Fatal attraction: carnivorous plants roll out the red carpet to lure insects

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We provide the first experimental test of the hypothesis that the coloration of carnivorous plants can act as a signal to lure insects and thus enhance capture rates. An experimental approach was needed to separate effects of the visual appearance of plants from those of traits that may correlate with appearance and also affect capture rates. We compared insect capture rates of pitcher plants with artificially coloured red and green pitchers in a paired design, and found that plants with red pitchers captured significantly more flying insects. Thus, we present the first experimental evidence of visual signalling in carnivorous plants. Further, it has previously been suggested that carnivorous plants use contrasting stripes or UV marks on their pitchers to lure insects; our results emphasize that insect traps do not need to sport contrasting colours to be attractive; it might be sufficient to be different from the background.

Keywords: plant–animal interactions; visual signalling; insect vision; anthocyanins; traps

1. INTRODUCTION

The multiple, independent evolution of carnivory in plants is considered an adaptation to nutrient-poor habitats (Ellison & Gotelli 2001). In these habitats, the availability of animal prey is a key factor for plant fitness as it enhances biomass, flower and seed production (Moran & Moran 1998). To increase capture rates, plants might use a variety of deceiving signals to lure insects. While many carnivorous plants have nectaries and use olfactory signals, it has repeatedly been suggested that they also use visual signals to attract prey (Joel *et al.* 1985; Moran *et al.* 1999; Biesmeijer *et al.* 2005). This conjecture has, however, not been tested experimentally. Hence, and in contrast to the immense progress in understanding plant visual signals that are used for pollination and seed dispersal (Chittka *et al.* 2001; Schaefer *et al.* 2004), the design and efficiency of visual signals in carnivorous plants are poorly known.

The potential role of visual signalling in the remarkably diverse group of carnivorous plants is intriguing as the traps of all species examined in a large interspecific comparison sport visual characters that are considered to be attractive to insects (Biesmeijer *et al.* 2005). These include UV reflection and strong chromatic contrasts of radiating stripes on the traps (Joel *et al.* 1985; Biesmeijer *et al.* 2005). Remarkably, many unrelated plants sport red coloration, particularly on the structures used to capture prey. For example, pitcher plants from the genus *Nepenthes* sport no or very little UV reflectance but large inter- and intraspecific variation (from green to red) in the coloration of pitchers (Joel *et al.* 1985; Moran *et al.* 1999). Although the human eye perceives strong contrasts between red colours and the generally green background of most plants, red is considered dull or cryptic to most insects since their colour vision does not extend as far into the red as that of humans (Chittka *et al.* 2001). However, red colours are not invisible to insects (Chittka & Waser 1997), and the capture rates of *Sarracenia* pitcher plants correlated with the amount of red venation (Cresswell 1993; Newell & Nastase 1998). However, given that red veins are lined with nectaries (Cresswell 1993) and red is often not a strongly contrasting colour to insects, it is uncertain whether the nectaries or the red colour enhanced capture rates in these studies.

From the perspective of plant–animal communication, the development of red coloration on insect traps might be non-adaptive since the expression of anthocyanins, the pigments producing red hues, is often related to stress responses in plants (Schaefer & Rolshausen 2006). In particular, foliar anthocyanin production is often related to N and P deficiencies (Steyn *et al.* 2002). Consistent with this view, prey-deprived individuals of *Nepenthes rafflesiana* were characterized by smaller and fewer pitchers and by increased anthocyanin production (Moran & Moran 1998).

To test the adaptive value of red coloration in attracting prey, we conducted an experiment comparing capture rates in artificially coloured red and green pitchers. If red coloration is primarily a stress response, we expect no difference in the capture rates of individuals with red or green pitchers. In contrast, if red coloration is a visual signal functioning to lure insects, we expect that red pitchers would capture more insects than green ones do.

2. MATERIAL AND METHODS

We bought 20 same-aged *Nepenthes ventricosa* plants from a commercial supplier. In this species, originating from Southeast Asia, pitchers differed in their coloration from red to green. To exclude the effects of correlated selection, i.e. that traits associated with differential coloration (e.g. olfactory cues) may bias prey capture, we coloured pitchers artificially either completely red (experimental group) or completely green (control group) using a mixture of opaque white (Milan no. 306), yellow (Eberhard Faber no. 8801-1), and green and red paints (Buntlack, Obi). Because both colours consisted of a mix of acrylic and tempera paints, we minimized biases caused by different odours associated with the paint (albeit not entirely eliminating them). Therefore, if insects reacted differently to the colours, we assumed that this is primarily due to visual differences. Indeed, in a previous experiment, we used similar colours and found that aphids did *not* discriminate between these artificial colours and natural red and green plant coloration (Schaefer & Rolshausen 2007).

To measure natural and artificial pitcher colours, we used an Avantes 2048 spectrometer (Avantes, Eerbeek, The Netherlands) that was connected with a coaxial fibre cable to a Deuterium–Halogen lamp (Ava-lamp DHS) as a standardized light source.
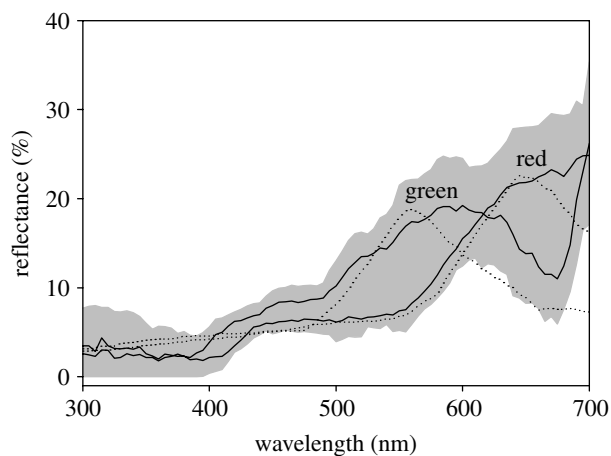
Figure 1. Mean reflectance spectra of *N. ventricosa* pitchers. The mean reflectance spectra of natural red and green pitchers are illustrated with solid lines, those of artificial colours with dotted lines. The shaded area represents the standard deviation of natural pitcher coloration.

Reflectance of the natural colours of 20 pitchers and of 10 artificial green and 10 artificial red pitchers was measured relative to a standard white reference tile (diffuse PTFE; WS-2). The probe was mounted inside a matt black plastic tube to exclude ambient light (Schaefer *et al.* 2007). The angle of illumination and reflection was fixed at 45°. Spectra were processed with AVASOFT v. 6.1 software and calculated in intervals of 5 nm from 300 to 700 nm. The artificial colours matched the natural colour variation found in *N. ventricosa* (figure 1).

We categorized plants into two groups according to the number of active pitchers. One group contained plants with one or two pitchers; the other group included plants with three to five pitchers. From each group, we randomly assigned plant individuals to the experimental (red) or control (green) group. There was no difference in the number of pitchers per plant between groups (mean±s.e.: experimental group: 2.9 ± 0.37 pitchers; control group 2.9 ± 0.27 pitchers; *t*-test, $n = 20$, $t = 0.0$, $p > 0.99$). It is well known that differences in the microhabitats might influence capture rates (Cresswell 1993). To minimize such effects, we positioned one red and one green plant in pairs at 40 cm distance from each other outside the Institute of Biology in Freiburg. This site was characterized by several freshwater pools; pairs of plants were placed at 4 m distances from other pairs and from ponds. We randomly determined the position of plants within a pair.

At the start of the experiment, we inspected the pitchers without removing the cap of the pitchers. We only found Collembola (in almost every plant). After 7 days since the start of the experiment, we removed the cap of the pitcher to examine the entire interior and counted all insects. Some of these insects might not have been visible at the start of the experiment. We therefore continued the experiment until day 15 when we extracted insects with forceps and identified the major taxonomic groups of prey. To use a conservative figure, we subtracted the number of insects caught on day 7 from the total number of insects on day 15 to obtain the number of insects that were caught during the last 8 days of the experiment. We used this number, which excluded all Collembola, to test for differences between groups with paired two-sided *t*-tests as data were normally distributed.

3. RESULTS

We found a total of 133 prey items in the pitchers. Fifty of these were caught during the last 8 days of the experiment with a mean capture rate of 2.5 (± 0.4 s.e.) prey items per plant. These prey items consisted of Diptera (58%), Homoptera and Acari (14% each), Hymenoptera (10%, mainly Symphyta which only occurred in red pitchers) and Araneae (4%). Only Diptera were common enough to test for differences in capture rates. Artificially coloured red individuals caught more Diptera (paired *t*-test,
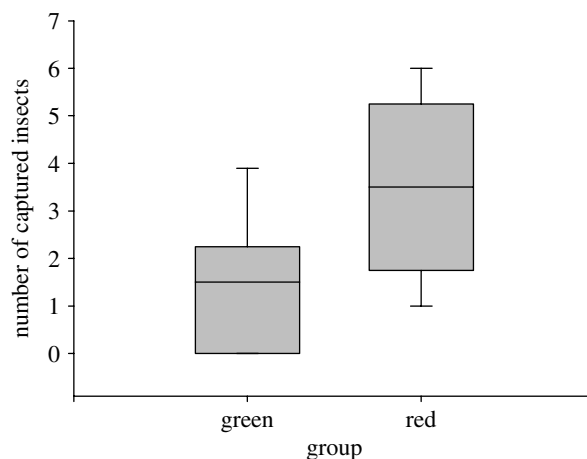

Figure 2. The number of insects caught per plant with either red or green pitchers over the course of 8 days. Illustrated are means, interquartiles, and 10th and 90th percentiles as whiskers.

$t = 3.25$, $p < 0.01$) and a higher overall number of insects than artificially coloured green individuals (paired *t*-test, $t = 2.98$, $p < 0.01$; figure 2).

4. DISCUSSION

Our experiment shows that carnivorous plants can increase their foraging success using visual signals. More specifically, we show that red coloration can be an adaptive trait for carnivorous plants as it increased the overall capture rates of insects, particularly that of Diptera. These results extend our understanding of the evolutionary ecology of carnivorous plants for two reasons. First, we present the first experimental evidence of visual signalling in carnivorous plants. Second, it has previously been suggested, based on correlations between capture rates and pitcher coloration, that carnivorous plants use UV signals or contrasting stripes to lure insects (Joel *et al.* 1985; Moran *et al.* 1999; Biesmeijer *et al.* 2005). The higher capture rates of unicoloured red pitchers in our experiment thus extend the array of potential visual signals that carnivorous plants might use. Our results emphasize that insect traps do not need to sport contrasting colours to be attractive; it might be sufficient to be different from the background.

The higher efficiency of red pitchers might be surprising at first glance, since it contrasts with the traditional belief that red coloration is an inefficient signal to insects. While humans can see colour farther into the red than most insects, some insects such as Symphyta also possess photoreceptors with peak sensitivity in the red. More importantly, red colours are not invisible to insects (e.g. Diptera) lacking such photoreceptors (Chittka & Waser 1997). Artificial red objects are even used for pest control owing to their success in luring fruit flies (Cytrynowics *et al.* 1982; Katsoyannos & Kouloussis 2001), which is consistent with our results of increased capture rates of Diptera by red pitchers. Even bees that cannot discriminate red colours based on differences in hue are able to distinguish them based on differences in luminance, i.e. the intensity of reflected red light. Likewise, bees might distinguish red colours produced by

anthocyanins from other colours based on differences in the blue or green part of the spectrum (Chittka & Waser 1997). In our experiment, the reflectance of natural and artificial red pitchers differs from that of natural and artificial green pitchers both in the green (520–570 nm) and in the red parts (greater than 610 nm) of the spectrum. It thus remains open whether insects perceived red pitchers as different because they reflected more red light or less green light and had a lower overall luminance. In both the cases, red pitchers are more different (i.e. red and dark) from the background of green foliage than green pitchers. We propose that red pitchers are more effective because they represent, in addition to the olfactory signals of nectaries, a visual stimulus that might direct insects to the trap.

We conclude that the multiple, independent evolution of carnivory in plants (with more than 600 species described to date) presents an ideal, but hitherto overlooked, model system to analyse signalling in plant–animal interactions. Elucidating the mechanisms that plants employ to capture insects will greatly enhance our understanding of the evolutionary ecology of carnivory. Moreover, in light of the increased capture rates of red pitchers the variability of pitcher coloration in *N. ventricosa* is puzzling. Carnivorous plants might thus be ideal to test hypotheses on insect vision as well as on the proximate mechanisms of plant coloration.

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