Females restrict the position of domatia and suffer more herbivory than hermaphrodites in *Myriocarpa longipes,* a neotropical facultative myrmecophyte

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Abstract

Domatia are hollow structures in plants occupied by ant colonies, in turn ants provide protection against herbivores. In plants, competition for resources has driven sex-related changes in the patterns of resource allocation to life-history traits and defence traits. The resource-competition hypothesis (RCH) proposes that female plants due to their higher investment in reproduction will allocate fewer resources to defence production, showing greater herbivore damage than other sexual forms. We hypothesise the existence of sex-related differences in defensive traits of domatia-bearing plants, being female plants less defended due to differences in domatia traits, such as size, number of domatia and their position, exhibiting more herbivore damage than hermaphrodite plants of *Myriocarpa longipes*, a facultative neotropical myrmecophyte. We found eight species of ants inhabiting domatia; some species co-inhabited the same plant, even the same branch. Our results are consistent with the predictions of RCH, as female plants had ant-inhabited domatia restricted to the middle position of their branches and exhibited greater herbivore damage in leaves than hermaphrodites. However, we did not find differences in domatia size and leaf area between sexual forms. Our study provides evidence for intersexual differences in domatia position and herbivory in a facultative ant–plant mutualism in *M. longipes*. We highlight the importance of considering the plant sex in ant–plant interactions. Differences in resource allocation related to sexual reproduction could influence the outcome of ant–plant interactions.

Introduction

Herbivory is a strong selective pressure in plants (Coley & Aide 1991, Coley & Barone 1996). To reduce the effect of herbivores, plants offer food or nesting sites to recruit insect defenders such as ants, which are natural predators of herbivorous arthropods (Del-Claro *et al.* 1996, Heil 2008, Mithöfer & Boland 2012, Oliveira & Freitas 2004). In this sense, some myrmecophytic plants possess specialised structures that facilitate ant nesting, called domatia. In turn, ants provide with nutrients and defence against herbivores (Beattie 1985, Fiala & Maschwitz 1992, Mayer *et al.* 2014). These hollow cavities are in many cases spontaneously produced by plants (Davidson 1993) and sometimes are induced by ants (Edwards *et al.* 2009).

It has been shown that defensive traits can differ between sexual forms (Cornelissen & Stiling 2005). However, evidence involving sex-related differences in indirect defences mediated by ants is limited and is mainly focused on extrafloral nectaries. For example, Beaumont *et al.* (2016) found a greater abundance of ants and herbivores in males than on female plants of *Adriana quadripartita*. Sandoval-Molina (2018) reported more ants foraging on extrafloral nectaries of female plants than in male plants in a dioecious population of *Opuntia robusta*, but in a trioecious population male plants had more ants than females and hermaphrodites. In the same study, he found that females were slightly more damaged than males in the dioecious population, while in the trioecious population males were equally damaged as females, but hermaphrodites were less damaged than the other sexes. However, nothing is known about intersexual differences in domatia traits and how they may change the outcome of ant–plant interactions.

One explanation for sex-related differences in defensive traits is the resource allocation related to the sexual expression. Eckhart and Seger (1999) suggested that sex-related differences in sexual reproduction should influence plant growth and anti-herbivore defences. The resource-competition hypothesis (RCH) explains that the competition for resources between the reproduction, growth and defence is stronger in female plants than in males or hermaphrodites (Delph 1999, Herms & Mattson 1991, Lloyd & Webb 1977). This is because female plants

invest more resources in reproduction (e.g., fruits and seeds) than the other sexes, as a result their growth is slower and invests less energy in plant defences, sustaining greater tissue damage (Herms & Mattson 1991). For this reason, females tend to have larger leaves than other sexual forms, increasing their photosynthetic rate to compensate for their resources requirements, as Nicotra *et al.* (2003) reported for *Siparuna grandiflora*.

Changes in domatia size in response to biotic and abiotic factors have been reported in literature, suggesting that domatia are an adaptive phenotypic plasticity trait (Kokolo *et al.* 2020). In some domatia-bearing plant species, herbivory was shown to change domatia size; even simulated herbivory is able to change domatium size at branch level (Young *et al.* 2003). For example, in *Cordia nodosa*, plants under herbivore pressure had larger domatia than plants excluded from herbivores (Frederickson *et al.* 2013). In *Cinnamomum camphora,* the position of the domatia varied according to the venation of the leaf (*Nishida et al. 2005*).

Literature on domatia and ant species inhabiting the genus *Myriocarpa* (Urticaceae) is scarce. Monro (2009b) mentioned that *M. longipes Liebm.* has domatia, but he did not provide any information on its location, position and ant species that live inside. Longino (2009, 2007) reported three species of ants nesting in the domatia of *Myriocarpa* plants, *Pheidole rhinoceros, Pheidole walkeri* and *Azteca brevis.* Previously, *M. longipes* was reported as a dioecious plant (Steinmann 2005), but we identified a gynodioecious population (hermaphrodites and females) in Los Tuxtlas Research Station, Veracruz, Mexico (see supplementary materials for details, S1 Figure 1). This sexual dimorphism provides an excellent opportunity to examine sex-related differences in domatia size, position and the outcome of ant–plant interactions. To our knowledge, there are no previous studies in any plant species that explore the differences related to plant sex in domatia traits and their potential effect reducing herbivory.

Here, we conducted a field survey to analyse differences in domatia size, position, ant species inhabiting, leaf area and herbivory between plants with different sexual phenotypes. We hypothesise the existence of sex-related differences in defensive traits, where female plants are expected to be less defended due to differences in domatia traits, such as size, number of domatia and position, exhibiting more herbivore damage than hermaphrodite plants. Thus, we tested the following question: 1) Do the size and position of domatia promote their greater occupancy by ants on female plants than on hermaphrodite plants? 2) Are the leaves of female individuals more damaged than those of hermaphrodite individuals? In addressing the above, this study builds towards understanding the effect of plant sex on the outcome of ant–plant interactions.

Methods

Study site and plant species

We carried out our observations in different years, between 2016 and 2019 at the Los Tuxtlas Research Station, Veracruz, Mexico (18°30′N and 95°03′W), a reserve operated by the Universidad Nacional Autónoma de México (UNAM). It is located in the southeast of the state of Veracruz, Mexico, and is surrounded by extensive lowland plains. Dominant vegetation in this region is tropical evergreen forest (Rzedowski 1986), comprising 940 species of vascular plants, divided into 545 genera and 137 families (Ibarra-Manríquez *et al.* 1997). For a more detailed description of Los Tuxtlas, see González Soriano *et al.* (1997). Our work was

conducted along the main access roads of the research station, on a transect of approx. 700 m of length where plants grow naturally in partially opened areas of the understory. We selected and GPS-marked 20 individuals of *Myriocarpa longipes* (8 hermaphrodites and 12 females), of similar height and with visible and fresh inflorescences. The selected plants were at a minimum distance of 5 m from each other along the road. We determined the sex of the plant using descriptions provided by Monro (2009a). Until now, this plant species was described as dioecious or monoecious (Steinmann 2005). However, we found a gynodioecious population growing in the study site.

Myriocarpa longipes (Urticaceae) is a small tree (8–12 m height) distributed from Mexico to South America (Colombia, Bolivia, Brazil). It has ovate to elliptical leaves, 12–55 cm long and 6.5–23 cm wide. It can be found in disturbed, undisturbed and secondary evergreen wet forest, from sea level to 2400 m (Monro 2009a, WFO 2020). This plant species has domatia on its branches inhabited by ants (Monro 2009b). Domatia in *M. longipes* are hollow structures in branches; its inner wall is lined with dead pith tissue that forms a spongy layer that is removed by the ants when they are inhabited (M. Sandoval-Molina, pers. obs.).

Ant species composition and domatia measurements

To determine the composition of ant species associated with domatia, from the 20 previously marked plants of *M. longipes*, we selected only 5 plants (3 hermaphrodites and 2 females). Using an extendable pole pruner, we removed two branches of approximately the same length (~2 m) from each plant, cutting at the bifurcation base of the main stem. We destructively sampled domatia using a knife and collected ants inhabiting each domatium and were preserved in 70° ethanol in Eppendorf tubes individually tagged. Then, ants were mounted and identified with the aid of taxonomic keys (Fisher *et al.* 2007, Mackay & Mackay 1989). We corroborated taxonomic identification using the ant species list of Los Tuxtlas Biological Station, Veracruz, Mexico (project ADMAC), and with the assistance of the entomological collection IEXA at the Instituto de Ecologia, A.C., Xalapa, México, where specimens were deposited.

Domatia position was classified into three categories: basal, middle and apical, according to their position along the branches. To explore differences in domatia size between sexual forms, we used a caliper to measure the length and width of the thickest section of the branch where the domatium is located (supplementary materials, S1 Figure 2). We also measured the thickness of the solid part of the stem, from the outside of the stem to the beginning of the domatium. With these measurements, we estimated the cross-sectional area of the hollow part of the stem and the stem area surrounding the domatium.

Leaf area and herbivory

To analyse the differences in leaf area and herbivory between female and hermaphrodite plants, on the 20 plants previously marked, we randomly removed between 3 and 10 leaves per plant. To control leaf age, we selected only fully expanded leaves, excluding the youngest leaves at the top of the branches. We analysed the percentage of leaf area removed by herbivores using the *BioLeaf* app (Machado *et al.* 2016). This software estimates the percentage of removed tissue in relation to the total leaf area. To measure leaf area between females and hermaphrodites, we selected three leaves with no evident signs of herbivory from each plant and were photographed using a smartphone camera. All the photographs were

taken at the same distance (approx. 50 cm) in a 90° angle, using a wooden structure built by us. Fully expanded leaves were placed in a millimeter paper as background and were photographed. We estimated the total area of the leaves using the *LeafArea* package in R that allows to analyse several photographs simultaneously in *ImageJ* software (Katabuchi 2015, R Core Team 2020).

Statistical analysis

To test for differences in cross-sectional domatia area between sexes, we performed a Wilcoxon test; for the stem area surrounding the domatium, we used a *t*-test. In addition, we carried out a Pearson Chi-squared to test for association between domatia position and sex, also between ant species and sex, and finally between domatia position and sex. Due to the small frequencies for some factors in the contingency tables (some observations < 5), *p*-values were calculated by a Monte Carlo simulation, using 5000 replicates.

To test for differences in herbivory, we fitted a generalised linear model (GLM) with gamma distribution and 'log' as a link function. We used the *arcsine* transformed percentage of herbivory as response variable (N = 95), this transformation helped to normalise the data and improved the normal distribution of the model residuals. To test for differences in leaf area, we fitted a GLM with Gaussian distribution and 'identity' as link function, using the leaf area as response variable (N = 60). In both models, plant sex was used as explicative variable. Model goodness of fit was checked by inspection of residuals using the *DHARMa* package (Hartig 2018), which uses a simulation-based approach to create readily interpretable scaled residuals from fitted models. Comparisons were done with post-hoc Tukey's test using the *emmeans* package (Lenth 2019). Plots were made using the *ggpubr* package (Kassambara 2019) and *ggplot* (Wickham 2016). We made all statistical analysis using R (R Core Team 2020, RStudioTeam 2020).

Results

We found that domatia were distributed along the branches of *M. longipes* of both sexes, but domatia inhabited by ants were restricted to middle position in females. We did not find differences in the number of ants-occupied domatia between females and hermaphrodites ($X^2 = 0.28$, $df = 1$, $p = 0.59$). We found that domatia occupied by ants are not commonly distributed along the branches ($X^2 = 6.14$, $df = 2$, $p = 0.05$), inhabited domatia were more frequently found in the middle section of the stem (N = 9) than at the base (N = 2) or top (N = 3) of the branches. Domatia were found in both young and old branches and occupied more than one stem internode; the longitude of domatia varied between 10 and 25 cm. The hole entrance to domatium occurred where leaves had previously grown at the base of the petiole (Fig. 1a, b). We found that domatia were hollow inside and were occupied by ants and found other taxonomic groups living inside domatia cavities, such as beetle larvae, myriapods and salamanders (*Bolitoglossa* sp.). Plants hosted eight ant species, with adults and larvae (Fig. 1c to h). Five species were found in female plants and six in hermaphrodites (Table 1): *Pheidole* sp. (Wheeler, 1938) (Fig. 1c), *Cephalotes scutulatus* (Smith, 1867) (Fig. 1d), *Camponotus* sp. (Mayr, 1861) (Fig. 1e), *Pachycondyla* sp. (Smith, 1858) (Fig. 1f), *Camponotus atriceps* (Smith, 1858) (Fig. 1g), *Dolichoderus lutosus* (Smith, 1858) (Fig. 1h), *Dolichoderus bispinosus* (Olivier, 1792) and *Azteca* sp. (Forel,1878). We found that ant species could co-inhabit the same plant, even on the same branch. We found two or more ant species living in different domatium on the same branch of the same plant.

Herbivory and domatia morphology

We found a significant difference in percentage of herbivory between sexes, female leaves were more damaged than hermaphrodites ($t = 2.00$, $df = 93$, $p = 0.04$; Fig. 2a; Table 2). We did not find differences in leaf area between female and hermaphrodite plants (supplementary materials, S1 Figure 3; Table 2).

Regarding domatia measurements considering all positions along the branch (supplementary materials, S1 Figure 4), we did not find differences in the cross-sectional area of the domatium between females (*mean ± sd*: 0.198 cm^2 ± 0.170) and hermaphrodites (*mean ± sd*: 0.117 cm^2 ± 0.117). When we compared only the domatia in the middle position of the branches, the females had slightly larger domatia than the hermaphrodites, but this analysis was not statistically significant. We did not detect differences in the stem area surrounding the domatium. We found a marginally significant association between the position of domatia and plant sex ($X^2 = 5.83$, $df = 2$, $p = 0.055$); domatia in females were positioned mainly in the middle of the branches, while in hermaphrodites domatia were found all along the branches (Table 1; Fig. 2b). We did not find a relationship between domatia position and ant species ($X^2 = 16.33$, $df = 14$, $p = 0.34$), nor between ant species and plant sex ($X^2 = 6.85$, $df = 7$, $p = 0.68$).

Discussion

The present study investigated the intersexual differences in domatia size, position, leaf area and herbivory between sexual forms of *M. longipes* in a gynodioecious population. According to our predictions, female plants had differences in domatia position and exhibited greater herbivore damage in leaves than hermaphrodites. Literature concerning differences in plant traits related to indirect defence between sexual forms is limited and has been focused on extrafloral nectaries bearing plants (Beaumont *et al.* 2016, Sandoval-Molina 2018). To our knowledge, this is the first study to show the existence of sex-related differences in domatia position, and its effect on herbivore damage in *M. longipes,* a facultative myrmecophyte.

In our study, female plants were less defended, with ant-inhabited domatia restricted to the middle section of the branches and showing higher levels of herbivory than hermaphrodite plants. It has been shown that domatia are a highly plastic trait (Kokolo *et al.* 2020) and may even vary at the branch level in the presence of herbivores (Young *et al.* 2003). Probably, as the female plants showed restrictions in the position of their domatia, the ants could not occupy them; as a result, fewer ants inhabited and were unable to defend the plant successfully against herbivores. In contrast, hermaphrodite plants had domatia all along their branches, housing a greater number of ants and reducing the damage produced by herbivores.

Domatia restricted to the middle position in females, similar leaf size and a higher proportion of damaged leaves in female individuals than in hermaphrodites suggest that our results are parallel with the predictions of the RCH. This hypothesis proposes that female plants, as result of its higher investment in reproduction, will allocate less resources to defence production, showing greater herbivore damage than hermaphrodites. This hypothesis would be tested if the reproductive allocation of the female had been greater than in the hermaphrodite individual. Another explanation for the magnitude of damage observed in female plants is a lower cost of vegetative tissue production, following the optimal defence hypothesis, should be less defended because the loss of less
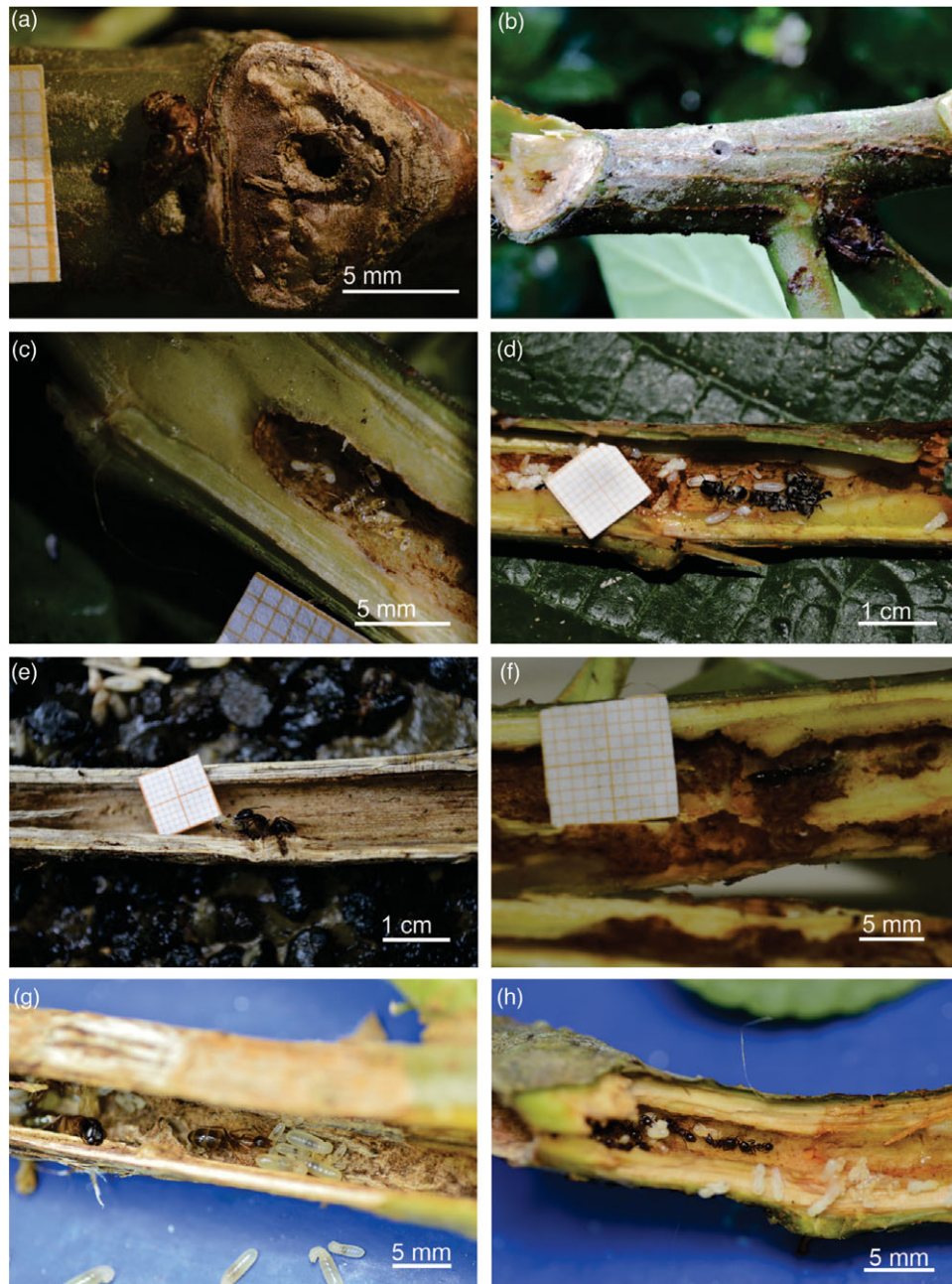
Figure 1. Field observations of ant–plant interactions in *Myriocarpa longipes*. (a) The ants enter the domatia through the hole at the base of the petiole, where the leaves have previously grown. (b) Portion of a stem showing the cross-section of the domatia. Dissection of the stem where the domatia are located, showing workers and brood of different ant species: (c) *Pheidole* sp., (d) *Cephalotes scutulatus*, (e) *Camponotus* sp., (f) *Pachycondyla* sp., (g) *Camponotus atriceps* and (h) *Dolichoderus lutosus*. Some domatia show melanised fungi inside.

energetically valuable tissue implies a lower loss in terms of fitness (McKey 1979, Rhoades 1979).

Female plants probably produced fewer chemical defences in their leaves, showing greater herbivore damage than hermaphrodites. However, little is known about secondary metabolites in *Myriocarpa* species. Niño *et al.* (2011) reported that the extract of *Myriocarpa stipitata* has alkaloids, terpenes, tannins and saponins, being the former related to inhibitory activity against yeast. Literature concerning intersexual differences in chemical defences production is limited (Dziedzic *et al.* 2020, Iszkuło *et al.* 2013, Janczur *et al.* 2021). There is only one study in a

cactus that showed that female plants produce lower concentrations of a secondary metabolite (4-hydroxybenzoic acid) than hermaphrodite plants (Janczur *et al.* 2021). The extent of damage observed in our study is likely the result of differences in secondary metabolites between sexual forms, but differences in chemical defences on *M. longipes* remain unexplored and further studies are required to confirm its effect in decreasing herbivory of plants.

Ant species composition inhabiting domatia could be also an important factor influencing the extent of herbivore damage. It is known that the intensity of herbivory exhibited by

Table 1. Ant species composition according to the position of the domatia between female and hermaphrodite plants of *Myriocarpa longipes*. In female plants, ant-inhabited domatia were restricted to the middle position of the branches; no ant species were found in domatia at the base and apical positions.

Plant sex	Domatia position	Ant species
Hermaphrodites	Base	*Pheidole* sp.
Hermaphrodites	Middle	*Camponotus* sp., *Pheidole* sp., *Dolichoderus lutosus*
Hermaphrodites	Apical	*Camponotus atriceps*, *Cephalotes scutulatus*, *Azteca* sp.
Females	Base	–
Females	Middle	*Camponotus* sp., *Pheidole* sp., *Dolichoderus bispinosus*, *Pachycondyla* sp., *Cephalotes scutulatus*
Females	Apical	–

Table 2. Model statistics for the percentage of herbivory and leaf area between female and hermaphrodite plants of *Myriocarpa longipes*. We showed results from generalised linear models with gamma and Gaussian error distribution. Bold numbers represent significant *p*-values <0.05.

Predictors	Herbivory				Leaf area			
	Estimates	Std. error	Statistic	*p*-value	Estimates	Std. error	Statistic	*p*-value
(Intercept)	0.243	0.020	−17.593	**<0.001**	256.063	14.452	17.718	**<0.001**
Sex (hermaphrodites)	0.775	0.099	−2.000	**0.048**	5.664	22.851	0.248	0.805
Sample size	95				60			
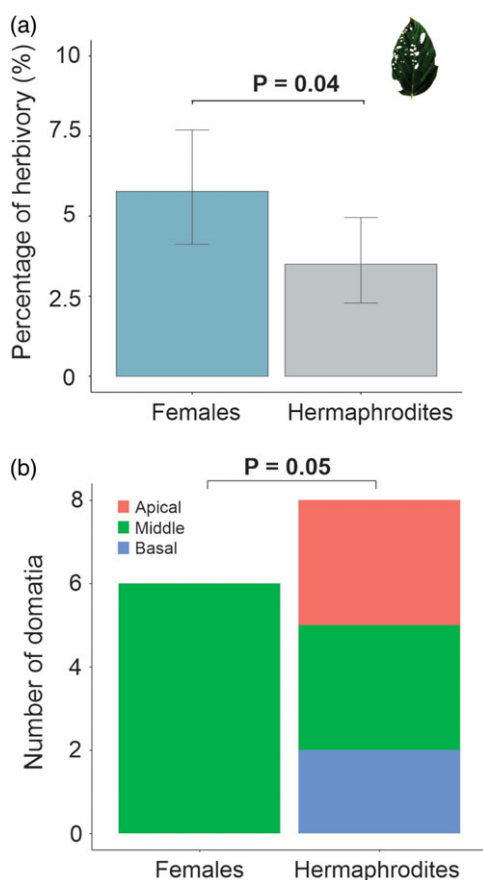
Figure 2. Percentage of herbivory and number of domatia of *Myriocarpa longipes*. (a) Differences in leaf damage percentage between females and hermaphrodites. (b) Number of domatia inhabited by ants on branches between different sexual forms. The *p*-value for the post-hoc contrast and Chi-squared are shown above the bar plots and boxplot, respectively. Error lines represent the 95% confidence intervals.

myrmecophytic plants depends on size or aggressiveness of its ant associates (Miller 2007, Rico-Gray & Thien 1989). This is because ant species vary in their foraging behaviour, patrolling ability, recruitment efforts and predatory activity, resulting in different levels of herbivory (Del-Claro *et al.* 2016, Fagundes *et al.* 2017). In our study, we found that ant species diversity varied within and between individuals of *M. longipes*, even within the same branches, and some ant species co-existed on the same plant. It is likely that ant species composition between sexual forms is affecting the extent of herbivore damage, but it is likely that we did not observe this effect due to our small number of dissected plant individuals (five plants). Further studies should increase the number of plants sampled to disentangle the effects of plant sex on domatia size and ant colonies, as well as its effect at the individual level.

It has been shown that domatia size can change in response to herbivory (Frederickson *et al.* 2013). Our results did not show differences in the cross-sectional domatia area between females and hermaphrodites when we considered all domatia positions. However, when we only considered domatia in the middle position, we found a trend that females may have larger middle domatia than hermaphrodites, but this difference was not statistically significant and also probably related to the small number of plants sampled (see supplementary materials, S1 Figure 4). This result suggests that probably even when females restricted the position of domatia, they responded to higher herbivory increasing domatium size in the middle position to house more ants to counter herbivory, but more studies are needed to confirm this effect.

Finally, when ants fail to defend plants, a penalty could be imposed on them, reducing the growth or survival of domatia (Edwards *et al.* 2006). It is likely that facultative ants inhabiting domatia of females are inefficient deterring herbivores, so plants are 'taking' the decision to penalise them restricting the available spaces for nesting. Even if that is reflected in an increase in the amount of herbivory damage. Another possibility is that if nesting

sites are being restricted in the female plants, the species composition of ants inhabiting the domatia should change and colonies of more aggressive ant species will be favoured, influencing the foraging behaviour of other insects, such as herbivores, and modifying the outcome of ant–plant interactions.

Conclusion

This work is consistent with the predictions of the RCH. Female plants exhibited greater herbivore damage to the leaves than hermaphrodites and had ant-inhabited domatia restricted to the middle position of their branches.

The results of this study suggest the existence of sex-related differences in domatia position and herbivory in a gynodioecious population of *M. longipes*. Few studies in the literature address intersexual differences in plant defences in the context of ant–plant interactions (Beaumont *et al.* 2016, Sandoval-Molina 2018). However, until now, nothing was known about domatia plants. On the basis of our findings, we highlight the importance of considering plant sex in ant–plant interactions studies. It is possible that differences in resource allocation related to sexual reproduction influence the outcome of these interactions.

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