Nectar, not colour, may lure insects to their death

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We experimentally demonstrate in the field that prey of the carnivorous plant *Sarracenia purpurea* are attracted to sugar, not to colour. Prey capture (either all taxa summed or individual common taxa considered separately) was not associated with total red area or patterning on pitchers of living pitcher plants. We separated effects of nectar availability and coloration using painted 'pseudopitchers', half of which were coated with sugar solution. Unsugared pseudopitchers captured virtually no prey, whereas pseudopitchers with sugar solution captured the same amount of prey as living pitchers. In contrast to a recent study that associated red coloration with prey capture but that lacked controls for nectar availability, we infer that nectar, not colour, is the primary means by which pitcher plants attract prey.

1. INTRODUCTION

Flowers use visual and chemical signals to attract insects for seed dispersal and pollination (Chittka & Kevan 2005). Carnivorous plants are hypothesized to use visual signals to attract prey (Juniper *et al.* 1989), but few studies support this hypothesis (Joel *et al.* 1985; Gloßner 1992; Cresswell 1993; Moran 1996; Schaefer & Ruxton 2008). Recently, Schaefer & Ruxton (2008) reported that prey capture by *Nepenthes ventricosa* increased with increasing red coloration on pitchers, but only flies were captured in sufficient abundance to permit statistical comparisons. However, the results of Schaefer & Ruxton (2008) may not be applicable to all pitcher plants. First, Schaefer & Ruxton conducted their experiment in Germany, far outside the native range (southeast Asia) of *Nepenthes*. Second, in its native habitat, *Nepenthes* captures predominantly ants and termites (Moran *et al.* 2001; Merbach *et al.* 2002), not flies. Finally, all pitcher plants (approx. 110 Nepenthaceae, Sarraceniaceae and Cephalotaceae species) possess extrafloral nectaries (Juniper *et al.* 1989; Vogel 1998), and extrafloral nectar production has been measured in all pitcher plants examined to date (Macbride 1818; Cresswell 1993; Dress *et al.* 1997; Deppe *et al.* 2000; Green & Horner 2007; Bauer *et al.* 2008; Bhattarai & Horner 2009). Insects, especially ants, forage for this nectar (Cresswell 1993; Deppe *et al.* 2000), and nectar production increases prey capture rates by decreasing

friction on pitcher lips (Macbride 1818; Bauer *et al.* 2008). Thus, links between pitcher coloration and nectar production on prey capture remain controversial (Green & Horner 2007; Bhattarai & Horner 2009).

We experimentally examined effects of red coloration and nectar availability (as sugar solution) in the field on prey capture by the pitcher plant *Sarracenia purpurea*. We first assessed prey capture by living pitchers ranging in colour from mostly green to mostly red. We then measured independent and interactive effects of colour and nectar using 'pseudopitchers' (50 ml painted tubes) with and without added sugar solution. We hypothesized that both real pitchers and pseudopitchers with nectar would capture more prey than pseudopitchers without nectar. We further predicted that because ants have bichromatic vision (Briscoe & Chittka 2001), real or pseudopitchers with contrasting venation (e.g. red veins on a green background) would capture significantly more ants than nearly monochromatic pitchers.

2. MATERIAL AND METHODS

We conducted this study during mid-summer, when insect abundance and prey capture are highest in New England (Fish & Hall 1978). In July 2008, we randomly identified 25 pitchers, each on a different *S. purpurea* plant, at Tom Swamp, a bog in Petersham, MA, USA (42°30′ N 72°12′ W). On 3 July 2008, we suctioned out all liquid and prey carcasses from pitchers (methods of Nastase *et al.* 1991), rinsed them and refilled them with distilled water. Three days later, pitcher contents were collected, prey counted and identified. The experiment was repeated on 11 and 14 July 2008. On 17 July 2008, pitchers were harvested, cleaned, flattened and photographed (Canon S3-IS digital camera) to determine each pitcher's area, proportional red colour and perimeter of red venation (using SigmaScan Pro 5.0; SPSS, Chicago, IL). Spectral reflectance (375–800 nm) of pitchers was measured around the pitcher and across its hood (Ocean Optics S2000 fiber-optic spectrometer, ISP-REF integrating sphere and OOIColor/OOIIrrad 2.05.00 software; Ocean Optics, Dunedin, FL, USA). Reference scans were made using Kodak (Rochester, NY, USA) black and white reference cards.

Seventy pseudopitchers (painted 50 ml plastic centrifuge tubes (BD Biosciences, San Jose, CA, USA)) were used to separate effects of colour and nectar on prey capture. Seven sets of 10 were assigned different colour treatments. One set was painted entirely green using 'Fresh Foliage' acrylic paint (Plaid Enterprises, Norcross, GA, USA); one set was painted entirely red (Plaid Enterprises 'Burgundy'); four sets were first painted green, then red 'veins' were added to produce 6–62% red colour, the range found on real pitchers; and one set of 10 was unpainted controls. Peak reflectances of the green and red paints were the same as those of pitchers (figure 1). While other surface characteristics besides colour (e.g. texture, glossiness, surface geometry; Motoyoshi *et al.* 2007) also may attract insects, we could not control for them here. To test for interactions between nectar colour in attracting prey, four lines of Karo Corn Syrup (ACH Food Companies, Memphis, TN, USA), cooked until thick, were streaked at random locations on one-half of each set of pseudopitchers. Like all plant nectars (Baker & Baker 1981), corn syrup includes a mixture of fructose and glucose. To closely resemble pitcher plants, pseudopitchers were embedded at an angle in the bog surface with more than 50 per cent of the tube visible above the *Sphagnum*. Psuedopitchers were located within 10 m of plants for which we assessed prey capture. Fifteen millilitres of 50 per cent ethanol was put inside each tube; trapped insects were collected after 48 h, counted and identified. Ethanol, with its lower surface tension than water, is widely used in insect traps and was used to limit escape of prey from pseudopitchers. Little evaporation of ethanol occurred over 48 h trapping periods. The entire experiment was repeated twice over a three-week span of time.

Data were analysed using robust general linear models (Maronna *et al.* 2006) to minimize the influence of extreme (outlier) points on the results. We used the function `glmrob` in the `robustbase`
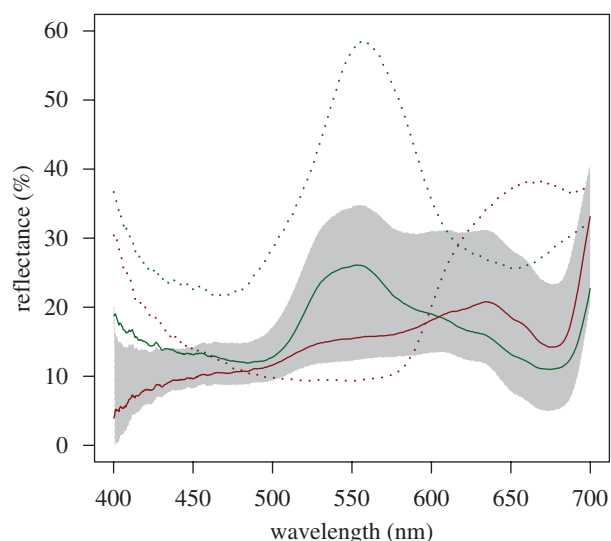
Figure 1. Spectral reflectance of living green and red *Sarracenia purpurea* pitchers (solid lines); 95 per cent confidence region of spectral reflectance of all 25 living pitchers (grey area) and reflectance of green and red paint (dotted lines).

library (v. 0.4-3) of the R software package (v. 2.7.2; R Development Core Team 2007). Because the number of prey captured takes on integer values, we used a Poisson link function when fitting the models.

3. RESULTS

Pitcher plants ranged from mostly green to mostly red (figure 2). Real plants and pseudopitchers with nectar caught nearly identical numbers of prey (357 and 344 arthropods, respectively), while pseudopitchers without nectar caught far fewer (62 arthropods). Ants predominated in prey of real plants (60%) and pseudopitchers with nectar (92%), with the remainder distributed among flies (27% versus 1%), springtails (8% versus 6%) and spiders (5% versus 1%). Pseudopitchers without nectar caught mostly springtails (71%) and a few ants (10%), flies (14%) and spiders (5%). As ants and flies have similar average dry mass (300 μg), and ants have greater nitrogen concentration than flies (14% versus 10%; Wakefield *et al.* 2005), ants provide most of the prey-derived nutrients to these pitcher plants.

Statistical results were identical whether we analysed all prey or only ants; prey capture rates averaged or summed across the three experimental runs; or proportional red areas or perimeters of red veins. Thus, we report analyses only for ants and flies captured by pitchers or pseudopitchers summed over all experimental runs as a function of proportional red area. The complete dataset is available at http://harvardforest.fas.harvard.edu/data/p11/hf114/hf114.html.

The number of prey captured was unrelated to the proportion of visible red area (figure 2) on real pitchers (ants: $p = 0.17$; flies: $p = 0.07$) or pseudopitchers with or without nectar (ants: $p = 0.21$; flies: $p = 0.11$). Presence of artificial nectar significantly increased ant capture ($p = 0.0003$) but not fly capture ($p = 0.99$) by pseudopitchers (figure 2). The relationships between red coloration and number of ants captured by

pseudopitchers or by real pitchers were virtually identical (figure 2). There was no significant 'paint effect'. Unpainted pseudopitchers without artificial nectar captured neither ants nor flies and unpainted pseudopitchers with artificial nectar captured the same amount of ants (but only one fly) as either real pitchers or painted pseudopitchers with artificial nectar ($p = 0.66$, ANOVA; figure 2).

4. DISCUSSION

Our experimental results illustrate that ants are attracted to sugar, not to colour, which suggests that pitcher plants use nectar, not colour, to attract prey. These results highlight the importance of studying prey capture in the field, of distinguishing among multiple possible attractants, and of employing experimental controls. While we do not suggest that nectar is the *only* attractant used by all carnivorous plants, and other factors such as volatile odours of decomposing prey may also attract prey (e.g. Bhattarai & Horner 2009), all pitcher plants examined to date produce copious nectar and primarily capture ants (Ellison & Gotelli 2009) that forage widely for carbohydrates (Bestelmeyer *et al.* 2000). By removing prey contents and rinsing pitchers with water before we measured prey capture, we minimized the potential contribution of decomposing prey odours as attractants; the low numbers of flies caught support this assertion. We recognize that ethanol in pseudopitchers could be an alternative attractant, but the lack of significant differences in prey capture between real pitchers and sugared pseudopitchers, and the strong differences in prey capture between pseudopitchers with and without sugar solution (but both including ethanol), suggests that the collecting fluid (water in pitchers versus ethanol) played a minor role in determining prey capture rates.

Many carnivorous plants exhibit contrasting patterns and nectar guides when photographed under ultraviolet light (Gloßner 1992), but there is no evidence that potential prey in the field actually see these patterns. Ants, the primary prey of all pitcher plants (Ellison & Gotelli 2009), see ultraviolet and green (Briscoe & Chittka 2001) and would see red as grey, weakly contrasting with the pitcher's green base colour. Such a weak contrast is unlikely to be a strong attractor, and in fact we found no association between red coloration and ant or fly capture by living pitchers. Because extrafloral nectaries are associated with pitchers' lips, veins (Płachno 2007) or ultraviolet patterns (Moran 1996), the role of colour and nectar in prey attraction can be confounded easily. Only by experimentally separating these two attractors using pseudopitchers could we identify the primacy of nectar in attracting prey. Other experiments have shown colour to be incidental to prey capture by *Sarracenia alata* (Green & Horner 2007; Bhattarai & Horner 2009). Seen in the light of all these findings, the variability in pitcher plant colour that puzzled Schaefer & Ruxton (2008) is not at all puzzling. No adaptive value for colour need be asserted or sought in the sweet shop of horrors that is *S. purpurea*.
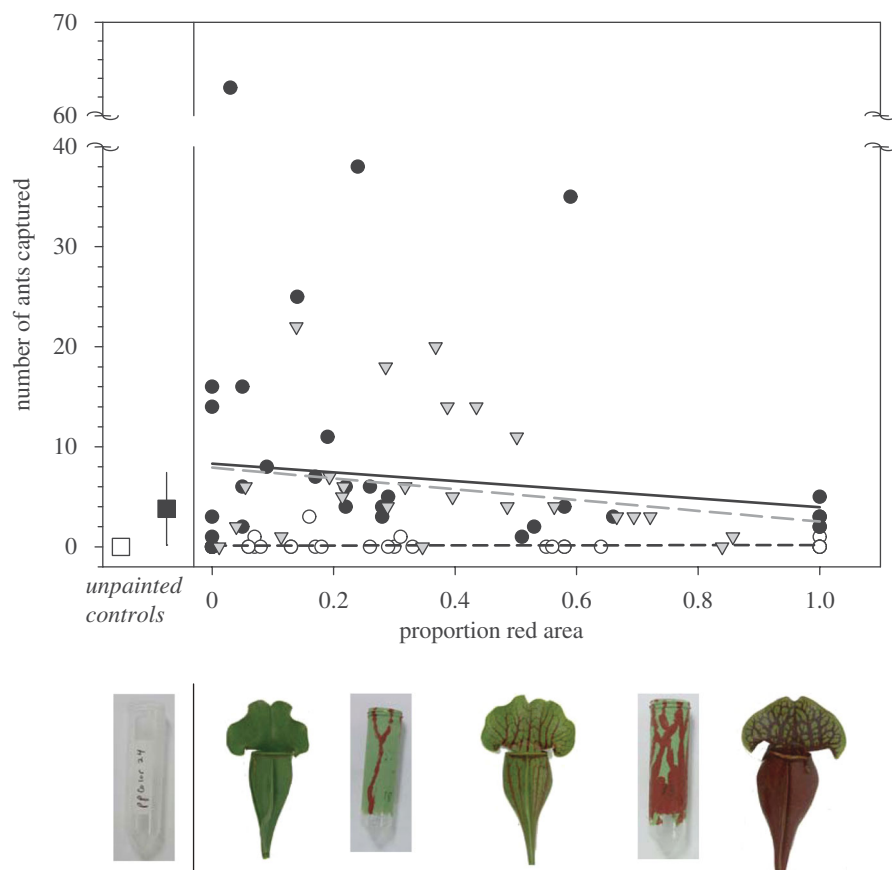
Figure 2. Relationship between ants captured and red area on 25 live *Sarracenia purpurea* pitchers (grey triangles and grey dashed line), 35 pseudopitchers with artificial nectar (filled circles and black solid line) and 35 pseudopitchers without artificial nectar (open circles and black dashed line) (examples shown below *x*-axis). Left of the horizontal line are the numbers (±1 s.d.) of ants captured by five unpainted controls with (filled squares) and without (open squares) artificial nectar.

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