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Tuning of color contrast signals to visual sensitivity maxima of tree shrews by three Bornean highland Nepenthes species

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Three species of Nepenthes pitcher plants (*Nepenthes rajah*, *Nepenthes lowii* and *Nepenthes macrophylla*) specialize in harvesting nutrients from tree shrew excreta in their pitchers. In all three species, nectaries on the underside of the pitcher lid are the focus of the tree shrews' attention. Tree shrews are dichromats, with visual sensitivity in the blue and green wavebands. All three *Nepenthes* species were shown to produce visual signals, in which the underside of the pitcher lid (the area of highest nectar production) stood out in high contrast to the adjacent area on the pitcher (i.e., was brighter), in the blue and green wavebands visible to the tree shrews. *N. rajah* showed the tightest degree of "tuning," notably in the green waveband. Conversely, pitchers of *Nepenthes burbidgeae*, a typical insectivorous species sympatric with *N. rajah*, did not produce a color pattern tuned to tree shrew sensitivity maxima.

Many plant species use visual signals to attract animals for purposes such as pollination and seed dispersal.[1-3] For example, to maximize the probability of pollen transfer from anther to stigma, insect-pollinated flowers utilize sensory cues and morphological structures tailored to the physiology and behavior of the target species.[4,5] The traps of carnivorous plants have evolved in response to a similar evolutionary pressure to that which shaped the flowers of animal- pollinated plants: the necessity to attract and retain the target animal at the site of maximum benefit to the plant. For example, in pitchers of *Nepenthes rafflesiana* Jack, the peristome (the collar-like structure surrounding the pitcher mouth, and the site of highest nectar production) stands out in high visual contrast to the pitcher body proper, in the UV, blue and green wavebands. These correspond to the visual sensitivity maxima of many of the targeted insect prey taxa.[6,7] However, not all *Nepenthes* deploy strictly carnivorous pitchers: recent studies have demonstrated that those of four Bornean species, *Nepenthes rafflesiana* var. *elongata* (recently renamed *Nepenthes baramensis* C. Clarke, J.A. Moran and Chi C. Lee[8]), *Nepenthes rajah* Hook. f., *Nepenthes macrophylla* (Marabini) Jebb and Cheek and *Nepenthes lowii* Hook. f., collect mammal excreta.[9-12] The latter three species attract mountain tree shrews (*Tupaia montana*); in return for the nectar provided by the pitchers, the animals deposit excreta, from which the plants derive nitrogen.[9,10] In these three species, the lid, rather than the peristome, is the site of greatest nectar production, and is therefore the "target" to which the tree shrews are required to orient themselves. This ensures that their hindquarters are positioned over the pitcher mouth, allowing capture of any excreta.[9,10] Here, the hypothesis was tested that pitchers of these species produce color signals that are tuned to the visual sensitivity maxima of their diurnal partner. Tree shrews are dichromats- they have two types of cone cell in the retina, which are sensitive to blue light (S cones) and green light (L cones).[13,14]

Specifically, the degree of color contrast between the area of greatest nectar production (the lid) and the closest adjacent area of the pitcher proper (i.e., the "background"), was quantified. In *N. rajah* and *N. macrophylla*, this was the peristome; in *N. lowii*, the peristome is vestigial,[9] so the flared inner wall of the pitcher was scanned instead (**Fig. 1**). For comparison, pitchers of *Nepenthes burbidgeae* Hook. f. ex. Burb. were analyzed. This species is sympatric with *N. rajah*, but does not attract tree shrews, catching invertebrates in typical *Nepenthes* fashion (JM, pers. obs. **Fig. 1A**). Thus, it was predicted that *N. burbidgeae* would not produce a color contrast signal tuned to tree shrew visual sensitivity maxima.

In all three of the tree shrew- attracting species, the pitcher lid was found to be brighter in the blue and green wavebands than the adjacent area of peristome (inner wall of the pitcher body for *N. lowii*), i.e., contrast values relative to background were positive (**Figs. 2 and 3**). This was not the case for the typical, insectivorous *N. burbidgeae*, in which the lid was darker than the peristome in both wavebands (i.e., negative contrast values; **Figs. 2 and 3**). Thus, in the three tree shrew-specialized species, the lid, the site of the highest nectar production, was brighter than the adjacent background in the wavebands corresponding to the

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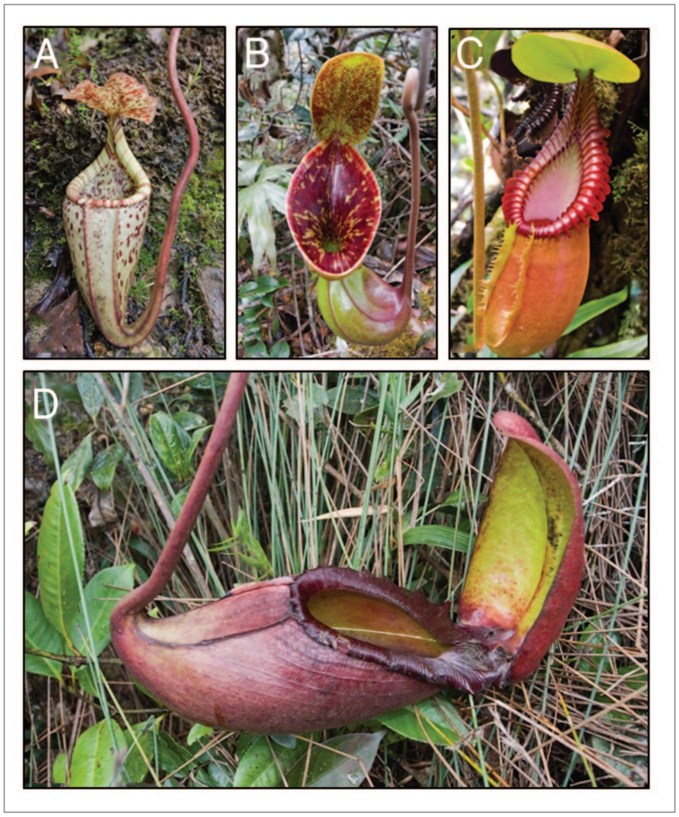

Figure 1. Pitchers of species used in the study. A) *Nepenthes burbidgeae*. B) *Nepenthes lowii*. C) *Nepenthes macrophylla*. D) *Nepenthes rajah*.

sensitivity maxima of the two types of cone present in the retina of the target animal.[13,14] However, within this group, there were differences. *N. rajah* showed a contrast peak (*ca.* 560 nm), which corresponded almost exactly with the sensitivity region of the L cones in the tree shrew retina; *N. macrophylla* showed a similar pattern, although the contrast peak occurred at a slightly shorter wavelength (*ca.* 535 nm) than the L cone sensitivity maximum. *N. lowii* showed the least tight "fit" between contrast peak and L cone sensitivity, with the former occurring at a longer wavelength (*ca.* 610 nm). For the waveband corresponding to the S cones, all three species showed positive contrast values, although to a lesser degree than for the L cone waveband, i.e., the contrast signal was more highly tuned to the latter, in all three species. In terms of overall "tuning" of the visual signal to the visual sensitivity of tree shrews, the tightest tuning was exhibited by *N. rajah*, while *N. macrophylla* and *N. lowii* showed less tight tuning, and the insectivorous *N. burbidgeae* exhibited none at all (**Fig. 3**).

It is important to note that tree shrews are not the only mammalian visitors to one of the study species: summit rats (*Rattus baluensis*) have been shown to feed at, and defecate in, pitchers of *N. rajah*.[11,18] Further, it has been demonstrated that *N. rajah* pitchers produce volatiles that attract small mammals.[11] The possible role of volatiles in attraction of tree shrews to *N. lowii* and *N. macrophylla* pitchers awaits further investigation.

Materials and Methods

Fieldwork was undertaken in Sabah, Borneo in February 2011 at the Mesilau landslip, Mount Kinabalu [06° 03' N, 116° 36' E, 2050 m asl; *N. rajah* (n = 31 plants) and *N. burbidgeae* (n = 10)], and Mount Trus Madi [05° 33' N, 116° 31' E, 2500 m asl; *N. macrophylla* (n = 28) and *N. lowii* (n = 33)]. One mature, fully-opened pitcher was selected per plant. A reflectance scan, *ca.* 0.3 cm^2 in area, was then taken of the underside of the lid, and an adjacent area of peristome (*N. rajah*, *N. macrophylla* and *N. burbidgeae*) or the flared inner wall at the upper part of the pitcher (*N. lowii*). Scans were taken under natural light at 1-nm intervals from 400 to 700 nm using a spectroradiometer (USB4000, Ocean Optics Inc.) and fiber optic probe (BIF200-UV/VIS, Ocean Optics). A second scan was then taken of a Spectralon® white standard (WS-1, Ocean Optics). The dark signal was subtracted from each reflectance measurement, after which the reflectance values were divided by the white standard values to provide a normalized reflectance index. Color contrast (C) between lid and peristome (inner wall for *N. lowii*) was calculated at 1-nm intervals across the scanned waveband as follows:

$$C = (I_l - I_p) / I_p$$

where I_l and I_p are the reflected radiant flux values (W m^{-2} nm^{-1}) for lid and peristome (inner wall for *N. lowii*), respectively, at a given wavelength.[15] Tree shrew retinas contain two types of cone cell. The S cones ("Short waveband") are sensitive to blue light (428 to 445 nm), whereas the L cones ("Long waveband") are sensitive to green light (549 to 558 nm).[13,14] No data exist for visual sensitivity in *T. montana*; available data from a congener, *Tupaia belangeri*, were used as a proxy. Area under curve (AUC) was calculated for each contrast curve in the wavebands corresponding to the sensitivity regions of tree shrew S and L cones, using the trapezoidal rule.[16] The sensitivity waveband of the S cones is 18 nm in width (428–445), whereas that of the L cones is 10 nm (549–558). Therefore, to allow meaningful comparison between the two, a correction factor of 1.8 was applied to all AUC values for the latter. Data were analyzed using SigmaPlot v.12 (Systat Software Inc.). AUC data were tested for homoscedasticity and normality using Levene's and Kolmogorov-Smirnov/Lilliefors tests, respectively. As transformation failed to make the data meet the assumptions of a parametric test, the nonparametric Kruskal-Wallis test was used in lieu of one-way ANOVA.[17] This was followed by all-pairwise comparisons using Dunn's test.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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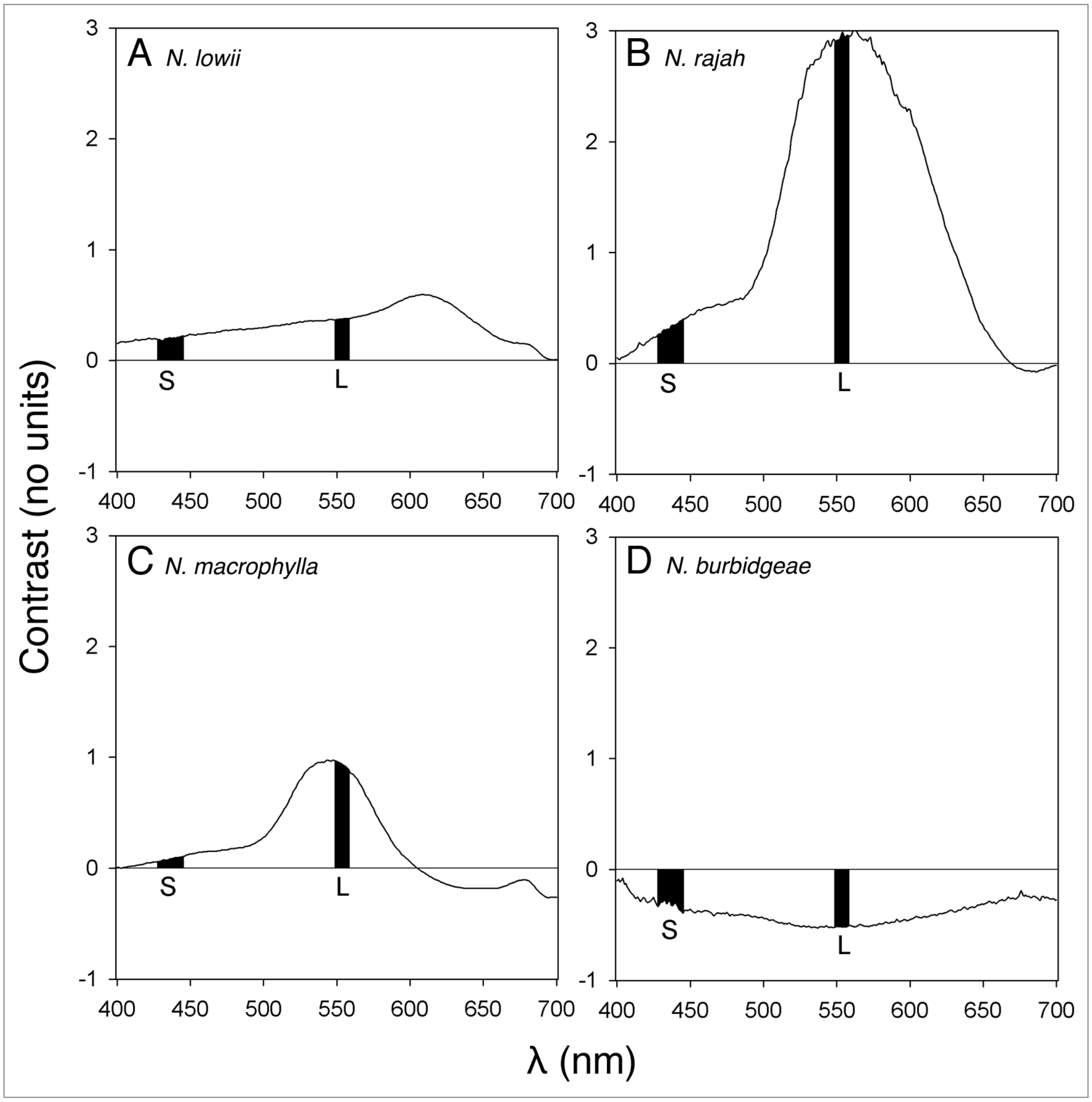

Figure 2. Color contrast patterns (no units) across the waveband 400–700 nm, between pitcher lid and peristome (pitcher lid and flared inner section of pitcher body for *Nepenthes lowii*). (A) *Nepenthes lowii* (n = 33). (B) *Nepenthes rajah* (n = 31). (C) *Nepenthes macrophylla* (n = 28). (D) *Nepenthes burbidgeae* (n = 10). The black bars denote the visual sensitivity regions of the S and L cones (sensitive to blue and green light, respectively) of tree shrew retinas.

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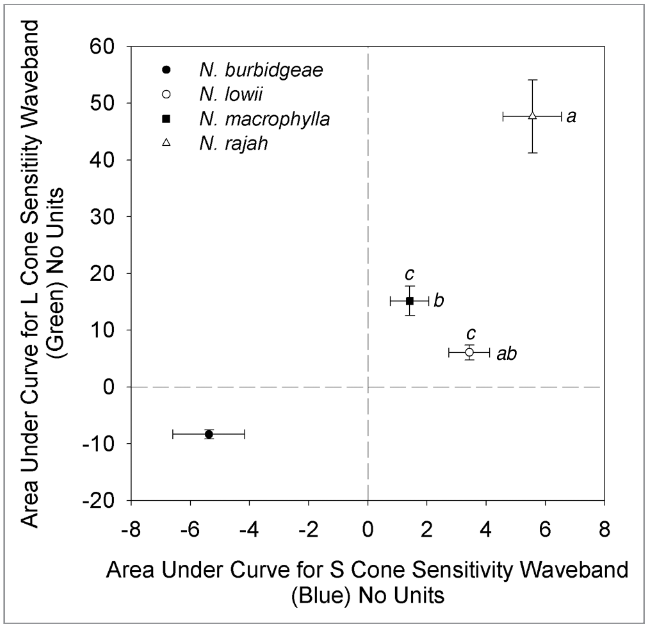

Figure 3. Area under curve (AUC; no units) for color contrast between pitcher lid and peristome (pitcher lid and flared inner section of pitcher body for *Nepenthes lowii*) in wavebands corresponding to visual sensitivity regions of tree shrew S cones (sensitive to blue light; x axis) and L cones (sensitive to green light; y axis). Closed circle, *Nepenthes burbidgeae* (n = 10); open circle, *Nepenthes lowii* (n = 33); closed square, *Nepenthes macrophylla* (n = 28); open triangle, *Nepenthes rajah* (n = 31). Points represent mean values, bars represent 1 SE. Species sharing the same lower case italicized letter are not statistically different (p > 0.05, Dunn's test) with respect to L cones (above vertical bars), or S cones (to right of horizontal bars), respectively.

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