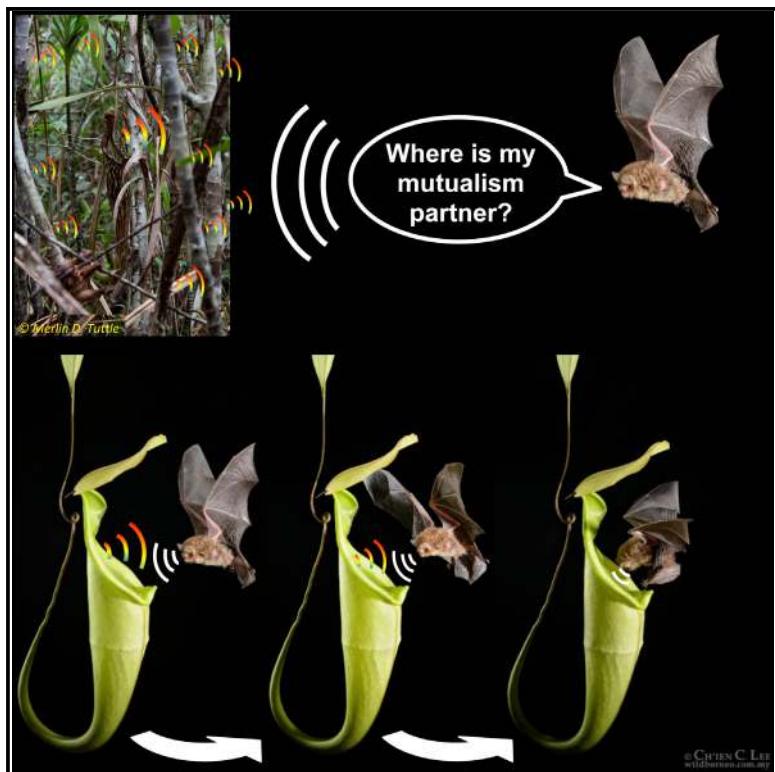


Current Biology

Bats Are Acoustically Attracted to Mutualistic Carnivorous Plants

Graphical Abstract



Authors

Michael G. Schöner,
Caroline R. Schöner, Ralph Simon, ...,
Sébastien J. Puechmaille, Liaw Lin Ji,
Gerald Kerth

Correspondence

schoenerm@uni-greifswald.de

In Brief

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

Highlights

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

Bats Are Acoustically Attracted to Mutualistic Carnivorous Plants

Michael G. Schöner,^{1,4,*} Caroline R. Schöner,^{1,4} Ralph Simon,^{2,4} T. Ulmar Grafe,³ Sébastien J. Puechmaille,¹ Liaw Lin Ji,³ and Gerald Kerth¹

¹Zoological Institute and Museum, Ernst-Moritz-Arndt-Universität Greifswald, Johann-Sebastian-Bach-Straße 11–12, 17489 Greifswald, Germany

²Department of Sensor Technology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Paul-Gordan-Straße 3–5, 91052 Erlangen, Germany

³Department of Biology, Faculty of Science, Universiti Brunei Darussalam, Tungku Link, Gadong 1410, Brunei Darussalam

⁴Co-first author

*Correspondence: schoenerm@uni-greifswald.de
<http://dx.doi.org/10.1016/j.cub.2015.05.054>

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

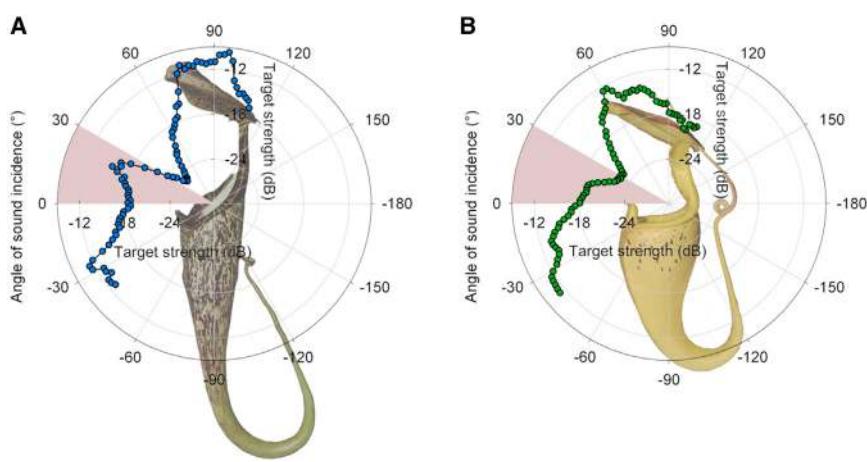
How mutualisms evolve or how these interactions are maintained is still not sufficiently understood [12]. Particularly, if

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



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

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

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

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

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.

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 *Glossophagidae* calls often consist of multiple harmonics and are

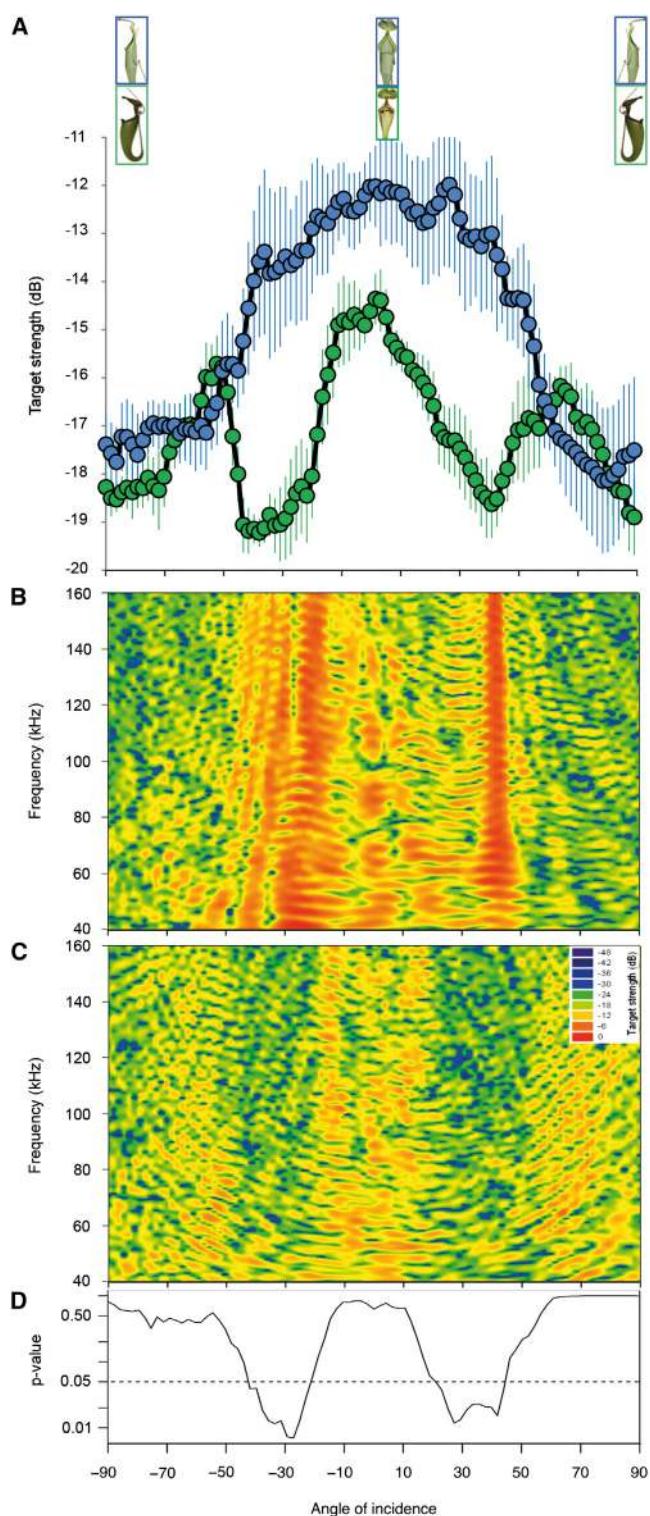


Figure 2. Echo Reflectance of *N. hemsleyana* and *N. rafflesiana* Pitchers in the Azimuth Plane

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

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

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

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

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

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

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

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

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

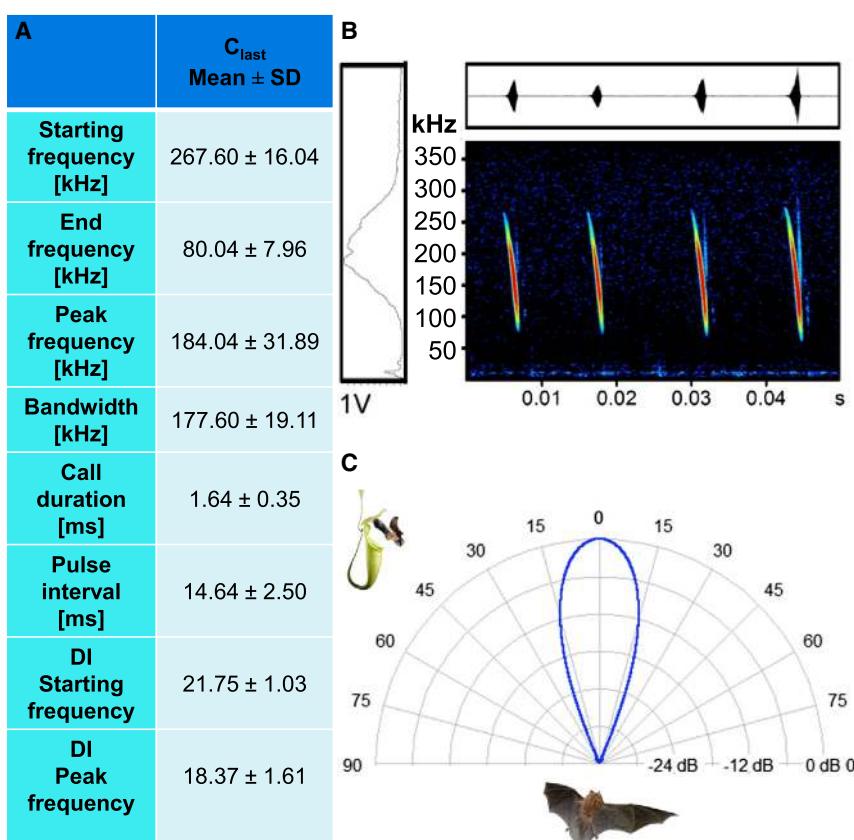


Figure 3. Echolocation Calls and Call Directionality of *Kerivoula hardwickii*

(A) Call parameters (n of all analyzed calls = 25) of the last five calls of a pitcher approach (C_{last}) and the referring call directionality (measured as directivity index [DI]).

(B) Spectrogram, power spectrum, and oscillogram of the echolocation calls of *K. hardwickii*.

(C) Beam shape of the calls of *K. hardwickii*. The high mean peak frequencies in C_{last} resulted in a very high call directivity (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

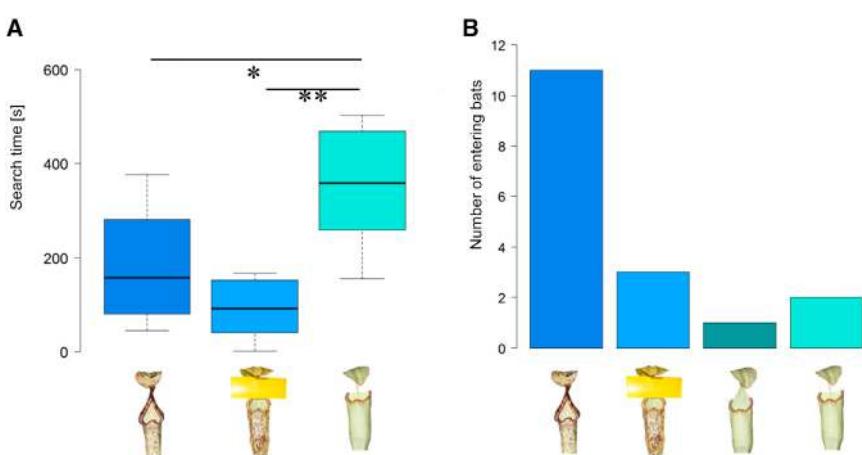


Figure 4. Behavioral Responses of *K. hardwickii* to Reflector Modifications

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 1/4" free-field microphone (G.R.A.S. Sound & Vibration) and a custom-built condenser speaker (Sensory Technology, University of Erlangen). This setup allowed measurements within a frequency range of 40 to 160 kHz. Measurements were taken at a distance of 20 cm and from different angles around the pitcher's orifice (defined as 0°) for the elevation plane (−40° to +60°) and azimuth (±90°, Figure S1A) in increments of 1.8° [9].

Echolocation Call Analyses

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

Behavioral Experiments

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

SUPPLEMENTAL INFORMATION

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

AUTHOR CONTRIBUTIONS

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

ACKNOWLEDGMENTS

We thank E.K.V. Kalko for support in planning the experiments; P. Braun and L. Dombrowski for assistance in the field; and E. Weise, E. Donke, and Q. Suhaini

for video analysis. We also thank M.D. Tuttle and C.C. Lee for providing us with photographs. R. Specht, S. Greif, H.R. Goerlitz, and K. Hochradel helped in acoustic analysis and interpretation. C. Klein provided *Nepenthes* pitchers for pre-ensonification tests. S. Dool, T. Kingston, S. Parsons, J. van Schaik, B. Fenton, and two anonymous referees commented on the manuscript. The German Academic Exchange Service (DAAD), the German Research Foundation (DFG: KE 746/5-1), and the Universiti Brunei Darussalam (RG/1[105] and RG/1[193]) funded this project. The Forestry Departments of Brunei Darussalam and Sarawak granted us permits to work in the field.

Received: February 13, 2015

Revised: May 4, 2015

Accepted: May 27, 2015

Published: July 9, 2015

REFERENCES

1. Bascompte, J., and Jordano, P. (2007). Plant-animal mutualistic networks: the architecture of biodiversity. *Annu. Rev. Ecol. Evol. Syst.* 38, 567–593.
2. Bronstein, J.L. (2001). The exploitation of mutualisms. *Ecol. Lett.* 4, 277–287.
3. Ryan, M.J., Fox, J.H., Wilczynski, W., and Rand, A.S. (1990). Sexual selection for sensory exploitation in the frog *Physalaemus pustulosus*. *Nature* 343, 66–67.
4. Ryan, M.J. (1998). Sexual selection, receiver biases, and the evolution of sex differences. *Science* 281, 1999–2003.
5. Schaefer, H.M., and Ruxton, G.D. (2009). Deception in plants: mimicry or perceptual exploitation? *Trends Ecol. Evol.* 24, 676–685.
6. Chittka, L., Shmida, A., Troje, N., and Menzel, R. (1994). Ultraviolet as a component of flower reflections, and the colour perception of Hymenoptera. *Vision Res.* 34, 1489–1508.
7. von Helversen, D., and von Helversen, O. (1999). Acoustic guide in bat-pollinated flower. *Nature* 398, 759–760.
8. Hossaert-McKey, M., Soler, C., Schatz, B., and Proffit, M. (2010). Floral scents: their roles in nursery pollination mutualisms. *Chemoecology* 20, 75–88.
9. Simon, R., Holderied, M.W., Koch, C.U., and von Helversen, O. (2011). Floral acoustics: conspicuous echoes of a dish-shaped leaf attract bat pollinators. *Science* 333, 631–633.
10. Grafe, T.U., Schöner, C.R., Kerth, G., Junaidi, A., and Schöner, M.G. (2011). A novel resource-service mutualism between bats and pitcher plants. *Biol. Lett.* 7, 436–439.
11. Schöner, C.R., Schöner, M.G., Kerth, G., and Grafe, T.U. (2013). Supply determines demand: influence of partner quality and quantity on the interactions between bats and pitcher plants. *Oecologia* 173, 191–202.
12. Jandér, K.C., and Herre, E.A. (2010). Host sanctions and pollinator cheating in the fig tree-fig wasp mutualism. *Proc. Biol. Sci.* 277, 1481–1488.
13. Müller, R., and Kuc, R. (2000). Foliage echoes: a probe into the ecological acoustics of bat echolocation. *J. Acoust. Soc. Am.* 108, 836–845.
14. Yovel, Y., Franz, M.O., Stilz, P., and Schnitzler, H.-U. (2011). Complex echo classification by echo-locating bats: a review. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* 197, 475–490.
15. Moran, J.A. (1996). Pitcher dimorphism, prey composition and the mechanisms of prey attraction in the pitcher plant *Nepenthes rafflesiana* in Borneo. *J. Ecol.* 84, 515–525.
16. Marshall, A.G. (1983). Bats, flowers and fruit: evolutionary relationships in the old world. *Biol. J. Linn. Soc. Lond.* 20, 115–135.
17. Boonman, A., Bumrungsri, S., and Yovel, Y. (2014). Nonecholocating fruit bats produce biosonar clicks with their wings. *Curr. Biol.* 24, 2962–2967.
18. Douangboubpha, B., Bumrungsri, S., Satasook, C., Wanna, W., Soisook, P., and Bates, P.J.J. (2015). Morphology, genetics and echolocation calls of the genus *Kerivoula* (Chiroptera: Vespertilionidae: Kerivoulinae) in Thailand. *Mammalia*. Published online January 23, 2015. <http://dx.doi.org/10.1515/mammalia-2014-0004>.

19. Schmieder, D.A., Kingston, T., Hashim, R., and Siemers, B.M. (2010). Breaking the trade-off: rainforest bats maximize bandwidth and repetition rate of echolocation calls as they approach prey. *Biol. Lett.* 6, 604–609.
20. Siemers, B.M., and Schnitzler, H.-U. (2004). Echolocation signals reflect niche differentiation in five sympatric congeneric bat species. *Nature* 429, 657–661.
21. Lazure, L., and Fenton, M.B. (2011). High duty cycle echolocation and prey detection by bats. *J. Exp. Biol.* 214, 1131–1137.
22. Jakobsen, L., Ratcliffe, J.M., and Surlykke, A. (2013). Convergent acoustic field of view in echolocating bats. *Nature* 493, 93–96.
23. Brinkløv, S., Jakobsen, L., Ratcliffe, J.M., Kalko, E.K.V., and Surlykke, A. (2011). Echolocation call intensity and directionality in flying short-tailed fruit bats, *Carollia perspicillata* (Phyllostomidae). *J. Acoust. Soc. Am.* 129, 427–435.
24. Skolnik, M.I. (2001). *Introduction to Radar Systems*, Third Edition. (Boston: McGraw Hill).
25. Simon, R., Knörnschild, M., Tschapka, M., Schneider, A., Passauer, N., Kalko, E.K.V., and von Helversen, O. (2014). Biosonar resolving power: echo-acoustic perception of surface structures in the submillimeter range. *Front Physiol* 5, 64.
26. Clare, E.L., Goerlitz, H.R., Drapeau, V.A., Holderied, M.W., Adams, A.M., Nagel, J., Dumont, E.R., Hebert, P.D.N., and Fenton, M.B. (2014). Trophic niche flexibility in *Glossophaga soricina*: how a nectar seeker sneaks an insect snack. *Funct. Ecol.* 28, 632–641.
27. Brinkløv, S., Kalko, E.K.V., and Surlykke, A. (2009). Intense echolocation calls from two ‘whispering’ bats, *Artibeus jamaicensis* and *Macrophyllum macrophyllum* (Phyllostomidae). *J. Exp. Biol.* 212, 11–20.
28. Gould, S.J., and Lloyd, E.A. (1999). Individuality and adaptation across levels of selection: how shall we name and generalize the unit of Darwinism? *Proc. Natl. Acad. Sci. USA* 96, 11904–11909.

Current Biology

Supplemental Information

Bats Are Acoustically Attracted to Mutualistic Carnivorous Plants

Michael G. Schöner, Caroline R. Schöner, Ralph Simon, T. Ulmar Grafe, Sébastien J. Puechmaille, Liaw Lin Ji, and Gerald Kerth

Supplemental figures

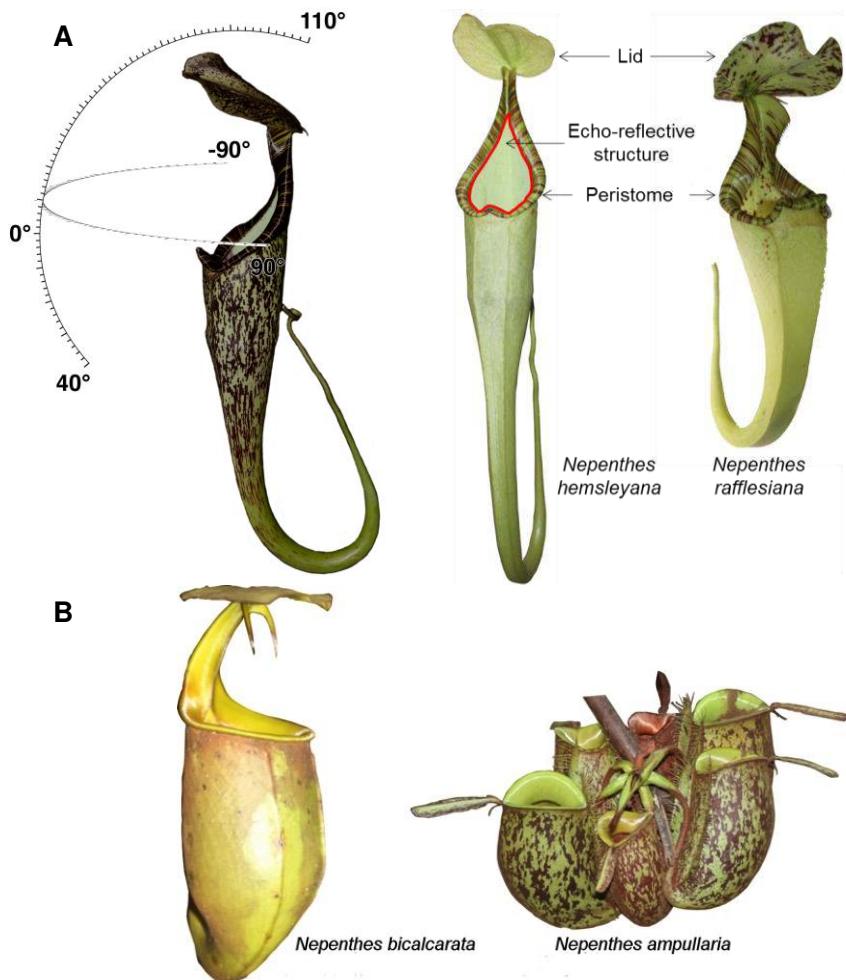


Figure S1, Related to Figure 1 and 2. *Nepenthes hemsleyana* and other sympatric *Nepenthes* species.

(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).

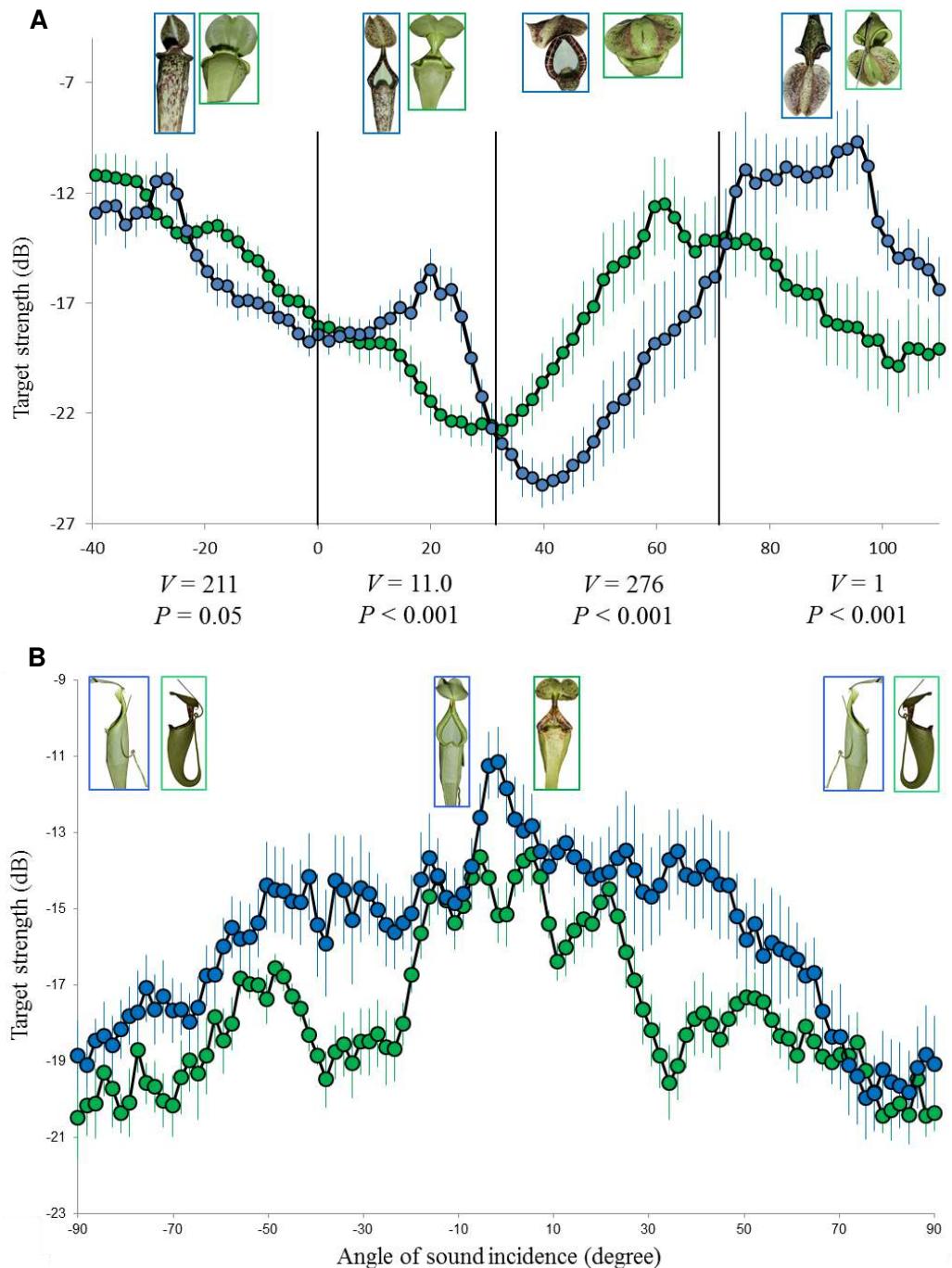


Figure S3, Related to Figure 1 and 2. Target strength of *N. hemsleyana* and *N. rafflesiana*.

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 | | 1 unmodified and 3 modified pitchers | |
| | | | | |
| | | | | |
| | 11 | 3 | 2 | |
| unmodified | unmodified | unmodified | unmodified | |
| | 3 | 4 | 3 | |
| enlarged | enlarged | 1/3 removed | 1/3 removed | |
| | 1 | 2 | 2 | |
| partly removed | partly removed | 1/2 removed | 1/2 removed | |
| | 2 | 1 | 1 | completely removed |
| completely removed | completely removed | completely removed | completely removed | |

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

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

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

Supplemental Experimental Procedures

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

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

Experimental ensonifications: We ensonified *N. hemsleyana* and *N. rafflesiana* pitchers with a biomimetic sonar head consisting of a custom built condenser speaker with a membrane made of Electro Mechanical Film and a $\frac{1}{4}$ " free-field microphone Type 40BF in combination with the preamplifier 26AB, which was connected to the power module 12AA (all from G.R.A.S. Sound & Vibration, Denmark). Using a continuously replayed MLS (Maximum Length Sequence) for ensonification allowed us to retrieve IR (impulse responses) through deconvolution of echo and original MLS. The frequency response of the speaker allowed measurements between 40-160 kHz (sound pressure levels at 1 m distance: approximately 95 ± 6 dB) [S3] covering *K. hardwickii*'s peak and end frequency range. We measured from a distance of 20 cm from different angles ($1.8^\circ/\text{step}$) around the pitcher's orifice (defined as 0°) for the elevation (-40 to $+60^\circ$) and the azimuthal plane ($\pm 90^\circ$; Figure S1A). For the azimuthal measurement the sonar head was directly ensonifying the backwall structure between the lid and the pitcher's orifice (Figure 2A). During a further azimuthal measurement the sonar head was tilted 5° downwards pointing into the pitchers cavity (results of this measurements are shown in Figure S3B). For the

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

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

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

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

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

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

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

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

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

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

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

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

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

Behavioral Experiments: We caught bats in harp traps or *Nepenthes* pitchers and marked them with PIT-tags for individual identification [S3] to ensure that each bat was tested only once. Experiments were filmed (Sony HDR-CX560VE) in a flight arena (3.5 m \times 3.5 m, height 2.5 m) and conducted in the early morning hours around dawn (5:00 to 7:00), which is the normal time when bats are searching for new roosts (personal observation during radio-tracking studies). We fed and released the bats within 12 hours of capture into their original habitat. Pregnant and lactating females as well as juveniles were excluded from the experiments. To be sure that

fragrance definitely has no influence on the bats' choice, we had emptied all experimental pitchers and washed them before starting the experiment.

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

Furthermore, we conducted experiments in which each bat could choose between one unmodified and three pitchers with modified reflector (6 male, 12 female bats; 44 different pitchers; Experiment 2, Table S1B), the lid (5 males, 6 females; 30 pitchers), or the peristome (5 males, 5 females; 25 pitchers; Experiment 3, Table S1C). For these experiments we used each pitcher to test up to three different bats (1.41 ± 0.62 mean \pm s.d.). We randomly arranged the unmodified and the three modified ($1/3$, $1/2$ or complete lid or peristome removed) pitchers within the flight arena (distance to each other = 0.5 m; height = 1.5 m). Each bat was tested only once per type of experiment but due to the limitation of individuals 12 of the 25 bats were tested in different types of experiments. One bat in each of the experiments regarding the reflector and the lid and two

bats in the peristome experiment made no choice within the maximum time span of 20 min per trial so that we had to exclude them from the analyses of the bats' final pitcher choices.

Individuals without background knowledge on the experiments analyzed the videos. Because of camera problems two experiments had to be analyzed based on direct observations during their performance. For the statistical analysis we pooled the approaches to the partly and completely removed reflectors into a 'modified reflector' treatment as there was no difference between them ($P = 0.15$).

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

Supplemental References

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