Variation in colour signals among *Sarracenia* pitcher plants and the potential role of areoles in the attraction of flying Hymenoptera

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Abstract:

Sarracenia insectivorous plants show a diversity of visual features in their pitchers but their perception by insects and their role in attraction, have received little attention. They also vary in prey composition, with some species trapping more flying Hymenoptera, such as bees. To test the hypothesis of a link between visual signal variability and prey segregation ability, and to identify which signal could attract flying Hymenoptera, we characterised, the colour patterns of 32 pitchers belonging to four taxa, modelled their perception by flying Hymenoptera, and examined the prey they trapped. The pitchers of the four taxa differed in colour patterns, with notably two long-leaved taxa displaying clear areoles, which contrasted strongly in colour and brightness with the vegetative background and with other pitcher areas in the eyes of flying Hymenoptera. These taxa trapped high proportion of flying hymenoptera. This suggests that contrasting areoles may act as a visual lure for flying Hymenoptera, making plants particularly visible to these insects. Prey capture also differed according to pitcher stage, morphology, season and visual characteristics. Further studies on prey visitation are needed to better understand the link between prey capture and attraction feature.

ADDITIONAL KEYWORDS: brightness contrast-carnivorous plant-colour contrast-colour perception modelling-insect vision-plant insect interaction-prey attraction-prey capture-trap morphology-visual signals

INTRODUCTION

Angiosperms, the most diverse group of plants, underwent explosive radiation during the Cretaceous in a coevolutionary process with insect pollinators (Grimaldi, 1999; Van der Niet & Johnson, 2012). These flowering plants have thus evolved different combinations of nectar rewards, and sensory signals, such as visual and olfactory cues, which attract a diversity of pollinators (Kevan & Baker, 1983; Dobson, 1994; Lunau & Maier, 1995; Chittka & Raine, 2006). Visual and olfactory signals often operate synergistically in attracting pollinators (Raguso, 2008). Some plants, such as orchid species, have even evolved 'dishonest' signals that deceive pollinators, by exploiting their sensory biases (Schaefer & Ruxton, 2009) and/or by using deceit mimicry of nectar or sexual mate (Dafni, 1984; Schiestl, 2010), without any reward. Carnivorous plants are particularly efficient at exploiting the sensory biases of generalist insect-pollinators because their leaves often resemble flowers in their visual and olfactory traits such as colour patterns (Moran *et al.*, 1999; Schaefer & Ruxton, 2008; Moran *et al.*, 2012b), even in the UV range (Joel *et al.*, 1985; Moran, 1996; Kurup *et al.*, 2013; Golos, 2020), nectar guides (Dress *et al.*, 1997; Bennett & Ellison, 2009), and perfume (Jaffé *et al.*, 1995; Jürgens *et al.*, 2009; Di Giusto *et al.*, 2010). The most adorned plants regarding these floral features attract a wide range of flower-visiting insects (Joel, 1988; Di Giusto *et al.*, 2010). Part of the attracted insects are then trapped with an arsenal of leaf traits, as diverse as snap-traps (Forterre *et al.*, 2005), viscoelastic mucilage (Gaume & Forterre, 2007; Adlassnig *et al.*, 2010) and slippery surfaces (Gaume *et al.*, 2004; Bauer *et al.*, 2013). They are digested by endogenous and/or exogenous proteolytic enzymes, and ultimately supplement carnivorous plants with essential nutrients, which often lack in the soil where they grow (Ellison & Adamec, 2018).

One group of carnivorous plants, the so-called pitcher plants, includes the well-known Sarraceniaceae from the Americas and the Nepenthaceae from Southeast Asia. These plants exhibit several morphological and physiological adaptations, such as pitcher-like leaves of different sizes and shapes (Cresswell, 1993; Bhattarai & Horner, 2009; Gaume *et al.*, 2016), different attractive visual or olfactory signals (Moran *et al.*, 1999; Di Giusto *et al.*, 2010) and capture devices (Bonhomme *et al.*, 2011). However, it is difficult to

disentangle the contribution of the different features since they are often combined to form whole trapping syndromes, which target specific guilds of animals or characterise specific diets (Pavlovič *et al.*, 2007; Gaume *et al.*, 2016).

Compared to olfactory cues (Jaffé *et al.*, 1995; Jürgens *et al.*, 2009; Di Giusto *et al.*, 2010), the visual cues of pitcher plants as perceived by insects have received no attention to our knowledge and their role in attraction is the subject of controversy. For instance, pitcher red colouration has been proposed to play an important role in prey attraction by some authors (Newell & Nastase, 1998; Schaefer & Ruxton, 2008) but not by others (Green & Horner, 2007). In an experiment with *Sarracenia purpurea* (Linnaeus), extrafloral nectar, often associated to red colouration in carnivorous plants (Bennett & Ellison, 2009; Gaume *et al.*, 2016), was shown to account for prey attraction, rather than the red colouration itself (Bennett & Ellison, 2009). Yet, colour signals should not be overlooked since they operate at larger distances than nectar and are thus needed to attract flying insects. Moreover, many pitcher plants sport a large colour diversity with contrasting patterns and not just bicolor patterns with nuances of green and red (Moran *et al.*, 1999), which suggests that visual signals are important components of the attractive system in these plants. For instance, clear white areoles present in some carnivorous plants (Schnell, 2002; McPherson, 2006) have been shown to function as light lures helping insect capture in *Sarracenia minor* (Walter) (McGregor *et al.*, 2016), and in the Sumatran *Nepenthes aristolochioides* (Jebb & Cheek) (Moran *et al.*, 2012a). Yet, according to Schaefer & Ruxton (2014), these areoles only play a role in attracting insects to pitcher plants, but have no role in prey capture. The role, if any, of clear areoles in attracting prey – a prerequisite for capture in carnivorous plants – remains to be clarified. As visual contrasts are important features of attraction in pollinating systems (Spaethe *et al.*, 2001), we hypothesise that clear areoles are better perceived by flying prey since they are more conspicuous against the green vegetation background and the remaining parts of the plant. We test this hypothesis by exploring the variation in colouration in four *Sarracenia* pitcher plants of different pitcher morphology and visual characteristics, especially regarding the areoles, and by modelling how these colours are perceived by flying Hymenoptera. We secondly investigated whether clear areoles play a role in attracting

this kind of insects to the pitcher by examining the relationship between the composition in prey trapped by pitchers and the visual signals they displayed, as seen by flying Hymenoptera.

MATERIAL AND METHODS

PLANT TAXA AND GROWING CONDITIONS

We considered four *Sarracenia* taxa differing in the visual aspect and leaf shape (Fig. 1). Two were natural species: *S. purpurea* subsp. *venosa* (Rafinesque) and *S.* x *mitchelliana* = *S. purpurea* x *S. leucophylla* hybrid. *S. purpurea*, which does not bear clear areoles, mostly traps ants and comparatively few flying hymenopterans (Cresswell, 1991; Heard, 1998; Newell & Nastase, 1998; Bhattarai & Horner, 2009). The two others were horticultural hybrids with clear areoles: *S.* x Juthatip soper = (*S. leucophylla* x *S. purpurea*) x *S. leucophylla* and *S.* x *leucophylla* = *S. leucophylla* x *S.* x Juthatip soper. The long-leaved *Sarracenia leucophylla* (Rafinesque) characterized by clear areoles has been observed to trap a large amount of bees in the field (Gibson, 1983a) and its horticultural hybrids have been observed to trap wasps and hornets in large amounts (Meurgey & Perrocheau, 2015). Plants were grown in polyculture in a 6 m² container filled with a peat mixture consisting of 2/3 blond peat and 1/3 sand on a background of clay balls. Containers were placed outdoors in the experimental station of AMAP (Montpellier, France) in a sunny place. They were regularly watered with demineralized water to match their natural moist and mineral-poor environment.

PITCHER MORPHOLOGICAL MEASUREMENTS AND PREY CAPTURE ANALYSIS

Pitchers were marked the day they opened and four pitcher stages were thus scored (1: young, 2: midle, 3: mature, 4: old) corresponding to 5±1, 15±3, 40±5, and 60±10 days after pitcher opening, respectively. We measured a total of 32 pitchers on 21 plants (8 pitchers per taxon). We took a first set of measurements for 24 pitchers of different stages belonging to 5-6 plants of each of the four taxa in mid-August 2016 (summer), and a second set of 8 pitchers of stage 2 belonging to two plants of each of the four taxa in October 2016

(autumn). The plants measured in autumn were different from those measured in summer. We measured pitcher length and aperture width (see Fig. 1D) before taking colour measurements (see below). These measurements were compared among taxa using one-way ANOVAs. We then collected pitcher content and preserved it in 70% ethanol. Claire Villemant identified prey individuals to the species level when possible, and at least always to the family or super-family level, depending on the stage of the digestive process. It was always possible to make the distinction between flying Hymenoptera (e. g. social or solitary bees and wasps, hornets, parasitoid wasps and sawflies) and crawling Hymenoptera (ants).

PITCHER COLOUR MEASUREMENT AND ANALYSIS

To study the visual signals of the carnivorous plants, we measured the reflectance spectra of the 32 pitchers using spectrophotometry. The light produced by a Deuterium-Halogen light source (Avalight) was conducted to the sample through an optic probe (FCR-7UV200-2-45-ME, terminated by a quartz transparent window cut at 45° guaranteeing a constant distance between the sample and the light beam). The light reflected by the sample was conducted through the probe to a spectrometer (Ocean Optics USB 2000). Measurements were taken between 300 and 700 nm, which included the range of flying Hymenoptera sensitivity, considered to be between 300 nm and 650 nm, as in honeybees (De Ibarra *et al.*, 2014). In each of the 32 pitchers, we took 4 to 5 repeated spectral measurements of the following six areas: areoles of the body, veins of the body, peristome, areoles of the operculum, veins of the operculum and tube (Fig. 1).

Sarracenia pitcher plants trap mainly bees, wasps and hornets as flying Hymenoptera (Gibson, 1983a; Farnsworth & Ellison, 2008; Meurgey & Perrocheau, 2015), and all have similar trichromatic vision (Peitsch *et al.*, 1992) with similar spectral sensitivity (Peitsch *et al.*, 1992; Briscoe & Chittka, 2001; De Ibarra *et al.*, 2014). Hence, we chose the hornet *Vespa crabro* (Linnaeus) to model how *Sarracenia* colours are perceived by flying Hymenoptera, using Vorobyev & Osorio's discriminability model (Vorobyev & Osorio, 1998) to compute the visual contrasts produced by a pitcher colour seen against a green grass background or

against another pitcher colour. We took the D65 standard illuminant as the irradiance spectrum of an open habitat, the reflectance spectrum of a colour area of a *Sarracenia* pitcher plant, the mean reflectance spectrum of green grass as a visual background (Gomez, personal data) for whenever the contrast against the background was computed. We built flying Hymenoptera photoreceptor absorptance curves by using photoreceptor peaks measured for hornet by Peitsch *et al.* (1992), that is 336, 436 and 536 nm for the S, M, and L photoreceptors respectively. We assumed a neural noise and ωi-values of 0.13, 0.06 and 0.12 for S, M, and L receptors respectively, as in Vorobyev & Osorio (1998). We assumed that brightness was detected by L receptors (Lehrer, 1993).

We first computed the colour and brightness contrasts displayed by the various pitcher areas against the mean green grass background. These contrasts are used to quantify how visible the pitcher plant stands out against its natural background, and has been used to better understand pollinator attraction to plants, as in orchids for instance (Streinzer *et al.*, 2009; Streinzer *et al.*, 2010). These authors computed colour and brightness contrasts to understand attraction at short and long distances respectively, as Hymenoptera use colour signal at close range and brightness signal to detect small objects or objets at larger distances, respectively (Giurfa *et al.*, 1996; Dyer *et al.*, 2008). Second, we computed the colour and brightness contrasts between any two pitcher areas. Aguiar *et al.* (2020) considered an equivalent of colour contrast against the background of sepals, petals and labellum of tropical orchids pollinated by bees, but also the colour contrast between each floral piece and showed the importance of intrafloral colour patterns in pollinator perception, which could serve as a guide to the flower centre. Colour patterns between pitcher areas could similarly help attracting prey to the trap (Moran, 1996). Regarding pitcher areas, areoles of the pitcher body and areoles of the operculum had similar colouration and yielded similar contrasts, they were thus pooled into a common 'areoles' category. Likewise, the veins of the pitcher body and the veins of the operculum had similar colouration and yielded similar contrasts, and were pooled into a common 'veins' category. Hence the variable pitcher area had four levels: areoles, veins, peristome, and tube. All computations were performed with the R package pavo version 2 (Maia *et al.*, 2019).

STATISTICAL ANALYSES

We analysed variation in plant colouration and variation in prey capture using a mixed model approach as it is well suited to repeated observations. All statistics were performed using the software R version 4.0.3 (R Core Team, 2020). Model assumptions were checked by plotting residuals versus fitted values.

First, we explored to which extent the colour contrast and the brightness contrast displayed by a pitcher area against the green background varied with plant taxon, pitcher stage and pitcher area. We took the colour contrast or the brightness contrast of all spectral measurements as the dependent variable, a normal error distribution, and Plant identity and Pitcher identity nested within Plant identity as random effects. We tested plant taxon, pitcher stage, season, pitcher area, and all relevant two-way and three-way interactions when possible (for season, only the simple effect could be included). Both dependent variables were square-root transformed to match residual normality.

Second, considering the contrasts between any two pitcher areas, we explored to which extent the colour contrast and the brightness contrast displayed by a pitcher area against another varied with plant taxon, pitcher stage and the pitcher area considered. We took the colour contrasts or the brightness contrasts between pitcher areas as the dependent variable, a normal error distribution, and Plant identity and Pitcher identity nested within Plant identity as random effects. We tested plant taxon, pitcher stage, season, pair of areas (each combination of two areas contrasted with each other), and all relevant two-way and three-way interactions when possible (for season, only the simple effect could be included). Both dependent variables were square-root transformed to match residual normality.

Finally, we tested whether the number of prey trapped was linked to plant taxon, pitcher stage, pitcher morphology and season. The total number of prey individuals and the total number of crawling Hymenoptera trapped were analysed using Generalized Linear Models (GLM) assuming a Poisson distribution with a log-link function. We tested, as explanatory variables, Plant taxon, Pitcher Stage, Pitcher length, Aperture width, the ratio Pitcher length / Aperture width, and Capture area (as defined in Bhattarai & Horner (2009) i.e. the

197 area of a circle of diameter Aperture width) and Season. For morphological variables, which were partly
198 correlated (for instance the ratio Pitcher length / Aperture width was negatively correlated with Pitcher length
199 : correlation-coefficient = -0.83, t = -8.27, p-value < 0.001), we chose to include in the same model only the
200 least correlated variables, that is Pitcher length and Aperture width (which were not correlated : correlation-
201 coefficient = -0.20, t = -1.15, p-value = 0.261). For this analysis, an outlier was identified and excluded (a *S.*
202 x *leucophylla* pitcher with 153 prey individuals including 54 ants).

203 Furthermore, we tested whether the number of flying Hymenoptera prey trapped was also linked to
204 visual signals. The number of flying Hymenoptera was analysed using similar Poisson regression models. As
205 Plant taxon and Pitcher stage absorbed the variation in visual signals, they were not included in the analysis.
206 Season was retained as it was likely to affect the abundance of prey available. As the visual signals measured
207 were numerous compared to the sample size, we focused on the variables of interest, i.e. the contrasts related
208 to areoles and also included in the analysis only the least correlated contrasts (<50%, see Supporting
209 Information Fig. S1). Hence, we tested the effects of Season, Pitcher length, Aperture width, Areoles
210 brightness contrast with background, Areoles-Peristome colour contrast, Areoles-Peristome brightness
211 contrast, Areoles-Tube colour contrast.

212 For all models, we selected the best model with the lowest AIC, and we did that for the fixed and
213 random effects separately. In all mixed models, only Pitcher identity was retained as a random effect.
214 Backward selection of models and type III tests were carried out. For each significant factor, we estimated
215 the regression coefficients and post-hoc tests were carried out between any two factor levels, when necessary.

216

217 **RESULTS**

218 PITCHER MORPHOLOGY AND COLOURATION

219 Plant taxa differed both in morphology and colouration (Fig. 1, Supporting Information Fig. S2, Fig. S3). *S.*
220 x *leucophylla* and *S.* x Juthatip soper tended to have long pitchers with a narrow aperture (Fig. S3) and well-

defined clear areoles, which respectively appeared white and pink to a human eye (Fig. 1, Fig. S3). The other two taxa, *S.* x *mitchelliana* and *S. purpurea*, had short pitchers with a large aperture (Fig. S3), and areas defined as areoles had a green colouration to a human eye, which was similar to that of the pitcher tube (Fig. 1, Fig. S2). In all taxa, the tube generally reflected light in the middle wavelengths while the peristome and veins reflected more in the long wavelengths, appearing respectively green and red to a human eye (Fig. 1, Fig. S2).

CONTRASTS WITH A GREEN BACKGROUND

The visual contrasts – colour contrast and brightness contrast – produced by pitcher areas against a green background as seen by flying Hymenoptera varied with taxon and stage (Table 1). More specifically, to flying Hymenoptera, peristome and tube showed no significant difference in colour contrast between taxa (Fig. 2A, (Supporting Information Table S1), and veins and tube showed no significant difference in brightness contrast between taxa (Fig. 2B, Supporting Information Table S2). Conversely, areoles and veins differed in colour contrast and areoles and peristome differed in brightness contrast between taxa (Fig. 2, Table S1, S2). The veins contrasted more in colour with a green background in S. x *mitchelliana* than in *S.* x *leucophylla*. The peristome contrasted more in brightness with a green background in *S.* x *leucophylla* than in *S.* x Juthatip soper. The areoles contrasted more in colour with a green background in *S.* x *leucophylla* and S. x Juthatip soper than in *S.* x *mitchelliana* and *S. purpurea* while *S.* x *leucophylla* had the most contrasting areoles of all four taxa. Hence, areoles accounted for most of the visual differences observed between taxa as they differed both in colour and brightness among taxa (Fig. 2). Yet, the area that contrasted most strongly against a green background was not always the same from one taxon to another (Table S1, S2): for the two long-leaved taxa, *S.* x *leucophylla* and *S.* x Juthatip soper, areoles were the most contrasting area either for colour or for brightness (Table S1, S2). Conversely, the most contrasting area for colour was the veins for *S.* x *mitchelliana* while it was the peristome for *S. purpurea* (Table S1, S2).

The change in colour and brightness contrasts with pitcher stage was not similar in all taxa (Table 1). However, areoles were the area most contrasting in colour at stage 1 (~5 days after pitcher opening) and at stage 2 (~15 days after pitcher opening) while the peristome was the area most contrasting in colour at stage 3 (~40 days after pitcher opening) and at stage 4 (~60 days after pitcher opening) (Supporting Information Fig. S4A, Table S1). Regarding brightness, areoles were the most contrasting pitcher areas against background at stage 1, 3 and 4 but only the second most contrasting areas at stage 2, veins being the most contrasting areas for this stage (Fig. S4B, Table S2). Season also directly affected the colour but not brightness contrasts with background, the colour contrasts being more pronounced in autumn than in summer (Table 1, Table S1).

CONTRASTS AMONG PITCHER AREAS

The visual contrasts – colour contrast and brightness contrast –between pitcher areas seen by flying Hymenoptera varied with taxon and stage (Table 2). More specifically, as seen by flying Hymenoptera, areoles produced a higher colour contrast with any other area in *S.* x *leucophylla* than in the 3 other taxa (Fig. 3A). These contrasts were also generally higher for *S.* x Juthatip soper than for *S.* x *mitchelliana* and *S. purpurea* but this was not the case for the areoles-veins contrast, which was not significantly different between *S.* x Juthatip soper and *S.* x *mitchelliana* (Fig. 3A). Areoles displayed a generally higher brightness contrast against any other area in *S.* x *leucophylla* than in the 3 other taxa but this was not the case for the areoles-veins contrast, which was not significantly different between *S.* x *leucophylla* and *S.* x *mitchelliana* and for the areoles-tube contrast, which was not significantly different between *S.* x *leucophylla* and *S.* x Juthatip soper (Fig. 3B). Overall, colour contrasts values between any two pitcher areas were always higher when they involved the areoles than when they involved any other areas for these two taxa (Supporting Information Table S3, Table S4). Yet, the pair of areas that displayed the strongest contrasts was not always the same from one taxon to another (Table S3, Table S4). For the two long-leaved taxa, *S.* x *leucophylla* and *S.* x Juthatip soper, the strongest contrast for colour or brightness was produced by areoles against peristome (Table S3, Table S4). For *S.* x *mitchelliana* the strongest colour contrast was produced by veins against tube

and peristome against tube and the strongest brightness contrast was produced between any red areas (peristome or veins) against any green area (areoles or tube). For *S. purpurea*, the strongest colour or brightness contrast was produced by areoles against peristome and peristome against tube, the brightness contrast being higher between areoles and peristome than between tube and peristome.

The differences in contrast produced by each pair of areas depended on the pitcher stage (Table 2, Supporting Information Fig. S5) but areoles and peristome always produced the highest contrast at stages 2 and 3 regarding colour and at stages 1, 2 and 3 regarding brightness (Table S3, S4). At stage 4, the most contrasting pairs of areas regarding colour and brightness contrasts involved the peristome against both areoles and tube (Table S3, S4). Overall, pitcher stages did not show many differences (Table 2), but differences occurred according to the pair of areas considered (Table 2, Fig. S5). Pitchers of stage 1 showed a weaker brightness contrast than pitchers of other stages, and the contrast displayed by areoles or tube against peristome or veins was always the lowest for that stage (Table S4). Regarding contrasts produced by areoles against peristome, stage 2 was the highest for colour but stage 3 was the highest for brightness. Moreover, stage 2 displayed the highest brightness contrast between areoles and veins, while the most brightness contrasting pairs of areas of stages 3 and 4 were peristome and tube. Finally, colour and brightness contrasts between areas varied with season and were generally more pronounced in autumn than in summer (Table 2, S3, and S4).

PREY CAPTURE AND PITCHER FEATURE

Prey spectra varied with plant taxon (Supporting Information Fig. S6). The prey trapped by pitchers were mostly composed of flying Hymenoptera and Diptera in *S.* x *leucophylla* (35 ± 8 %, 23 ± 10%); flying Hymenoptera, ants (crawling Hymenoptera) in *S.* x Juthatip soper (39 ± 7%, 39 ± 8%); ants and Diptera in *S.* x *mitchelliana* (32 ± 13 % and 21 ± 8%) and ants in *S. purpurea* (36 ± 16 %). Flying Hymenoptera trapped were mostly solitary bees (93 ± 6%) with a clear dominance of sweet bees (Halictidae).

The total number of prey individuals and crawling Hymenoptera caught in pitchers depended on plant taxon and pitcher stage. Likewise, pitcher morphology affected the total number of prey individuals (Supporting Information Table S5). Pitchers of *S.* x *leucophylla* and *S.* x Juthatip soper trapped the highest total number of prey individuals while pitchers of *S.* x *mitchelliana* and *S. purpurea* trapped the lowest number of prey individuals (Fig. 4, Table 3). As regards crawling Hymenoptera, *S.* x Juthatip soper pitchers trapped more individuals than other taxa (Fig. 4, Table 3). Pitchers of stage 1 trapped the lowest number of prey individuals and crawling Hymenoptera individuals while pitchers of stage 3 trapped the highest total number of prey individuals followed by pitchers of stage 2 (Table 3). Stage 4 trapped more crawling Hymenoptera individuals than other stages (Table 3). Regarding morphology, the total number of prey individuals increased with aperture width and pitcher length (Table 3).

The number of flying Hymenoptera individuals caught in pitchers depended on season, visual signals and pitcher morphology (Table S5). Regarding morphology, the number of flying Hymenoptera individuals increased with pitcher length (Table 3). Pitchers also trapped less flying Hymenoptera individuals in autumn than in summer (Table 3). In terms of visual signals, the colour contrast between areoles and tube had a positive effect on the number of flying hymenopterans caught in pitchers while the colour contrast between areoles and peristome and the areoles brightness contrast with background had a negative one (Table 3).

DISCUSSION

PITCHER COLOURATION AND ATTRACTION OF FLYING HYMENOPTERA

Beyond the broad range of colouration patterns within pitchers and between plant taxa, our findings support the hypothesis that the clear areoles – a characteristic of the two long-leaved taxa related to *S. leucophylla*, *S.* x *leucophylla* and *S.* x Juthatip soper – may potentially help *Sarracenia* pitcher plants attracting flying Hymenoptera. (i) Clear areoles contrasted strongly both in colour and brightness with the other parts of the pitcher and were the areas that contrasted the most against the green background, making them the most conspicuous area in the eyes of flying Hymenoptera. Flying Hymenoptera, such as honeybees and

bumblebees, use both colour and brightness signals for flower detection (Giurfa *et al.*, 1996; Giurfa & Vorobyev, 1998; Dyer *et al.*, 2008). Brightness contrast is used for small or distant objects (subtending a small visual angle in the eye) while colour contrast is used for large or close objects (subtending a large visual angle in the eye) (Giurfa *et al.*, 1996; Dyer *et al.*, 2008). Hence, bright areoles may play a role as a long-distance attractant for flying Hymenoptera, because they make the upper part with the aperture of the trap more visible compared to the rest of the pitcher. (ii) Furthermore, the two taxa that sported clear areoles also trapped more prey in total and trapped a majority of flying hymenoptera compared to the two smallest-leafed taxa without clear areoles, *S.* x *mitchelliana* and *S. purpurea*, that trapped a majority of ants. (iii) In addition, the colour contrast between areoles and tube explained an important part of the variation in the number of flying Hymenoptera trapped, the number of captures increasing with this contrast. On the other hand, we observed a negative effect of areoles brightness contrast and areoles-peristome colour contrast. Such a seemingly contradictory effect can be explained by the fact that colour and brightness contrasts are not particularly correlated. The pitchers that contrasted strongly in colour between areoles and tube are not the ones that contrasted the most in brightness for their areoles or in colour between areoles and peristome. In any case, the effect of the areoles-tube contrast outweighed the other two negative effects combined and is thus the most reliable effect. Considering the spectrum of attracted insects instead of the spectrum of trapped insects would have helped to better assess the relative contribution of the interacting effects, since only a few insects attracted are bound to be trapped (Joel, 1988; Newell & Nastase, 1998). However, attraction is a prerequisite to capture and our results support the hypothesis that areoles help attraction by contrasting highly in colour against the tube, a result coherent with that found by Schaefer & Ruxton (2014). *Sarracenia* form dense groups of plants (Gibson, 1983b) and areoles are often seen against a background of other pitcher tubes. Areoles would thus lure flying insects to the deadly traps, as also shown by McGregor *et al.* (2016) and Moran *et al.* (2012a). Although we cannot exclude a role of areoles as light traps, in this study we suggest that areole high visual contrasts may help long-range attraction, a potential role, which has rarely been considered previously. Most of the insects caught in the traps of carnivorous plants are hymenopterans and dipterans

344 (Juniper *et al.*, 1989; Ellison & Gotelli, 2009). While Hymenoptera cannot detect long wavelengths (red) and
345 use brightness to detect red colours since the latter poorly reflect at shorter wavelengths, appearing them dark
346 (Martínez-Harms *et al.*, 2010), many flies have a tetrachromatic or even pentachromatic vision (Kooi *et al.*,
347 2021) and they are able to see red wavelengths both in colour and brightness. While most studies regarding
348 carnivorous pitcher plants have overlooked how pitcher colours are perceived by potential prey (Newell &
349 Nastase, 1998; Green & Horner, 2007; Schaefer & Ruxton, 2008; Bennett & Ellison, 2009), our results
350 underline the importance of red colouration, already considered in these previous studies, in enhancing the
351 visual contrast even seen by flying Hymenoptera. Indeed, for all plant taxa, whether for colour or brightness,
352 the peristome produced one of the most important contrast either against the tube or against the areoles. The
353 red peristome, which borders the mouth of the carnivorous pitcher, is a strategic area (Moran, 1996; Bohn &
354 Federle, 2004; Kurup *et al.*, 2013) and any contrasting patterns that concerns this area is of high advantage
355 in terms of plant fitness, as it is likely to favour insect guidance to the deadly trap. In the two short-leaf plants
356 *S.* x *mitchelliana* and *S. purpurea*, the red peristome provides similar brightness and colour contrast with
357 green areoles or with green tube. In the two long-leaved plants, *S.* x *leucophylla and S.* x Juthatip soper, the
358 red peristome contrasts more in brightness and in colour with white-pinkish areoles than with green tube,
359 which increases pitcher visibility. Areoles when distinct in colouration from the pitcher tube, add to pitcher
360 visibility and are associated to higher prey capture in the two long-leaved plants compared to the two short-
361 leaf plants.

362 In our study, all measured pitcher areas show very poor UV-reflection in all taxa (Fig. S2), thus
363 showing virtually no contrast in the UV range. Whether a UV contrast may exist locally between highly-
364 absorbing (like the UV-induced fluorescence documented in Sarraceniaceae by Golos (2020)) and highly-
365 reflecting areas remains to be studied. Such highly-localised UV-contrasts may help orientation, as suggested
366 in Kurup *et al.* (2013), but their role, if any, in attraction and capture is controversial and remains to be
367 elucidated (Jansen, 2017).

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INFLUENCE OF OTHER PITCHER TRAITS ON PREY CAPTURE AND COMPOSITION

Besides colouration, our results show that pitcher morphology likely plays a role in prey capture. Pitchers with a longer size and a larger aperture trapped significantly more prey in total. This is consistent with the results of Cresswell (1993) and Heard (1998) on *S. purpurea* and of Green & Horner (2007) and Bhattarai & Horner (2009) on *S. alata*, who showed that larger pitchers trapped more prey. Bhattarai & Horner (2009) supposed that the positive relationship often observed between pitcher size and prey capture was due to a greater quantity of attractants produced by larger pitchers and not merely a larger area of capture. Indeed, the capture area as defined by Bhattarai & Horner (2009) did not better explain variation in our models than aperture width. Larger pitcher apertures also mean larger surface areas of lid, which was the most nectar-rewarding area of the pitchers in the studied plants (TH, JP and LG personal observations). Nevertheless, previous studies were limited to a single taxon. By considering different taxa, we showed that the effect of pitcher length was still more important than the effect of aperture width. The hypothesis of Bhattarai & Horner (2009) is therefore plausible on an intraspecific scale, but on an interspecific scale, the shape of the trap must also play an important role in prey capture.

Pitcher length was also positively linked to the number of flying Hymenoptera individuals, which suggests that taller pitchers may be more efficient at retaining flying Hymenoptera and/or may be more attractive, for instance by their colour signals as shown above. The conspicuousness of taller pitchers can arise from their size and/or their colouration: here clear areoles were only present in long-leaved taxa. Both aspects – size and colouration – can be under selection. Such multi-component signals are frequent in the contexts where plant appearance is under selection. For instance, in a prey-pollinator conflict regarding sundew carnivorous plants, El-Sayed *et al.* (2016) showed that in addition to different colouration between flower and trap, spatial separation is necessary to attract pollinator to the flowers rather than to the traps. Likewise, in *Nepenthes* liana, which explore both terrestrial and aerial strata, two pitcher types "lower" and "upper" are produced, each one targeting a different group of prey, the "upper" type trapping more flying insects than the "lower" type (Moran, 1996; Di Giusto *et al.*, 2008; Gaume *et al.*, 2016). Similarly, in long-

leaved *Sarracenia* plants, pitchers are likely more accessible to flying insects. In *Nepenthes rafflesiana* (Jack.), this is also explained by the emission by "upper" pitchers of volatile compounds, which attract a diversity of flying insects (Di Giusto *et al.*, 2010). Whether *Sarracenia* taxa differ in their emission of odour attractive to prey remains to be studied.

VARIATION IN VISUAL SIGNALS AND PREY CAPTURE OVER PITCHER LIFE SPAN

Our study also show that both colour contrasting patterns and prey capture changed during pitcher life span. We observed an increase in capture at stage 2 and a peak at stage 3 for the number of prey individuals. Although our sampling is weak for stages 1 and 4, mixed models control for unbalanced observations and show that capture increases over the first part of pitcher life (stages 1 to 3) and later (stage 4) decreases, which implies that when aging, pitchers allocated their resources more to the digestive process and less to attraction and capture. Heard (1998) observed a similar peak in prey capture in 12-33-day-old pitchers (corresponding to stages 2 and 3 in our study) in *S. purpurea*. Several studies on pitcher plants have reported that the accumulation of ammonia released by dead prey when prey capture is too important leads to digestion disruption and pitcher putrefaction (Clarke & Kitching, 1995). Increase in ammonia with prey capture has been recorded for *Sarracenia* (Wakefield *et al.*, 2005), suggesting prey capture should not exceed a threshold. Enzyme activity increases with pitcher age in *Sarracenia* (Luciano & Newell (2017); hydrolase secretion increases after pitcher opening for *S. purpurea* but stops in absence of prey according to Gallie & Chang (1997). Hence, younger stages are likely specialized in attracting and capturing prey, a hypothesis that is supported by the variation in colouration that we observed. (i) Areoles are the most contrasting area for colour in younger stages (1 and 2) while peristome is the most contrasting area for colour in older stages, (ii) areoles-peristome contrast is stronger at stage 2 for colour and stronger at stage 3 for brightness and areoles-veins brightness contrast is stronger at stage 2 (iii) areoles against peristome produced the highest contrast on pitchers for colour at stages 2, 3 and for brightness at stages 1, 2, 3, while at stage 4 areoles do not contrast more than tube against peristome for colour and brightness.

Variation in colouration with pitcher age has been mentioned by Bhattarai & Horner (2009) and has been already shown in *S. alata* by Horner *et al.* (2012). Leaf colour plasticity is already known in pitcher plants: nitrogen supply or food intake has been shown to affect foliar reflectance pattern (Moran & Moran, 1998; Yoon *et al.*, 2019) or chlorophyll activity (Farnsworth & Ellison, 2008). Therefore, it remains an open question whether the change in colouration of pitchers as they age is a consequence of prey capture. We also observed a seasonal variation in pitcher colouration with more pronounced contrasts in autumn, although our design allows us to this only for stage 2 pitchers. In parallel, pitchers seem to capture more flying hymenoptera individuals in summer than in autumn. Bees representing the majority of flying hymenoptera trapped by our pitchers. Like many other Hymenoptera, bees are thermophile species and are more active in summer than in autumn. Summer good weather conditions maximize time spent foraging (Vicens & Bosch, 2000). As season did seem to have neither an effect on the total number of prey individuals nor on the total number of ants, an increase in the capture of other non-Hymenoptera prey is likely to have compensated for the observed decrease in the capture of flying Hymenoptera (see Supporting Information Fig. S7). An increase in the capture of non-Hymenoptera flower visitors for instance may result from a reduced availability of flowers redirecting them toward nectar-producing pitchers - or from pitcher attraction towards different groups of insects with various visual ability - or capture efficiency, a question that deserves proper study. Yet, as we focused on the relation between areoles and flying Hymenoptera and used a specific vision model for that purpose, it is difficult to consider the effect of visual signals on the whole prey spectrum. For instance, ant workers, which represent a large part of the prey spectra of *Sarracenia* are often dichromate (Aksoy & Camlitepe, 2018). It would therefore be interesting to model their vision to better understand pitcher colours as perceived by a completely different type of prey, especially since their capture variation among stage and taxon differed a lot from that of flying Hymenoptera. Moreover, carnivorous plants have been suggested to lure prey by other attractants such as nectar (Bennett & Ellison, 2009) and odours (Jürgens *et al.*, 2009). For example, Bhattarai & Horner (2009) showed that volatiles from *Sarracenia* pitchers attract a majority of flying insects but few crawling ones, suggesting an important role of odours in the attraction of flying prey.

Studies of these other attractants could help us to better understand the variation in prey capture and complete our knowledge on the attraction strategy of *Sarracenia* pitcher plants.

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The authors declare no competing interests.

SUPPORTING INFORMATION:

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644 **TABLES:**

645 **Table 1.** Factors of variation in colour and brightness contrasts with a green background. Only
646 the best models are presented. Both dependent variables were square-root transformed to match
647 residuals normality. "Chisq" values refer to type III Wald chi-square tests. Symbols describe
648 various levels of p-values *: p<0.05, **: p<0.01, and ***: p<0.001. Contrasts are detailed in
649 ESM, Tables S1 (colour contrast) and S2 (brightness contrast). " - " means that the variable
650 was not retained in the best model. " : " refers to the interaction between variables.

	Colour contrast				Brightness contrast			
Explanatory variables	Chisq	Df	p-value		Chisq	Df	p-value	
Intercept	80.11	1	<0.001	***	74.33	1	<0.001	***
Plant taxon	9.69	3	0.0214	*	1.38	3	0.7107	
Area	32.28	3	<0.001	***	15.68	3	0.0013	**
Pitcher stage	3.06	3	0.3823		5.27	3	0.1532	
Season	31.79	1	<0.001	***	-	-	-	
Plant taxon: Area	72.44	9	<0.001	***	26.96	9	0.0014	**
Plant taxon: Pitcher stage	11.20	9	0.2622		17.55	9	0.0407	*
Area: Pitcher stage	45.83	9	<0.001	***	40.48	9	<0.001	***
Plant taxon: Area : Pitcher stage	103.08	27	<0.001	***	136.96	27	<0.001	***

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662 **Table 2.** Factors of variation in colour and brightness contrasts between two any pitcher areas. The best model
663 is presented. Both dependent variables were square-root transformed to match residual normality. "Chisq"
664 values refer to type III Wald chi-square tests. *: p<0.05, **: p<0.01, and ***: p<0.001. Contrasts are detailed
665 in ESM, Table S3 for colour contrast and S4 for brightness contrast. " : " refers to the interaction between
666 variables.

	Colour contrast				Brightness contrast			
Explanatory variables	Chisq	Df	p-value		Chisq	Df	p-value	
Intercept	132.85	1	<0.001	***	55.23	1	<0.001	***
Plant taxon	13.14	3	0.0043	**	2.55	3	0.4662	
Pair of areas	347.46	5	<0.001	***	259.38	5	<0.001	***
Pitcher stage	2.12	3	0.5481		4.09	3	0.2520	
Season	56.42	1	<0.001	***	7.03	1	0.0080	**
Plant taxon: Pair of areas	553.75	15	<0.001	***	174.22	15	<0.001	***
Plant taxon: Pitcher stage	30.21	9	<0.001	***	6.45	9	0.6941	
Pair of areas: Pitcher stage	241.65	15	<0.001	***	199.66	15	<0.001	***
Plant taxon: Pair of areas : Pitcher stage	1114.93	45	<0.001	***	674.58	45	<0.001	***

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680 **Table 3.** Effects of covariates included in the best linear models presented in Table S5 to explain the
681 variations in the number of prey individuals, the number of flying Hymenoptera individuals and the number
682 of crawling Hymenoptera individuals caught in the pitchers. We used a GLM with a Poisson distribution. P-
683 values were adjusted for multiple comparisons with the Bonferroni correction, *: p<0.05, **: p<0.01, and
684 ***: p<0.001. For qualitative variables, we tested the contrast between two levels of factor or between 2
685 gathered levels of factor. For instance, we tested whether the number of prey individuals in S. x *leucophylla*
686 was higher than the number of prey individuals in S. x Juthatip soper and it was significantly different (p-
687 value<0.001). For quantitative variables, which were centred and scaled before analysis to assess relative
688 effect sizes, we showed the estimated regression coefficients of the model.

Dependent variable	Explanatory variables	Contrast on factor	Coefficient (± standard error)	z-ratio	adjusted p-value
Number of prey individuals	Plant taxon	*S.* x *leucophylla* > *S.* x Juthatip soper	-0.07 (±0.11)	-0.60	1.0000
		S. x *leucophylla* > *S.* x *mitchelliana*	0.85 (±0.20)	4.34	<0.001 ***
		S. x *leucophylla* > *S. purpurea*	1.21 (±0.30)	4.02	<0.001 ***
		S. x Juthatip soper > *S.* x *mitchelliana*	0.92 (±0.16)	5.76	<0.001 ***
		S. x Juthatip soper > *S. purpurea*	1.27 (±0.27)	4.79	<0.001 ***
		S. x *mitchelliana* > *S. purpurea*	0.36 (±0.24)	1.47	0.8454
	Stage	Stage 1 > (2, 3, 4)	-1.64 (±0.22)	-7.42	<0.001 ***
		Stage 2 > (1, 3, 4)	0.39 (±0.13)	2.99	0.0112 *
		Stage 3 > (1, 2, 4)	0.92 (±0.11)	8.65	<0.001 ***
		Stage 4 > (1, 2, 3)	0.33 (±0.18)	1.84	0.2635
	Aperture width		0.20 (±0.07)	3.09	0.0020 **
	Pitcher length		0.57 (±0.08)	6.76	<0.001 ***
Number of flying Hymenoptera individuals	Season	autumn > summer	-2.75 (±0.53)	-5.17	<0.001 ***
	Areoles-Tube colour contrast		0.92 (±0.24)	3.86	<0.001 ***
	Areoles brightness contrast		-0.34 (±0.12)	-2.81	0.0050 **
	Areoles-Peristome colour contrast		-0.47 (±0.08)	-5.77	<0.001 ***
	Pitcher length		0.78 (±0.13)	6.19	<0.001 ***
Number of crawling Hymenoptera individuals	Plant taxon	*S.* x *leucophylla* > *S.* x Juthatip soper	-1.44 (±0.26)	-5.55	<0.001 ***
		S. x *leucophylla* > *S.* x *mitchelliana*	-0.12 (±0.32)	-0.39	1.0000
		S. x *leucophylla* > *S. purpurea*	1.07 (±0.45)	2.40	0.0997
		S. x Juthatip soper > *S.* x *mitchelliana*	1.32 (±0.23)	5.62	<0.001 ***
		S. x Juthatip soper > *S. purpurea*	2.51 (±0.39)	6.38	<0.001 ***

		S. x *mitchelliana* > *S. purpurea*	1.19 (±0.43)	2.76	0.0351 *
	Stage	Stage 1 > (2, 3, 4)	-1.91 (±0.58)	-3.27	0.0043 **
		Stage 2 > (1, 3, 4)	0.30 (±0.26)	1.18	0.9547
		Stage 3 > (1, 2, 4)	0.59 (±0.25)	2.41	0.0629
		Stage 4 > (1, 2, 3)	1.02 (±0.28)	3.63	0.0011 **

FIGURES:

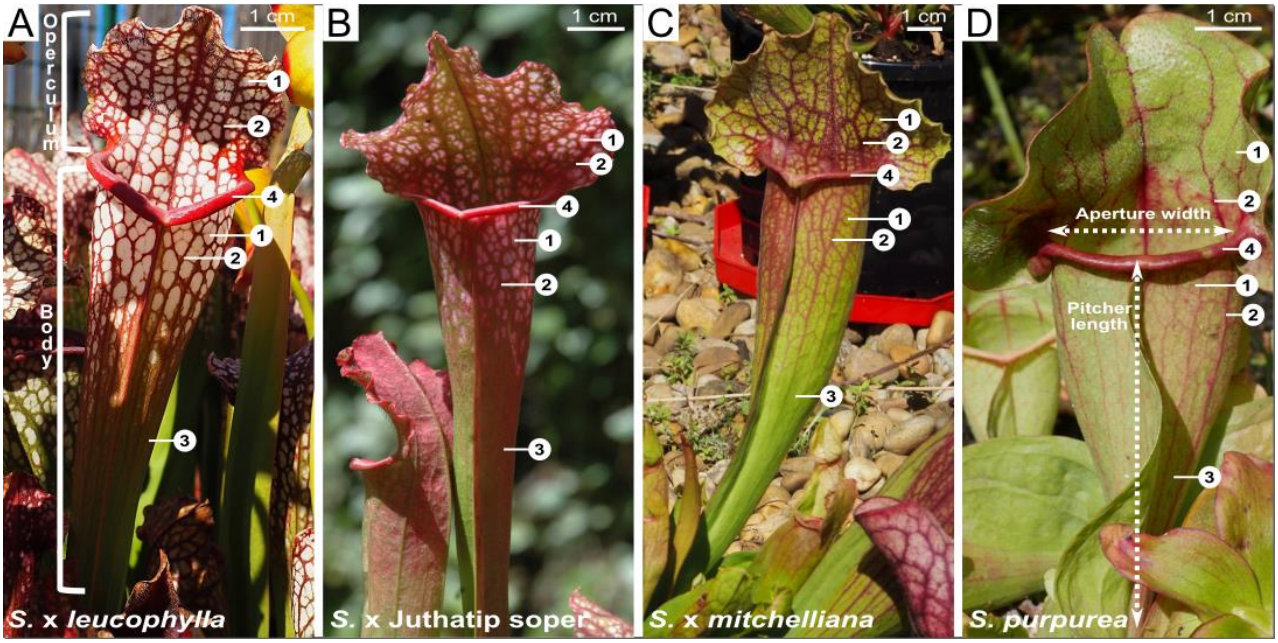


Figure 1. Pitchers of the four *Sarracenia* taxa: (A) *S.* x *leucophylla*, (B) *S.* x Juthatip soper, (C) *S.* x *mitchelliana*, (D) *S. purpurea* and their different areas: areoles (1) and veins (2) on the operculum, areoles (1), veins (2) and tube (3) on the pitcher body, and the peristome (4). For all species, areoles are defined as the areas between the veins, in the pitcher body or in the operculum. Areoles appear white (A), pink (B), or green (C, D) to a human eye. Aperture width and pitcher length, shown in (D) were measured in all pitchers.

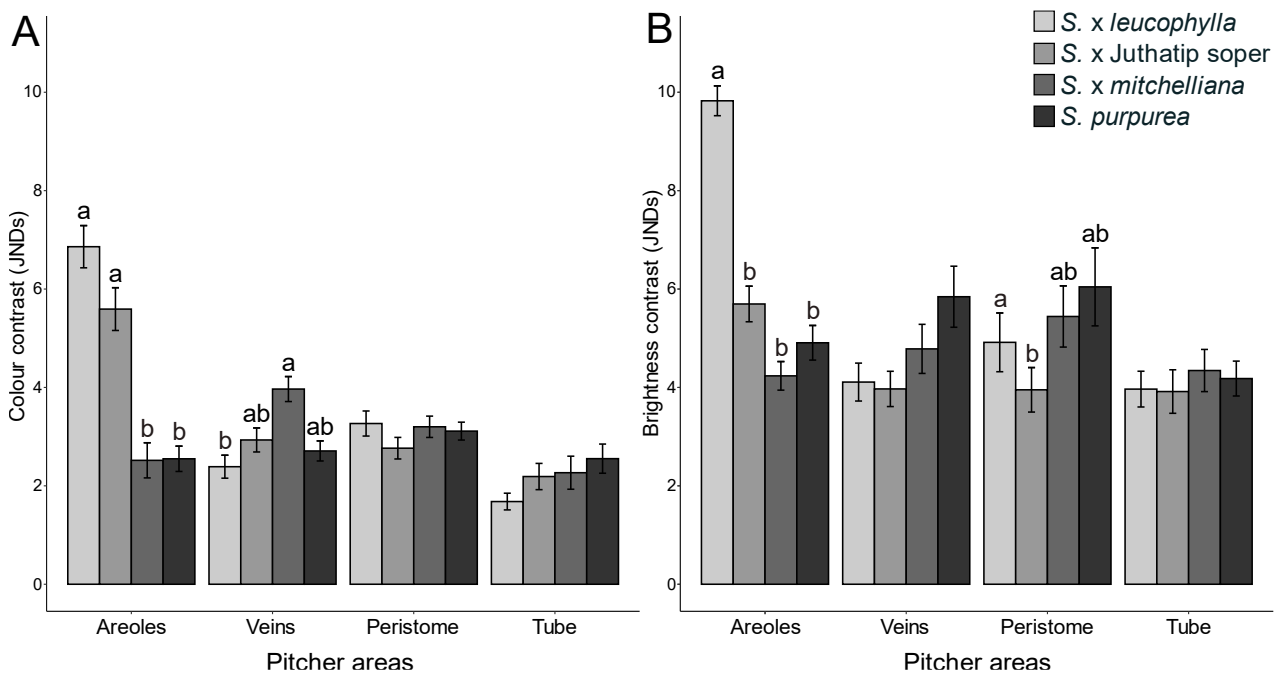
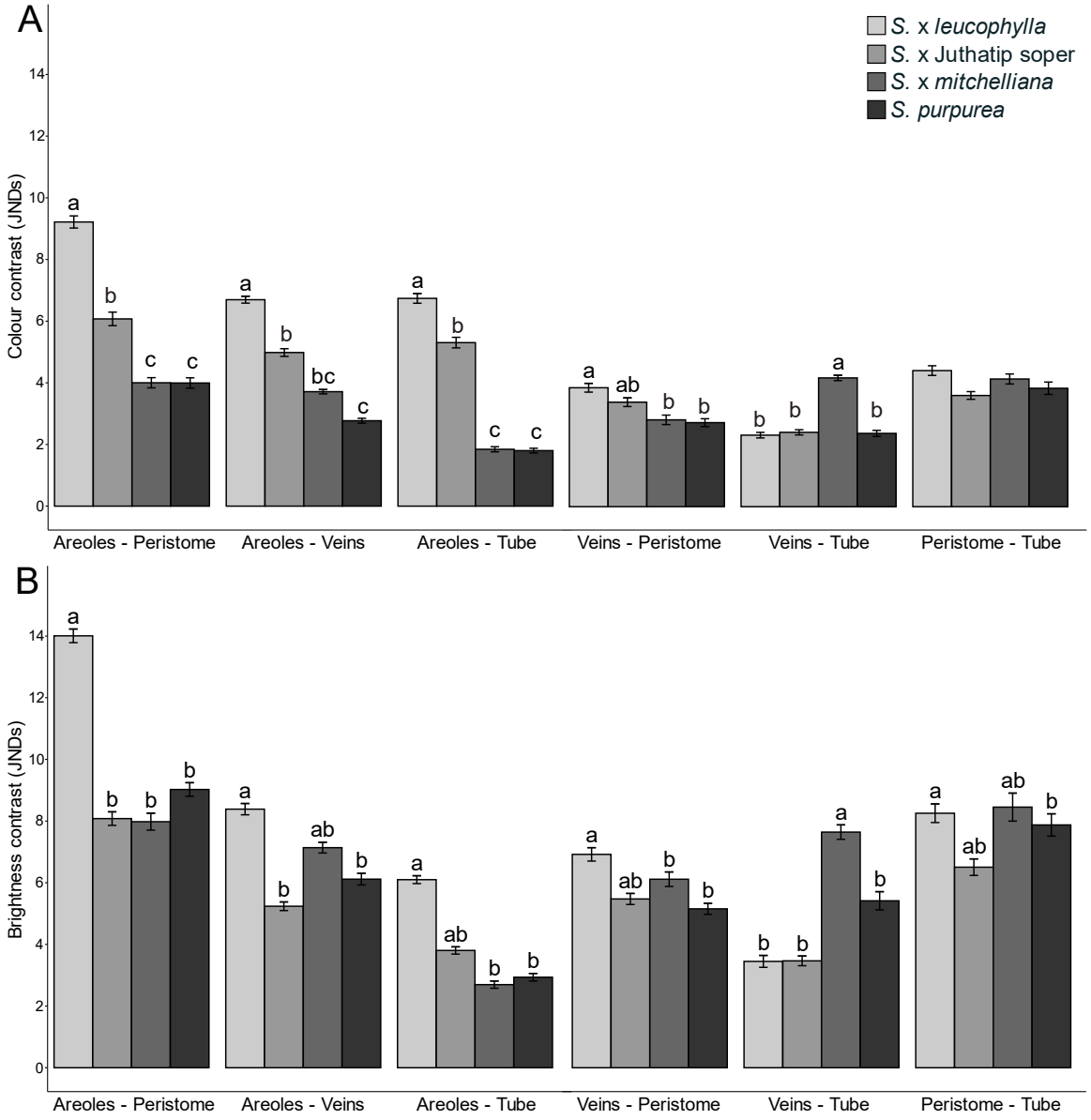
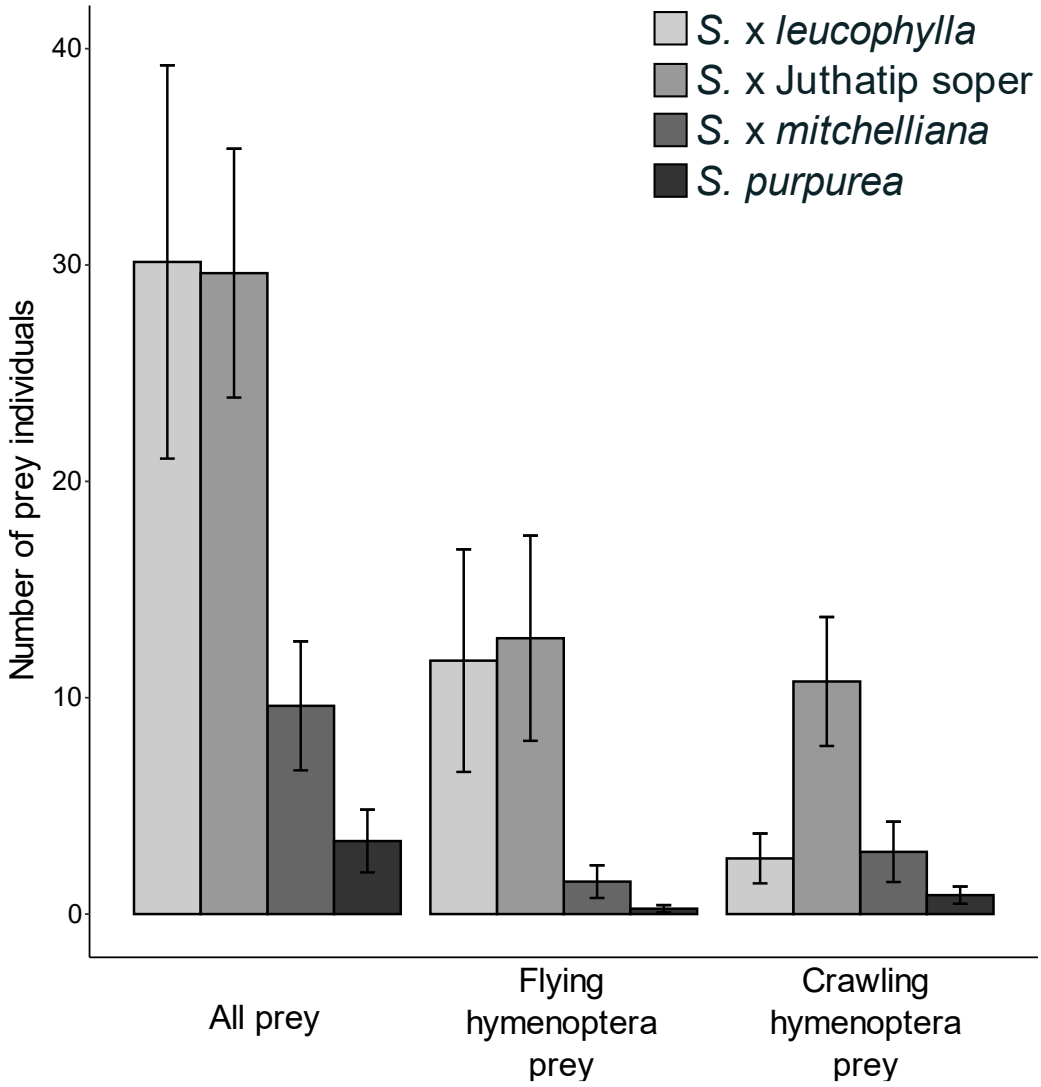


Figure 2. Variation in colour contrast (A) and brightness contrast (B) displayed by the different pitcher areas against the green background, as perceived by flying Hymenoptera. Mean values are presented with their associated standard errors. Contrasts are expressed in Just Noticeable Differences (JNDs). Different letters above bars show statistically significant differences in means between plant taxa for the contrasts of each specific area, no letter meaning that differences are not statistically different ($p>0.05$).



748

749 **Figure 3.** Variation among plant taxa in colour contrast (A) and brightness contrast (B) between any two
750 pitcher areas (areoles, peristome, veins, or tube), as perceived by flying Hymenoptera. Mean values are
751 presented with their associated standard errors. Contrasts are expressed in Just Noticeable Differences
752 (JNDs). Different letters above bars show statistically significant differences in means between plant taxa for
753 the contrasts of each specific area, no letter meaning that differences are not statistically different ($p>0.05$).



754

755 **Figure 4**. Variation among plant taxa in the number of prey individuals captured. Mean values are presented
756 with their associated standard errors. We consider all prey, flying Hymenoptera prey and crawling
757 Hymenoptera prey (ants).