelabitianum*, *P. albiflora*, *P. strobilifera* but used each of the latter three species just for one day). We found 21 potential parent-offspring pairs or full-siblings (TrioML > 0.45). All pairs roosted in the same roost type except of one, which involved a male and a female (Chi-square test for given probabilities: $\chi^2 = 17.19$, *df* = 1, $P < 0.0001$; Figure 4).

Supplementary Figure S1: Approaches of *Kerivoula hardwickii* to potential roosts. a)-c) 14 bats found roosting in furled leaves in the wild could choose between furled leaves of different plant species (*Alpinia ligulata* (Al), *Boesenbergia grandis* (Bg), *Musa muluensis* (Mm)). d)-e) 41 bats found roosting in pitchers of *Nepenthes hemsleyana* or *Nepenthes bicalcarata* could choose between pitchers of different *Nepenthes* species (*N. hemsleyana* (Nh), *N. bicalcarata* (Nb), *Nepenthes ampullaria* (Na), *Nepenthes rafflesiana* (Nr)) and a plastic tube (Pt). f)-g) 21 bats found roosting in pitchers and 47 bats found in furled leaves in the wild could choose between a furled leaf, three pitchers (Nh, Nb, Na) and the plastic tube. For statistics see Supplementary methods. Red colour indicates significance after sequential Bonferroni correction.



Supplementary Table S1: Study sites, available and occupied roost types and monitored roosts and bats. Percentages indicate the share of roosts of a given type/species that were available. Percentages in brackets indicate the share of chosen roosts in a study site. Furled leaves were available in all study sites and checked for bats although we did not quantify them in all sites (indicated by "n.a."). In the table we did not distinguish between furled leaves of different plant species as the bats showed no significant preferences for one of the furled leaves' species.



Study site	**Occuring (and occupied) *Nepenthes* species**	**Occuring (and occupied) furled leaves**	**Captured bats**	**Total monitoring time [days]**
Labi 31, Brunei	*N. hemsleyana*: 86% (100%) *N. bicalcarata*: 12% (0%) *N. ampullaria*: 2% (0%)	n.a. (0%)	63	65
Labi 17, Brunei	*N. hemsleyana*: 13% (26%) *N. bicalcarata*: 87% (74%)	n.a. (0%)	30	41
Andulau, Brunei	*N. hemsleyana*: 100 % (100%)	n.a. (0%)	3	19
Saw Mill, Brunei	*N. hemsleyana*: 11% (24%) *N. bicalcarata*: 46% (76%) *N. ampullaria*: 38% (0%)	n.a. (0%)	16	36
Badas, Brunei	*N. hemsleyana*: 3% (0%) *N. bicalcarata*: 69% (100%) *N. ampullaria*: 28% (0%)	n.a. (0%)	23	43
Teraja, Brunei	0% (0%)	100% (100%)	4	12
Headquarter, Sarawak/Malaysia	*N. hemsleyana*: 1% (0%) *N. ampullaria*: 1% (0%)	98% (100%)	82	37
Airport, Sarawak/Malaysia	*N. hemsleyana*: 4% (9%) *N. bicalcarata*: 6% (3%) *N. ampullaria*: 11% (0%)	79% (88%)	43	37
Camp 5, Sarawak/Malaysia	0% (0%)	100% (100%)	27	5
Long Iman, Sarawak/Malaysia	*N. hemsleyana*: 5% (21%) *N. ampullaria*: 57% (79%)	38% (0%)	35	5

Supplementary Table S2: F_{ST} values of the different monitored *Kerivoula hardwickii* populations (lower part of the matrix:) and corresponding *P*-values for all pairs of populations (upper part of the matrix). Bold values indicate significance after sequential Bonferroni correction.

	Labi 31	Andu -lau	Saw Mill	Badas	Labi 17	Teraja	Camp 5	Air-port	Head-quarter	Long Iman
Labi 31	-	0.024	**0.001**	**0.001**	**0.001**	0.009	**0.001**	**0.001**	**0.001**	**0.001**
Andulau	0.037	-	0.017	0.008	0.072	0.038	**0.001**	**0.001**	**0.001**	**0.001**
Saw Mill	0.013	0.052	-	0.020	0.021	0.011	**0.001**	**0.001**	**0.001**	**0.001**
Badas	0.017	0.058	0.011	-	0.026	0.008	**0.001**	**0.001**	**0.001**	**0.001**
Labi 17	0.025	0.007	**0.003**	**0.001**	-	0.090	**0.001**	**0.001**	**0.001**	**0.001**
Teraja	0.035	0.091	0.044	0.043	0.020	-	0.024	0.020	0.020	0.023
Camp 5	0.028	0.06	0.029	0.031	0.025	0.038	-	**0.002**	**0.001**	**0.001**
Airport	0.027	0.064	0.023	0.027	0.019	0.061	0.010	-	**0.001**	**0.001**
Head-quarter	0.030	0.063	0.028	0.035	0.031	0.039	0.011	0.016	-	**0.001**
Long Iman	0.037	0.065	0.038	0.042	0.030	0.033	0.012	0.017	0.015	-

Supplementary Table S3: Sequences and characteristics of the used microsatellite primers. Abbreviations: F = forward primer, R = reverse primer, H_O = observed heterozygosity, H_S = heterozygosity within populations, H_T = total heterozygosity

Inheritance	Repeat motif	Primer sequence (5'-3')	Primer (µM)	Size range (bp)	n	H_O	H_S	H_T
autosomal	$(AC)_{14}$	F: NED-TACTGAAGGCCCTGGGAAG	0.625	223-255	15	0.867	0.859	0.879
		R: GTTT-GGGAACACCTGATACATGCTAAG						
autosomal	$(TG)_{18}$	F: FAM-CCAGCTTGTCCCATCTTACAC	0.625	172-206	17	0.903	0.882	0.897
		R: GTTT-GCTATGAGCCTCCAAACTGC						
autosomal	$(AG)_{11}$	F: FAM-GCCCATGAACTTTGCATCTTAC	0.250	110-130	11	0.480	0.498	0.520
		R: GTCACAATCCCTGCCAGTTC						
autosomal	$(AG)_{10}$	F: FAM-TCTTCCTTAATGGCAGGACTTC	0.625	235-266	14	0.821	0.842	0.875
		R: GTTT-GGAGGTCAGGGTTCAATTCTC						
autosomal	$(CA)_{16}$	F: VIC-GCTTGGCAAACCATCACC	0.375	105-142	22	0.871	0.849	0.879
		R: GGCTCTGAATGTGGGTTCAC						
autosomal	$(CA)_{10}$	F: VIC-CGCCAGCAGATCCTAGAGAC	0.625	236-258	11	0.777	0.728	0.775
		R: GTTT-CCTCGATCTAACCACTGTATTTGAC						
autosomal	$(AC)_{11}$	F: PET-CCATAAGAGGGAGGAATGAGG	0.250	98-118	9	0.655	0.669	0.658
		R: GTTCAGCATGAGTGTATATGAGTGTG						
autosomal	$(AC)_{11}$	F: PET-TGCCACAATCACACATTCTATG	0.375	279-289	6	0.695	0.696	0.735
		R: AGGTCTGGAGCAAAGACACTTC						
autosomal	$(AC)_{15}$	F: VIC-CTCACGCTACTCCAGGAAGG	0.375	172-198	11	0.844	0.817	0.843
		R: GTT-TAACATCTGCCATGTACCCATC						
autosomal	$(TG)_{13}$	F: PET-ACTGGGCAATGTCCAAAGAC	0.625	178-210	28	0.935	0.926	0.952
		R: GT-TTCTTTGCTGTGGGAGCAG						
autosomal	$(GT)_{14}$	F: VIC-TCCTCATCAAGATATGAACATTGG	0.250	133-151	9	0.851	0.793	0.807
		R: GTT-TCAAGAAAGTGAGCTATGAAGCAG						
autosomal	$(AC)_{17}$	F: FAM-AATGAGACAGCAAAGCAAGAAAC	0.375	188-220	17	0.943	0.890	0.907
		R: AGTTGGATTCCCAGTCATGG						
autosomal	$(AC)_{15}$	F: FAM-TCTATCTCCAATGTAACTCCAAAGC	0.375	268-290	16	0.801	0.794	0.854
		R: GTGCCAGAAGCATCTGCTAAG						
autosomal	$(AC)_{14}$	F: VIC-GGCTCAAATTGTGCTAAATGG	0.250	203-237	17	0.814	0.806	0.825
		R: GTT-TACTGGGTGGCTGCAGAAG						
autosomal	$(AC)_{17}$	F: PET-TTCCGGAAGAGTCTAGGATGG	0.625	232-262	19	0.893	0.903	0.918
		R: CGCACTGTCCAATCTCAGG						
autosomal	$(AC)_{20}$	F: FAM-CAAGCCTCTTATGCAACTAGGG	0.250	94-121	20	0.866	0.864	0.886
		R: GTT-TGCCTGTATCTGGGAGCAG						

4.4 Contribution to Publications

Manuscript 1

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.

TUG developed the concept of the study and wrote the first draft of the manuscript. YSL conducted the data measurements of *Nepenthes*. **MGS** developed the study design for the bat-related part of the publication and measured the bats and analysed these data, and contributed to the measurement of *Nepenthes* and the analysis of these data. CRS contributed to data collection and analysis. GK, DGT and MS advised on the study design. All authors revised and edited the manuscript.

Manuscript 2

Schöner, M.G., Schöner, C.R., Kerth, G., Suhaini, S.N. & Grafe, T.U. (*submitted*): Handle with care: Adhesive pads improve the ability of Hardwicke's woolly bat, *Kerivoula hardwickii* (Chiroptera: Vespertilionidae), to roost in a carnivorous pitcher plant.

MGS developed the study design, analysed the data and wrote the first draft of the manuscript. CRS advised on the data analysis. **MGS**, CRS and SNS collected data. CRS, GK and TUG advised on the study design. All authors revised and edited the manuscript.

Manuscript 3

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.

MGS, CRS and RS contributed equally to this manuscript. **MGS**, CRS, RS, TUG and GK developed the concept of the study. **MGS** conducted call recordings. RS conducted the ensonification. **MGS**, CRS and LLJ performed the behavioral experiments. **MGS**, CRS, RS, and SJP analyzed the data. **MGS**, CRS, RS, GK, SJP, TUG, and LLJ wrote the paper.

Manuscript 4

Schöner, M.G., Simon, R. & Schöner, C.R. (2016). Acoustic communication in plant-animal interactions. *Curr Opin Plant Biol* 32:88-95.

MGS developed the concept of this review paper. **MGS** and CRS conducted the literature research and wrote the first draft of the manuscript. RS prepared Figure 1. All authors revised and edited the paper.

Manuscript 5

Schöner, M.G., Schöner, C.R., Kerth, G., Liaw, L.J. & Grafe, T.U. (2016): Bats attend to plant structures to identify roosting sites. *Acta Chiropterol* 18:443–440.

MGS developed the study design, analysed the data and wrote the first draft of the manuscript. **MGS**, CRS and LJL collected data. GK and TUG advised on the study design. All authors revised and edited the manuscript.

Manuscript 6

Schöner, M.G., Schöner, C.R., Ermisch, R., Puechmaille, S.J., Tan, M.C., Grafe, T.U. & Kerth, G. (*submitted*): Stabilization of a bat-plant mutualism.

MGS and CRS contributed equally to this manuscript. **MGS**, CRS, TUG and GK developed the concept of the study. **MGS**, CRS and RE collected data and performed the experiments. **MGS** processed genetic samples in the lab in Greifswald. **MGS**, CRS, and SJP conducted the genetic analysis. **MGS** and CRS wrote the first draft of the manuscript. All authors revised and edited the manuscript.

Prof. Dr. Gerald Kerth Michael Gerhard 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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Professional status

Since March 2017	Scientific research associate in the department Applied Zoology and Nature Conservation (funded by DBU)

Academic education

09/2012 – 07/2017	PhD candidate at the University of Greifswald
2012 – 2013	Teaching degree: Philosophy/Ethics
2007 – 2012	Teaching degree: Biology
2004 – 2011	Teaching degree: German, History
2004 – 2010	Magister Artium: Medieval German Philology, German Literature, Medieval History
	Topic of the thesis: „Staufische Herrscher als Minnesänger und ihre Beziehung zur volkssprachlichen Liedkunst“ (Grade: 1.0)
26/09/2006	Pre-diploma: German
13/02/2007	Pre-Diploma: History
2003 – 2004	Upper vocational school Kitzingen; University-entrance diploma

Former Professions

2015 – 2017	Coordinator of the biological research training group “RESPONSE” (funded by DFG)
2016	Scientific associate in the project “*inter*Studies” (BMBF)
2015 – 2016	Management of the coordinated teacher education group of the University of Greifswald
2014 – 2015	Scientific research associate at the University of Erlangen-Nuremberg (BMBF-Project “Bats and Wind Farms”)
2011 – 2014	Scientific assistant at the University of Greifswald
2008 – 2010	Student research assistant at the University of Würzburg
1999 – 2003	Office clerk at HATICO GmbH, Tirschenreuth

Received awards

25.06.2016	Lothar Kämpfe publication award
15.03.2015	1st and 2nd Student poster award at the 4th International Berlin Bat Meeting
03.03.2013	1st Student poster award at the 3rd International Berlin Bat Meeting

Scholarships

01/2014 – 08/2014	Scholarship for PhD candidates of the German Academic Exchange Service (DAAD)
08/2013 – 08/2013	DAAD-Scholarship for conference costs
07/2012 – 12/2012	DAAD-Scholarship for PhD candidates
06/2009 – 08/2009	DAAD-Scholarship for students

Fund raising

2016	DFG-project “Convergent multispecies interactions in coprophagous pitcher plant species” (co-author; successful grant of 288,000 €)
2016	BMU-project „Fledermäuse und Naturschutz – Mit FUN in die Wildnis (Co-applicant; successful grant of 390,000 €)
2016	BMBF-project “Qualitätsoffensive Lehrerbildung: LEHREN in MV“ (co-author; successful grant of 861,000 €)
2014	DFG research training group “GRK 2010 Biological RESPONSEs to novel and changing environments” (co-author; successful grant of 3,680,000 €)
2011	DFG-project “Interactions between bats and pitcher plants” (co-author; successful grant of 160,000 €)

Articles with peer review and under review

- **Schöner, M.G.**, Schöner, C.R., Ermisch, R., Puechmaille, S.J., Tan, M.C., Grafe, T.U. & Kerth, G. (*submitted*): Stabilization of a bat-plant mutualism.
- **Schöner, M.G.**, Schöner, C.R., Kerth, G., Suhaini, S.N. & Grafe, T.U. (*submitted*): Handle with care: Adhesive pads improve the ability of Hardwicke’s woolly bat, *Kerivoula hardwickii* (Chiroptera: Vespertilionidae), to roost in a carnivorous pitcher plant.
- Yilamujiang, A., Zhu, A., Ligabue-Braun, R., Bartram, S., Witte, C., Hedrich, R., Hasabe, M., Schöner, C.R., **Schöner, M.G**., Kerth, G., Carlini, C.R. & Mithöfer, A. (*submitted*): Coprophagous features in carnivorous *Nepenthes* plants: a role for ureases.
- Schöner, C.R., **Schöner, M.G.**, Grafe, T.U., Clarke, C.M., Dombrowski, L., Tan, M.C. & Kerth, G. (2016): Ecological outsourcing: a pitcher plant benefits from transferring pre-digestion of prey to a bat mutualist. *J Ecol*, early view.
- **Schöner, M.G.**, Schöner, C.R., Kerth, G., Liaw, L.J. & Grafe, T.U. (2016): Bats eavesdrop on plant structures to identify roosting sites. *Acta Chiropterol* 18:433-440.
- **Schöner, M.G.**, Simon, R. & Schöner, C.R. (2016): Acoustic communication in plant-animal interactions. *Curr Opin Plant Biol* 32:88–95.
- Struebig, M.J., Huang, J.C.-C., Mohamed, N.Z., Noerfahmy, S., Schöner, C.R., **Schöner, M.G.** & Francis C.M. (2016): Forest surveys extend the range of the Krau woolly bat (*Kerivoula krauensis*) in the Malay-Thai Peninsula, Borneo and Sumatra. *Mammalia* 81:211-215.
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- 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.
- **Schöner, M.G.**, Schöner, C.R., Simon, R., Grafe, T.U., Puechmaille, S.J., Liaw, L.J. & Kerth, G. (2015): Bats are acoustically attracted to mutualistic carnivorous plants. *Curr Biol* 25:1911–1916.
- 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.
- 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.
- Schöner, C.R., **Schöner, M.G.** & Kerth, G. (2010): Similar is not the same: Social calls of conspecifics are more effective in attracting wild bats to day roosts than those of other bat species. *Behav Ecol Sociobiol* 46:2053–2063.

Monographs

- **Schöner, M.G.** & Schöner, C.R. (2014): Staufische Herrscher als Minnesänger und ihre Beziehung zur volkssprachlichen Liedkunst. Mit einer Neuedition der Lieder Konradins. Vorwort von Sieglinde Hartmann. Göppinger Arbeiten zur Germanistik, Göppingen.

Other articles

- **Schöner, M.G.** & C. R. Schöner (2013): Batty and Pitty. A bat story for children. Illustrated by Claudia Spitzkopf and Robin Schöfer. http://www.seabcru.org/outreach/brunei-outreach-materials.
- **Schöner, M.G.** & Schöner, C.R. (2013): Symbiotischer Untermieter gesucht. Hardwicke-Wollfledermäuse schlafen in fleischfressenden Pflanzen. *Hundkatzepferd. Das Fachmagazin für den Tierarzt* 6:2–4.
- Schöner, C.R. & **Schöner, M.G.** (2012): Living inside a deadly trap. Woolly bats use carnivorous pitcher plants as roosts. *Bats* 30:2–3.
- **Schöner, M.G.** & Schöner, C.R. (2012): Fledermausporträt: Hardwicke-Wollfledermaus, *Kerivoula hardwickii* (Horsefield, 1824). *Nyctalus* 17:400–404.

Research trips abroad

01/2017 – 03/2017	Biological field research in Costa Rica
02/2016 – 02/2016	Biological field research in Malaysia
04/2014 – 09/2014	Biological field research in Brunei and Malaysia
09/2013	Biological field trip to Bulgaria
08/2013	Biological field trip to Costa Rica
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

Biological field research in Germany

2009 – 2010	Monitoring of diverse bat colonies
2008 –2009	Internship: Roosting behavior of the bat species *Plecotus auritus*
2008 – 2009	Internship: Long-term conservation project of black adders

Scientific services

Referee for:	Acta Chiropterologica, Journal of Zoology, Scientia Bruneiana, Zoologischer Anzeiger
Conferences	Organisation of the 21st Meeting of the DZG section Evolutionary Biology and Ecology

Teaching experience

since 2013	Supervision of Practical Courses, Hiddensee
since 2011	Supervision of several Bachelor and Master thesis and internships
2013	Seminar: International Conventions
2013	Tutorial: Animal behaviour
2012 – 2013	Seminar: Frontiers in Conservation
2008 – 2010	Tutorials: Medieval High German

Cooperation with other researchers

Dr. Oliver Behr, University of Erlangen; Prof. Dr. Gloriana Chaverri, University of Costa Rica; Dr. Charles Clarke, James Cook University Cairns; Prof. Dr. Ulmar Grafe, University of Brunei; Prof. Dr. Gerald Kerth, University of Greifswald; Dr. Axel Mithöfer, MPI Jena; Dr. Martina Nagy, University of Erlangen; Dr. Rachel Page, Smithsonian Tropical Research Institute; Dr. Andrej Pavlovic, University of Olomouc; Dr. Sébastien Puechmaille, University of Greifswald; Prof. Dr. Katharina Riedel, University of Greifswald; Dr. Ralph Simon, University of Erlangen; Dr. Matthew Struebig, University of Kent; Dr. Merlin Tuttle, University of Texas; Prof. Dr. Alex Widmer, ETH Zürich; Dr. Daniela Zühlke, University of Greifwald

Media

Photographers:	Ch'ien C. Lee (http://www.wildborneo.com.my/), Dr. Merlin Tuttle (https://merlintuttle.smugmug.com/Low-Resolution/Roosting/), Christian Ziegler (http://www.christianziegler.photography/)
Magazines (extract):	BBC Earth News (Jan. 2011, Apr. 2015), BBC Wildlife Magazine (Spring 2015), Discovery News (Jul. 2015), Geo (2/2014), LiveScience (Jul. 2015), National Geographic (Feb. 2011, Sep. 2015), Nature (Jul. 2015), New Scientist (Feb. 2015), Science (Jan. 2011, Jul. 2015), Science Alert (Jul. 2015), Stern (Dez. 2016), The New York Times (Sep. 2015), The Verge (Jul. 2015)
Newspaper (extract):	Bild, Borneo Bulletin, Brunei Times, Frankfurter Allgemeine Zeitung, Frankfurter Allgemeine Sonntagszeitung, Neue Züricher Zeitung, Süddeutsche Zeitung, Tageszeitung, Welt am Sonntag, The Epoche Times
Radio reports, -interviews:	Bayern 2 Wissenschaft und Forschung, BBC Science in Action, Deutschlandfunk, Europa FM (Rumania), Hessischer Rundfunk 1 Profile, Science AAAS podcast, Science Update, WDR5 Leonardo
TV:	National Geographic TV; BBC "Our Planet"; BBC "Nature's Weirdest Events"; EBS „Green Animals"
Exhibitions:	Sonderausstellung: Karnivore Pflanzen (Wilhelma Stuttgart); Veiled as vampires - the secret world of bats (Natural History Museum Helsinki); The Great Animal Orchestra (Bernie Krause; Cartier Foundation Paris)

Skills and further expertise, advanced trainings

Language skills:	English (fluent), French, Malay, Spanish (good command), Latin
Advanced trainings:	e.g., Project Management; Research Ethics; University Didactics; Career Development; Rhetorics; Conservation in the media (Schwerin); Summer School: Analysis and Visualisations of Ecological Data with GIS (Trier); Stable Isotopes (Berlin); Phenotypic Plasticity (Greifswald), Genetic Adaptation (Greifswald), Dispersal (Wooster Teerofen)
Field research:	typical ecological field methods; radio tracking; (micro-) habitat analyses; behavioral experiments; video und acoustic monitoring
Genetics:	establishment of primers, DNA isolation, PCR, gel electrophoreses, genotyping, analyses of population genetic data, mtDNA
Bat biology:	capturing methods, handling und morphological measurements; bioacoustics; thermoregulation; PIT-tag implanting
Botany:	*Nepenthes* and their ecology; growth experiments and analyses; PAM fluorometry, chlorophyll extraction
Software skills:	Microsoft Office, Citavi, R, Photoshop, ArcGIS, Typo 3, Open Project, diverse genetic softwares, bioacoustics (e.g., SASLab pro; BCAdmin), graphical networks
Further skills:	European driving licence, Scuba resort diver

Membership in scientific organizations

Since 2012	South East Asian Bat Conservation Research Unit (SEABCRU)
Since 2011	Brunei Nature Society (BNS)
Since 2010	Association for Tropical Biology and Conservation (ATBC)
Since 2009	DZG - Deutsche Zoologische Gesellschaft (German Zoological Society)
Since 2009	Medävistenverband e.V. (German Medieval Scientific Society)

Greifswald, 20th March, 2017

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