Effect of light environment on intra-specific variation in herbivory in the carnivorous plant *Pinguicula moranensis* (Lentibulariaceae)

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**ABSTRACT**

Identifying the factors that affect a plant’s probability of being found and damaged by herbivores has been a central topic in the study of herbivory. Although herbivory could have important negative consequences on carnivorous plants, their interaction with herbivores remains largely unexplored. We evaluated the effect of spatial variation in light environment (sunny, shade and full-shade sites) on the pattern of leaf herbivory and florivory of the carnivorous plant *Pinguicula moranensis*. Plants’ overall probability of leaf damage was high (74.24%). Mean herbivory was four times higher in the sunny and shade sites than the observed in the full-shade site. Nearly 8% of plants suffered damage to reproductive structures, although the probability of florivory was similar among sites. Discussion addressed the inter-site variation in mean herbivory considering the effect of light exposure and the impact that herbivory could have on fitness components of this carnivorous plant.

**1. Introduction**

Identifying the ecological factors that affect plants’ probability of being found and damaged has been a central topic in the study of herbivory (Dirzo 1984). For plants, light environment represents one of the most important components of spatial heterogeneity (Valladares 2003; Caldwell & Pearce 2012). Variation in exposure to light (and other correlated variables) influences the expression of defensive traits against herbivores, the abundance of herbivores, or their feeding behavior making difficult the establishment of predictions about the relationship between intensity of herbivory and light environment (Chacón & Armesto 2006; Muth et al. 2008). For example, higher damage has been reported in plants of shaded habitats (Maiorana 1981; Niesenbaum 1992). In contrast, higher herbivory has been observed in open sunny sites (Lincoln & Mooney 1984; Louda et al. 1987; Salgado-Luarte & Gianoli 2010).

Theoretical models advanced to explain the conditions favoring the evolution of carnivory have incorporated spatial heterogeneity in light environment as a main factor (Givnish et al. 1984; Benzing 1987). Empirical field data have corroborated this assumption, showing not only that patches or populations of carnivorous plants are exposed to contrasting light regimes, but showing that spatial variation in light affects the interaction between carnivorous plants and insects, such as prey (Zamora et al. 1998) and pollinators (Zamora & Gómez 1996). Consequences of this interaction with herbivores associated with spatial differences in light could also be expected. The loss of foliar tissue in carnivorous plants reduces both the photosynthetic capacity and the acquisition of mineral nutrients by diminishing prey capture. Thus, herbivory could be more significant than in non-carnivorous species (Atwater et al. 2006). In addition to leaf herbivory, damage to reproductive structures producing direct negative consequences could also occur. However, the interaction between carnivorous plants and their herbivores has received little attention. For example, being *Pinguicula* a widely distributed taxon with nearly 90 extant species, there are only 2 reports on herbivory, neither of which had the main goal of describing the role of ecological factors such as variation in light regime in intra-specific differences in leaf damage (Zamora & Gómez 1996; Alcalá et al. 2010).

Thus, considering *Pinguicula moranensis* plants’ natural exposure to environmental heterogeneity, the goal of this study was to evaluate intra-specific differences in leaf herbivory associated with spatial variation in light environment and to document the incidence of florivory in this species. Individuals of *Pinguicula* exhibit higher production of mucilage with higher retention capacity (Zamora et al. 1998). Thus, given that secreting glands reduced herbivory in field and laboratory conditions (Alcalá et al. 2010), it can be expected that plants in sunny habitats receive lower damage.

**2. Materials and methods**

**2.1. Study system**

*P. moranensis* is a rosetted, perennial herb with seasonally dimorphic leaves. During the drier months (November–May) plants develop a resistance rosette composed of tiny (9–40 mm) non-glandular leaves. In the rainy months, plants produce the capture rosette composed of larger glandular leaves (6–12 cm). These leaves are covered by stalked secreting glands and sessile digestive glands. The mucilage produced by the stalked glands allows plants to catch several groups of prey, mainly small flying insects. The highest flower production occurs during summer months, from June through October. The zygomorphic, hermaphroditic, spurred flowers are individually inserted in stalks up to 20 cm tall.
At the study site, grasshoppers and terrestrial mollusks have been observed feeding on *P. moranensis* rosettes. Damage to reproductive stalks and corollas of flowers has been also observed.

The study was conducted along the growing season of 2015 (June–September) in the northern portion of Cuernavaca City in the State of Morelos in Central Mexico (2170 m asl). The area is composed of a series of hillsides primarily covered by oak-pine forests. Along 500 m of a rocky wall there were about 2000 individuals of *P. moranensis* Kunth mostly concentrated in 3 main patches, ranging from 20 to 50 m². The first patch corresponds to a northeast oriented open site where plants received direct sunlight from 800 to 1400 (hereafter, ‘sunny site’). The second patch is also northeast oriented; however, direct sunlight is prevented due to the shade of trees located just opposite the wall (‘shade site’). The third patch is southwest-oriented, such that plants are never exposed to direct sunlight; the site is entirely shaded by mosses and several fern species (‘full-shade site’). Patches were separated from each other by at least 100 m. Three quadrats (17 × 3 m, 7 × 3 m and 12 × 3 m; length × height) were traced on the rocky wall, corresponding respectively with the sunny, shade and full-shade sites. Each quadrant was divided in 1-m² plots to facilitate the identification and mapping of all plants. Using random numbers, 50 individual plants were selected in each quadrant at the beginning of the growing season in early June.

### 2.2. Reproductive variables and herbivory measurement

The phenological status of individuals (vegetative–reproductive), leaf herbivory and folivory were measured in the three sites to evaluate differences as a function of environmental heterogeneity. Reproductive individuals were separated in flowering and fruiting plants. Observations were also made of the presence or absence of leaf damage, the location of damage (along the leaf margin, concentrated in the inner part of the leaf, or both) and damage to reproductive stalks or flowers.

Leaf herbivory was measured using digital image analysis according to the methods proposed by Sandrini-Nieto et al. (2007). Two frontal plane photographs were taken of each plant and the best image was selected to estimate the total leaf area. First, the total number of pixels contained inside the previously marked outline of the rosette was obtained using Adobe Photoshop CS2, ver. 9.0. Then, the area corresponding to holes and the damage along the leaf margin were measured and summed to estimate the corresponding percentage of leaf area lost by herbivory. Herbivory was measured in August; representing damage accumulated over 75 d. Finally, a database was constructed including the magnitude of leaf herbivory and the spatial coordinates of each individual plant within each site, to evaluate the spatial independence of herbivory among plants (i.e. herbivory more similar than expected among closer plants).

Abundance of the community of arthropods was utilized as an estimate of herbivore abundance. At the end of July, ten commercial traps (a surface of 11 × 9 cm covered with water resistant non-drying adhesive glue, Bell Laboratories Inc, Madison, WI) were fixed to the rocky wall in each of the three sites. Location of traps within each of the three quadrats was haphazardly determined. After eight days, traps were recovered and taken to the laboratory. Specimens were separated at order level with a stereoscopic microscope and counted.

### 2.3. Statistical analysis

To evaluate if the probability of an individual plant to be reproductive varied regarding to site differences in light environment (sunny, shade and full-shade), a likelihood ratio test was derived from a contingency analysis. Reproductive status was coded as a binary variable (0 = non-reproductive, 1 = reproductive; plant bearing a flower or fruit). At the time of the census, all plants produced only one reproductive stalk. In the model, reproductive status was introduced as an ordinal dependent variable, while the effect of light exposure was included as a nominal independent variable.

Contingency analysis was also utilized to evaluate the association between the probability of suffering herbivory and variation in light environment. All plants were individually catalogued as intact (0) or damaged (1), regardless of magnitude of damage. In addition, considering only plants with some degree of leaf damage, the association between type of damage and light exposure effect was evaluated. In this case, damage was categorized by individual plant as along the leaf margin (1), concentrated in the central part of the leaf (2) or both occurring in the same plant (3). Similarly to the leaf damage, a binary combination was used to evaluate the association between the probability of florivory (including damage to reproductive stalks and direct damage to flowers) and variation in light environment.

The effect of light exposure on the mean percentage of area lost by herbivory was evaluated using a one-way ANOVA. The angular transformation was performed on the dependent variable to fulfill ANOVA assumptions (Sokal & Rohlf 1995). Differences in arthropod abundance were statistically evaluated by means of a one-way ANOVA using the total number of specimens per trap as the dependent variable (log transformed to fulfill ANOVA assumptions) and the sites as the nominal independent variable. In the case of mean herbivory and arthropod abundance, untransformed means ±1 standard error, are shown. All the contingency analyses and those performed on mean values of herbivory and arthropod abundance were done using JMP software, ver 4.0.3.

Finally, to determine whether plants that were closer to each other tended to show more similar values of damage than expected at random independent autocorrelation analyses were performed (Brenes-Arguedas 2012). The SGS software (Degen et al. 2001) used to perform the autocorrelation analyses outputs distograms displaying the variation in the distance measurement (D block) according to the separation distance of pairs of individuals (distance classes). A permutation procedure using Monte-Carlo simulations was applied to test for significant deviation from the spatial random distribution. For each one of the spatial distance classes, the observed D values were compared with the distribution obtained after 1000 permutations. Then, a 95% confidence interval for the parameters was obtained. Thus, positive D values above the confidence limit represent statistically significant deviations of the random distribution (i.e. positive autocorrelation).
3. Results

Of the 150 initially marked plants, data were available for 132 individuals (sunny = 49, shade = 34, full shade = 49) because 18 plants detached from the rocky wall over the course of the study. Most of plants failed to produce flowers or fruits (73.46%). Consequently, the mean production of flowers and fruits per plant was low (0.1893 and 0.1515, respectively). The reduction in the proportion of flowering individuals (from 28% in the sunny site to 10% in the full-shade site) was marginally significant (Likelihood ratio test $\chi^2(2, 131) = 5.247, P = .0726$, Figure 1(a)). The reduction in the proportion of plants that fruited (from 38% in the sunny site to 5% in the full-shade site) was statistically significant (Likelihood ratio test $\chi^2(2, 131) = 8.033, P = .018$, Figure 1(b)). Nearly 8% of the total plants suffered damage to reproductive structures. The probability of florivory was statistically similar among sites ($\chi^2(2, 131) = 3.427, P = .1803$, Figure 2(a)). The probability of plants to suffer leaf damage was high (74.24%), with only 34 plants left undamaged. Differences among the sites in the probability of leaf damage were significant ($\chi^2(2, 131) = 4.84, P = .048$), showing that plants in the full-shade site were less likely to be damaged (Figure 2(b)). The type of herbivory differed significantly among sites ($\chi^2(2, 131) = 23.17, P < .0001$), and overall, damage along the leaf margin was more frequent (39.8%) than damage to the center of the leaves (27.55%). The probability that plants would have both types of damage decreased from 51.61% to 21.28% between the sunny site and the full-shade site (Figure 2(c)).

With respect to the percentage of herbivory, the overall mean value ±1 standard deviation was 2.41 ± 5.74%. However, around 10% of individuals showed damage ranging from 6.39% to 39.48%. The mean percentage of area lost by herbivory differed among sites ($F_{(2, 122)} = 6.2376, P = .0026$). The percentage of herbivory observed in the first two sites was statistically similar, being around four times higher than the herbivory suffered by plants in the full-shade site (Figure 3). No evidence of spatial autocorrelation in the magnitude of herbivory was detected, as D values were within the confidence limits at all three sites (Figure 4). Finally, arthropod abundance statistically differed among the three sites ($F_{(2, 27)} = 20.03, P < .001$). The mean number of specimens caught per trap inversely increased regarding light availability [sunny (16.9 ± 1.58), shade (29.1 ± 2.99) and full-shade site (36.8 ± 2.28)]. Most frequent specimens present in the artificial traps corresponded to Hymenoptera (ants, small wasps) and Diptera. In conjunct, the three groups accounted for between 71% and 77% from the total number of retained specimens in each site. Spiders corresponded to the second most frequent group present in traps (14–16%) per site. Specimens of Orthoptera, Homoptera, Coleoptera, Isoptera and Acarina were found at a low frequency.

4. Discussion

Individuals of *P. moranensis* showed a high probability of being found and consumed by herbivores. Once plants were found, they received an overall low mean damage, as observed in other studies (Zamora & Gomez 1996; Alcalá et al. 2010). Following Marquis (1992), we have showed that inter-individual differences in herbivory could be ecologically more significant than overall mean values of herbivory. This is because damaged ranged from 0% to nearly 40% of total leaf area. The extreme herbivory in some individuals could reduce the area available for catching prey and the fitness gains derived from carnivory (Aldenius et al. 1983; Zamora et al. 1997; Thorén & Karlsson 1998; Méndez & Karlsson 1999). Herbivory could also reduce the capacity of plants to obtain energy directly by the reduction in photosynthetic tissue and fitness components such as survival, growth and reproductive success, depending on the ability to compensate foliar damage (Crawley 1989; Marquis 1992; Berenbaum & Zangerl 1998; Delaney & Macedo 2001). Therefore, experimental studies inducing distinct levels of damage are necessary to understand the impact of herbivory on somatic and reproductive fitness in carnivorous plants.

The intra-specific variation in herbivory was related to spatial heterogeneity in light environment because plants at the two sites with higher light exposure received around four times more damage than plants in the full-shade site. It is necessary to point out that the absence of multiple patches of plants at the study site avoided the possibility of sampling environmental heterogeneity around each light treatment. As a consequence, other non-measured variables affecting susceptibility of plants to herbivory could partially account for the observed results. Providing that sampling strategy is not wholly affecting the relationship between spatial light variation and intra-specific differences in herbivory, our findings contrast with results showing that plants in shaded conditions exhibit higher damage (Maiorana 1981; Niesenbaum 1992; Muth et al. 2008; Guerra et al. 2010); but are in accordance with studies evidencing more severe...
Higher herbivore abundance in open, sunny sites is proposed as a frequent cause of intra-specific differences. However, in this study, arthropod abundance, that was used to estimate herbivore abundance, showed the opposite trend. In fact, the number of specimens caught per trap in the full-shade site was nearly twice as the observed at the sunny site. We suggest that two facts could be contributing to reduce the probability of plants to be found at the full-shade site, a key factor to explain their lower mean foliar damage. First, plants at the full-shade site tend to be more isolated from each other due to the density of plants is 1.6 times higher in the sunny site. Second, plants in the sunnier sites are more conspicuous because their color highly contrasts with the color of the rocky wall. Conversely, the rocky wall of the full-shade site is almost entirely covered by ferns and mosses, and in most cases individuals of *P. moranensis* are growing rounded or beneath other plants. Thus, despite the higher abundance of potential herbivores in the full-shade site, plants could be more difficult to find.

On other hand, independent of light environment, damage located along the leaf margin was more frequent than damage located in the center of the leaf. This feeding behavior could reduce herbivores’ likelihood of becoming trapped, facilitating their consumption of foliar tissue. Regarding potential herbivores, in the artificial traps, terrestrial mollusks were absent and grasshoppers were present but at low frequency (ca., 1%). Nearly 70% of the entrapped specimens corresponded to groups that can be considered as potential prey for *Pinguicula*, in particular dipterans, which frequently represent more than 50% of the entrapped prey in by plants in the field.

A spatial pattern of damage (i.e. aggregated pattern) could emerge under several conditions, for example, when herbivores have limited movement ability (Atwater et al. 2006). It can also be a consequence of the aggregated distribution of juvenile terrestrial mollusks after hatching from egg batches (Bohan et al. 2000). In other cases it has been observed that the variation in defense traits against herbivory are positively autocorrelated (Hakes & Cronin 2011). In this study no autocorrelation was detected, indicating that intensity of damage is not influenced by distance. This suggests that in this study system, there are no bottom-up (i.e. soil characteristics, plant traits) or top-down (i.e. behavior of herbivores) processes that could cause a spatial pattern of herbivory.

Reproductive potential of *Pinguicula* has been shown to vary depending on light environment. Zamora et al. (1998) showed that plants of *P. vallisnerifolia* were unable to obtain reproductive benefits from experimental addition of prey capture when growing in deep shade. In the present study, the percentage of flowering plants in the full-shade site was 30% lower than in sites with exposure to direct sunlight. According to theoretical models, plants that invest in carnivory in shaded sites could incur higher costs, since mineral nutrients gained through prey capture do not translate into fitness gains as it is expected to occur in sunnier sites (Givnish et al. 1984). Therefore, even though plants of *P. moranensis* in fully shaded sites are less prone to being consumed by herbivores, the foliar tissue that they do lose could be proportionally costlier due to the light deficiency.

**Figure 2.** Probability of damage to reproductive structures (a), to leaves (b) and according with position in leaf (c) in three patches of *P. moranensis* exposed to different light environment. Narrow vertical bars to the right of the main graph represent mean values across all sites (In (a) and (b), 1 = damaged plants, 0 = undamaged plants. In (c), 1 = leaf margin, 2 = central part of the leaf, 3 = both types of damage).

**Figure 3.** Mean values ±1 standard error for the percentage of area lost due to herbivory in three patches of *P. moranensis* plants exposed to different light conditions. Statistical differences indicated by Tukey’s Honest Significance Test are denoted by distinct letters.
In addition to leaf herbivory, damage produced by herbivores to reproductive structures was detected in this study. Nearly 8% of the flowering individuals suffer florivory. Damage from sucking insects causes progressive wilting of reproductive stalks whereas damage from biting insects leads to the immediate loss of flowers. Evidence indicates that florivory reduces female fitness by direct consumption of ovules, and it could reduce male fitness directly by the lost of pollen grains and indirectly by reducing the attractiveness of flowers (Krupnick & Weis 1998; Mothershead & Marquis 2000; McCall 2008; Cascante-Marín et al. 2009; Zangerl & Berenbaum 2009; Rodríguez-Rodríguez & Valido 2011; de Jager & Ellis 2013). Using morphometric techniques we have gathered information for *P. moranensis* indicating that flower shape is a trait under phenotypic selection (in preparation). Therefore, damage to flowers (including scrapings, loss of lobules, or loss of the entire corolla) has the potential to disrupt the interaction with pollinators in this species.

Damage produced by herbivores to reproductive structures could increase reproductive costs. For example, the occurrence of a trade-off between the investment in reproduction and resources devoted to vegetative organs evidences that reproduction is costly (Karlsson 1986; Worley & Harder 1996). In the outcrossing *P. villosa* L., a species somewhat similar to *P. moranensis*, the investment of resources to reproduction was five times higher than those invested in winter buds (Karlsson 1986). In our study species, the single flower of *P. moranensis* are large relative to total plant size, and fruits tend to produce between 700 and 800 seeds, so investment in reproduction could be high. Therefore, resources devoted to reproduction could be lost due to florivory, although they could be reallocated to somatic functions if damage occurs early in the growing season (Thorén et al. 1996). However, in this case damage occurred during July and August, when reproductive stalks reach their maximum length and flowers were fully formed. In this case, the opportunity for reallocation seems limited.

Overall, the evidence derived from this study indicates that individuals of *P. moranensis* suffer differential natural herbivory depending on light environment. However, differences are not mediated by insect abundance. Although mean herbivory is low, there was high inter-individual variation in herbivory, with some individuals suffering severe leaf damage. The consumption of reproductive stalks or the direct consumption of flowers produces an automatic negative impact on reproductive fitness difficult to compensate within the same growing season. Plants under shade conditions show lower probability of reproduction and similar probability of florivory, representing a particularly costly situation. Thus, while individuals of *P. moranensis* are able to colonize sites varying in light conditions, some patches or populations could experience negative biotic or abiotic conditions that do not favor the maintenance of the carnivorous habit.

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Disclosure statement

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