



Original article

Weak trophic links between a crab-spider and the effective pollinators of a rewardless orchid

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ABSTRACT

Sit and wait predators hunting on flowers are considered to be exploiters of plant–pollinator mutualisms. Several studies have shown that plant–pollinator interactions can be highly susceptible to the impact of a third trophic level, via consumptive (direct) and non-consumptive (indirect) effects that alter pollinator behavior and, ultimately, plant fitness. However, most flowering plants attract a wide array of flower visitors, from which only a subset will be effective pollinators. Hence, a negative effect of an ambush predator on plant fitness should be expected only when: (i) the effective pollinators are part of the predators' diet and/or (ii) the non-consumptive effects of predator presence (e.g. dead prey) alter the behavior of effective pollinators and pollen movement among individual plants. We analyzed the direct and indirect effects of a crab-spider (*Misumenops pallidus*), on the pollination and reproductive success of *Chloraea alpina*, a Patagonian rewardless orchid. Our results indicate that most of the flower visitors do not behave as effective pollinators and most effective pollinators were not observed as prey for the crab-spider. In terms of non-consumptive effects, inflorescences with and without spiders and/or dead-prey did not vary the frequency of flower visitors, nor pollinia removal or deposition. Hence, it is not surprising that *M. pallidus* has a neutral effect on pollinia removal and deposition as well as on fruit and seed set. Similar to other rewardless orchids, the low reproductive success of *C. alpina* (~6% fruit set) was associated with the limited number of visits by effective pollinators. Negative top-down effects of a flower-visitor predator on plant pollination may not be anticipated without studying the direct and indirect effects of this predator on the effective pollinators. In pollination systems where effective pollinators visited flowers erratically, such as in deceptive orchids, we expect weak or no effect of predators on plant fitness.

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1. Introduction

Plant–pollinator associations are among the most important and widespread mutualisms in nearly all terrestrial communities. The incidence of the interaction between flowering plants and animals can be very high. At least 67% of flowering plants primarily depend on insect pollinators and possibly near 90% use animals to various extents for sexual reproduction (Buchmann and Nabhan, 1996; Suttle, 2003). Thus, it is not surprising that such a predictable interaction would be exploited by predators. Yet, the impact of predation risk on flower visitors has been a neglected issue in pollination studies until the last decade (Dukas, 2001a; Dukas and

Morse, 2003). Recently several empirical studies assessing predation risk, directly mediated by the presence of an ambush predator or indirectly mediated by dead prey found in flowers, have consistently reported changes in pollinator density and behavior such as increasing avoidance or reducing the frequency and/or duration of visits to flowers (reviewed by Romero et al., 2013). Furthermore, behavioral changes in the flower visitors' assemblage have shown to translate into strong negative effects of predators on plant fitness, decreasing plant reproductive success by 17%–25% (Knight et al., 2006; Romero and Koricheva, 2011).

Notwithstanding, the net outcome of an ambush predator on the pollination mutualism depends on at least three main factors. First, pollinator traits: a recent review concluded that the strength of the predator effect does not depend much on predator taxa and foraging mode (sit-and-wait vs. active hunters), but pollinator-related variables were crucial (Romero et al., 2013). In particular,

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the behavioral response to predation risk was associated with pollinator taxa and body size, but not with lifestyle (social vs. solitary) (Romero et al., 2013, but see Clark and Dukas, 1994; Rodríguez-Gironés and Bosch, 2012). In general, Diptera showed a weaker behavioral response than Squamata, Lepidoptera and Hymenoptera (Romero et al., 2013, but see Yokoi and Fujisaki, 2009; Brechbühl et al., 2010), whereas large pollinators showed weaker responses to predation risk than small ones (Higginson et al., 2010; Rodríguez-Gironés and Bosch, 2012; Romero et al., 2013). Second, flower reward levels: empirical studies (Llandres et al., 2012) and theoretical models (Jones, 2010) have shown that pollinator susceptibility to predation interacts with resource intake, leading to a general trend of less vulnerable pollinators foraging more on richer flowers whereas more vulnerable (i.e. smaller) pollinators would forage on less rewarding flowers. Third, relative densities of mutualists and antagonists: the net effect of an ambush predator on the plant reproductive success will depend not only on its own density, but also on the relative density of effective pollinators versus herbivores or seed predators (Higginson et al., 2010; Romero and Koricheva, 2011). Hence, strong top-down effects of predators on flower reproduction should be mostly expected when pollinators are highly cognitive, have small body size, and flowers offer low rewards and/or herbivores and seed predators are not common or abundant.

Given that flower ambush predators frequently prey on all flower visitors but effective pollinators (sensu Ne'eman et al., 2009) are often a subset of the assemblage of flower visitors (e.g., Artz et al., 2010; Castro et al., 2013), only behavioral changes of these effective pollinators would translate into altered plant fitness. Accordingly, it is not surprising that the effect size of predators on flower visitors behavior is often more important (Romero et al., 2013) than their effect size on plant reproductive success (Knight et al., 2006; Romero and Koricheva, 2011). Then, a negative effect of an ambush predator on plant fitness should be expected only when: (i) the effective pollinators are part of the predators' diet and/or (ii) the non-consumptive effects of predator presence (e.g. dead prey) alter the behavior of effective pollinators and pollen movement among individual plants.

We tested these assumptions using a rewardless ground orchid, *Chloraea alpina* (Orchideaceae), its guild of insect visitors, and the crab-spider, *Misumenops pallidus* (Thomisidae). Orchids are a good model on which to explore these assumptions given that pollen is packed inside pollinia (Johnson and Edwards, 2000), making it easy to detect effective pollinators from floral visitors and track pollen movements within and between individual flowers and plants. The pollination biology of only a handful of Patagonian *Chloraea* species had been well studied (Clayton and Aizen, 1996; Lehnebach and Riveros, 2003; Humaña et al., 2008; Sanguinetti et al., 2012; Sanguinetti and Singer, 2014), but most work has focused on their breeding systems and fruiting success, with little emphasis on pollinator diversity and behavior. Given that *C. alpina*, as all *Chloraea* spp. studied to date, is a deceptive but pollinator-dependent terrestrial orchid, we expect a significant negative impact of an ambush predator on pollinators, and consequently, on plant fitness. Using natural populations we: (i) described the assemblage of flower visitors and effective pollinators of *C. alpina*, (ii) recorded the abundance of *M. pallidus* spiders and their prey selection patterns, (iii) evaluated the behavior of flower visitors in response to the presence of spiders and/or dead prey, and finally (iv) assessed the probability of pollination events and plant reproductive success in response to the presence of spiders and/or dead prey.

2. Methods

2.1. Study species

C. alpina Poepp. is a terrestrial non-autogamous, self-compatible, nectarless orchid that occurs in sandy and dry slopes across Patagonia (Correa, 1969; Clayton and Aizen, 1996). Our study was carried out in a large natural population of *C. alpina* covering an area of approximately 0.5 ha in the lower Challhuaco valley (41° 12' S; 71° 19' W), within the Nahuel Huapi National Park in north-western Patagonia, Argentina. This orchid is one of the first species flowering in the study area in early spring. Flowering plants produce usually one, rarely more, loose racemose inflorescences up to 40 cm height bearing one to nine yellow-orange flowers, with two friable pollinia each. The flowering season in the study area extends from October to November and encompasses 30 to 40 days. During that period, individual flowers remain open up to three weeks. In natural populations, only a small fraction (usually <10%) of all the senescent flowers have one or both pollinia removed and a smaller fraction (usually <5%) have at least part of a pollinium inserted into the stigmatic cavity (Clayton and Aizen, 1996). The presence of a well-developed rostellum precludes automatic, within-flower self-pollination. Thus, all pollination in this species is mediated by insects, but the guild of flower visitors that behave as effective pollinators was unknown. The capsule fruits ripen in approximately 20 days after flower senescence and each fruit produces numerous tiny seeds that are wind dispersed (Dressler, 1990).

M. pallidus F. Cambridge, is a small crab spider (4 ± 2 mm) of the family Thomisidae, that employs a sit-and-wait strategy to ambush flower-visiting insects. Species of this family use enlarged raptorial forelimbs, rather than a web, to capture prey (Legrand and Morse, 2000; Schmalhofer, 2001) and are active during daytime (Greenstone and Bennett, 1980). In the study area, females of *M. pallidus* preferentially use *C. alpina* flowers as foraging sites during early spring. In particular, although female spiders were also seen in few other flowering plants, especially *Anemone multifida* (Ranunculaceae), their occupancy rate in these other species was significantly lower (<2%; personal observation) than their presence and constancy in *C. alpina* (i.e. 2–25%, see results). Spiders are both insectivorous and arachnivorous, but insects comprise 75–90% of their diet (Nyffeler, 1999). *M. pallidus* shows a clear sexual dimorphism, where adult females are bigger and more active predators than males (Minervino, 1993). In addition, this species exhibits good camouflage abilities, as many other species in the family (Legrand and Morse, 2000; Schmalhofer, 2001; Thery and Casas, 2002). Furthermore, female crab-spiders spend much time in choosing the foraging site in comparison with other hunting arthropods, which suggests that the costs of moving between foraging sites in this group may be high (Greco et al., 1995; Kevan and Greco, 2001).

2.2. Orchid pollination and reproductive success

During the 2001 flowering season, we tagged and monitored 348 plants of *C. alpina*, representing all the flowering plants of this species within the study area. *C. alpina* populations showed a clumped distribution but flowering plants within the studied population were randomly distributed, with distance between neighboring plants ranging from few centimeters to 30 m. Each flower from each inflorescence within a plant was further identified by its relative vertical position and followed throughout its lifespan. We monitored each flower every three days from bud to senescence recording pollination events as either pollinia removal (r) from the anther, when the whole pollinia or at least one pollinium were absent from the anther, or pollinia insertion (i) when the whole

pollinia or at least a piece of a pollinium were present in the stigmatic chamber. Because of strong pollen limitation and full self-compatibility, these two pollination events can be used as good proxies of male and female pollination success, respectively (see also Nilsson, 1992). At the end of the 2001 flowering season, we recorded reproductive success by counting the total number of fruits per inflorescence, and we estimated total number of seeds per fruit by measuring fruit size (length and width; see data analysis).

Orchid pollination success during the 2001 flowering season was compared with data recorded for the same population from 1998 to 2003. In those years, before and after 2001, we estimated integrated pollination success over the flowering season by recording number of inflorescences, number of flowers and removal and/or insertion rates at the end of each flowering season using a random sample of 100–200 plants out of 300–600 individuals. The abundance of crab-spiders was roughly constant over all those years, being found in approximately 10% of the flowering plants in the population (unpublished data).

2.3. Flower visitor and pollinator assemblage

We described the assemblage composition and visit frequency of flower visitors by observing flowers during 200 periods of 10 min each, distributed over the 2001 flowering season (>33 h of observation) and during the peak of insect activity (9.30 am–5.00 pm). The average number of flowers observed at a time was 7.0 ± 0.3 (mean \pm SE) flowers from a total of 1 to 4 inflorescences. During each census, we recorded the identity and abundance of all the insects visiting the flowers. We considered a flower-visitor to be an effective pollinator of *C. alpina* if it carried pollinia or was observed removing and/or inserting pollen. In addition, we recorded presence–absence of spiders (as a measure of direct predation risk) and/or dead prey lying inside the flowers (as a measure of indirect predation risk), as well as total number of flowers on the patch, temperature, and time of day. Of the total of 200 observation periods, 132 (66%) included only inflorescences without spiders and/or dead prey, 52 (26%) included at least one inflorescence with at least one spider present but no dead prey, 9 (4.5%) included at least one inflorescence with dead prey but no spider, and 17 (8.5%) included at least one inflorescence with both, spiders and prey present.

2.4. Crab-spider abundance and diet

On each observation date, we also recorded the presence/absence of crab-spiders on each of the 348 marked *C. alpina* individual and the presence of dead flower visitors lying inside flowers. In order to determine and characterize the spider's prey assemblage, we recorded taxonomic identity, body size (total body length and thorax width) and body weight of the dead prey lying inside the flowers. Supplementary observations showed that the incidence of pollination events did not differ between flowers with manipulated and unmanipulated dead prey. Yet, to avoid errors in body size and weight measurements, due to desiccation and partial mass consumption by the spiders, these measurements were taken from captured individuals right after censuses of flower visitors. At the end of the flowering season, for those inflorescences that harbored crab-spiders at least once, we calculated the minimum number of days with presence of spider(s) and/or dead prey, and total number of dead prey per inflorescence.

2.5. Data analysis

Total pollination success of *C. alpina* was calculated as the proportion of flowers where we recorded at least one pollination event (i.e. removal or insertion of at least one or part of a pollinium) over the total number of flowers observed in each flowering season. Reproductive success was calculated as the number of fruits per inflorescence. In order to estimate seed set with the lowest impact on the natural population, we developed a function to relate fruit size and number of seeds. We collected 30 fruits from a nearby population and measured their size (length and width), calculated fruit volume, and counted the number of seeds per fruit. This resulted in the following equation relating fruit size and number of seeds: $y = 0.0232x - 13557$, $R^2 = 0.67$, $p < 0.01$ (M.A. Aizen unpublished data). Using this linear relationship we estimated the number of seeds per fruit in our population by measuring fruit size in the field.

Data on visit frequency were analyzed with a multiple regression using the number of flowers observed, time of day, temperature, presence or absence of spiders, and presence or absence of dead-prey as the variables explaining abundance of insects arriving during each 10 min observation period.

To establish the spider preferences for different prey species, we used Manly's Index of diet preference. This index estimates a predator's prey preference related to the available items (Manly et al., 1972). The estimator α_i represents the relative contribution of each item in the diet, under the assumption that all available items are equally abundant (Chesson, 1983). If the consumer's diet consists entirely of the i food type, therefore $\alpha_i = 1$. In contrast, if the i food type is not present in the diet, $\alpha_i = 0$. Assuming that the resource density was constant through the entire observation period, we used the non depletion equation:

$$\alpha_i = \frac{r_i/n_i}{\sum_{j=1}^m r_j/n_j}$$

where $i = 1, 2, \dots$ and m is the number of prey categories. The variable r is the abundance of a given prey item on the spider's diet (dead flower visitors found on flowers) and n represents its abundance in the population (i.e. abundance of each flower visitor during visit frequency surveys) (Chesson, 1983). Prey types which are consumed relative to their abundance in the environment will have $\alpha_i = 1/m$, $\alpha_i > 1/m$ indicates a preference for a particular prey type, and $\alpha_i < 1/m$ indicates selection against a specific item of prey.

In order to assess the effect of predation risk on plant fitness (i.e. pollinia removal, insertion and fruit production), we divided our database into the following categories: inflorescences in which no spiders or dead prey were observed ($n = 191$), those in which a spider was observed at least once ($n = 115$), or else those in which a spider and dead prey was observed at least once ($n = 75$) during the flowering season. These three categories represented our "treatments" called: none (control), spider, and spider + prey. We did not find inflorescences just with dead prey and no spider. We used a generalized linear model assuming a binomial distribution of residuals and a logit link function. The data were first fitted to an initial model and then least significant variables were progressively removed from the model through backward selection until a minimal appropriate model was obtained (i.e., a simplified model in which all terms are significant). Single factors or variables incorporated into significant interactions were maintained in the minimum adequate model (Crawley, 1993). We verified the ratio of residual deviance to degrees of freedom and no overdispersion was detected. The initial models fitted to the data were one for each of the three dependent variables Removal/Insertion/

Fruit = treatment*number of flowers per inflorescence, where treatment represents inflorescences either control, with spider, or with spider + prey and * represents the sum of the influences of two-way interactions between the treatment factor and the single variable number of flowers per inflorescence. Analyses were conducted by using the 'glm' function in the stats package of R statistical software. Finally, a multiple regression analysis was performed to assess whether the minimum number of days with presence of spider(s) and/or dead prey and the total number of prey per inflorescence can modify fruit and seed set. All data analyses were done using the R software environment (the R freeware statistical package, R Development Core Team, 2008).

3. Results

3.1. Orchid pollination and reproductive success

C. alpina pollination and reproductive success during the 2001 flowering season were low. Of 1422 individual flowers, 81% did not experience any pollination event (Fig. 1). From the 19% that showed at least one pollination event (i.e. pollinia removal, insertion or both), 93% exhibited removal of at least one of the pollinia, while 51% exhibited at least part of a pollinium inserted into the stigmatic cavity. In general, pollinia insertion without pollinia removal represents a rare event. The total number of fruits produced was 86, representing 6% of the total number of flowers. At the individual level, 40% of the 348 studied plants showed at least one event of pollen removal, but less than 27% had pollen deposited on the stigma of at least one flower, and 14% of the plants produced at least one fruit. Finally, the total number of seeds produced in the population, by those 86 fruits, was approximately 1,520,880, with an average value per fruit of 17,685 (± 1175) seeds. It is important to note here that the small difference in the proportion of flowers that showed a pollinia or part of a pollinium in the stigma of a flower (insertion event) versus those that set fruit (i.e. 9.7 vs. 6%, respectively) could be accounted by pollinium misplacement. Sometimes, stigmas were observed with a pollinium, or a piece of a pollinium, hinging from its border but probably not making real contact with the sticky stigmatic surface.

The pollination success observed in 2001 (6%) was consistent with the data collected from 1998 to 2003 (Fig. 1). The percent of flowers that experienced any pollen removal event was always less than 20% and those that showed insertion events were less than

10% of the total flowers per season (with the exception of 1998). Additionally, the proportion of male and/or female events was relatively constant among flowering seasons (Fig. 1).

3.2. Floral visitor and pollinator assemblage

The flowers of *C. alpina* were visited inconsistently. In 59% of the censuses, no flower visitors were observed arriving to any of the flowers of the patch. On the other hand, in those periods where we recorded visitors, the average number of insects per flower/10 min was 0.43. Hymenoptera accounted for 89% of the 272 flower visitors, while 7% were Diptera, and 4% were individuals belonging to the orders Lepidoptera and Coleoptera (Table 1). The most abundant families were Syrphidae (Diptera) at the beginning of the season and Halictidae (Hymenoptera) throughout the season. In particular, the most frequent species arriving or remaining on the flowers (76% of the insects observed during the visit frequency) was a bee of the family Halictidae, *Ruizantheda mutabilis* (Spinola 1851). This species was observed visiting *C. alpina* as a mating place, aggregating inside the flowers in large numbers (pers. obs.). However, it was never observed removing and/or inserting pollinia.

Of all flower visitors, only three species behaved as effective pollinators of *C. alpina* (Table 1). *Bombus ruderatus* (Fabricius, 1775) (Hymenoptera: Apidae) was the main effective pollinator introducing almost its entire body inside the flowers, allowing the removal and insertion of pollinia. This behavior was observed only a few times, but in all cases, the individuals visited two to four flowers within the same inflorescence before abandoning the patch. The other two species, *Svastrides melanura* (Apidae) and *Trichophthalma conmutata* (Nemestrinidae), were seen only once each with a pollinium attached to their thorax, outside a flower. In both cases, the pollinium hindered their ability to fly but visits by these insects, through dislodging a pollinium, may still be involved in orchid self pollination.

Table 1

Total number of individuals per insect taxa recorded during a total of 33 h of observations (visitor abundance) and found as prey inside *C. alpina* flowers (prey abundance) during the 2001 flowering season. Insect taxa in bold highlight effective pollinators from all floral visitors (i.e. individuals observed carrying pollinia). Manly's Index for the prey captured by *M. pallidus* is represented by alpha, which shows the relative contribution of every item in the diet, under the assumption that all the items are equally abundant. The values of α can vary from 0 to 1, i.e. from unacceptable prey to exclusive prey item. Mean ($\pm 1SE$) dry weight (mg) and body size (mm) are also provided.

Insects	Visitor abundance	Prey abundance	α	Weight (mg)	Body size (mm)
<i>Lepidoptera</i>	5	2	0.04	10.15 \pm 0.35	8.5 \pm 1.0
<i>Coleoptera</i>	7	0	—	6.25 \pm 2.31	4.46 \pm 1.47
<i>Diptera</i>					
Syrphidae	11	6	0.05	10.26 \pm 2.77	9.53 \pm 0.19
Bombilidae	0	3	—	0.66 \pm 0.05	4.79 \pm 0.28
Nemestrinidae					
<i>Trichophthalma conmutata</i>	1	1	0.100	24.50 \pm 0.0	11.26 \pm 0.0
Other Families	7	7	0.100	3.09 \pm 1.95	7.81 \pm 3.20
<i>Hymenoptera</i>					
Halictidae	24	2	0.008	7.85 \pm 0.65	5.94 \pm 0.33
<i>Ruizantheda mutabilis</i>	206	60	0.029	3.68 \pm 0.25	7.30 \pm 0.09
Apidae					
<i>Svastrides melanura</i>	1	0	—	40.91 \pm 0.0	7.91 \pm 0.0
<i>Bombus ruderatus</i>	3	0	—	61.43 \pm 3.65	15.41 \pm 2.74
Formicidae	1	6	0.601	0.23 \pm 0.02	4 \pm 0.13
Other Families	6	4	0.067	3.83 \pm 1.26	5.58 \pm 0.91
Thomisidae	0	1	—	1.00 \pm 0.0	3.01 \pm 0.0

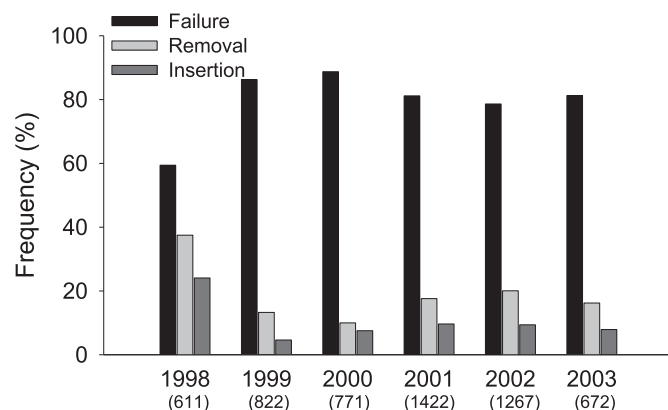


Fig. 1. *Chloraea alpina* (Orchidaceae) pollination success along six flowering seasons. The percentages represent number of flowers presenting pollinia removal, insertion, or no success over total number of flowers tallied per season. Sample size is indicated below each year. Note that percentages do not add strictly to 100% given that flowers that have both male (removal) and female (insertion) reproductive events are included in both bars.

The multiple regression analysis showed that while the presence of a spider ($t = 0.72$, $p = 0.47$) and/or dead prey ($t = 0.15$, $p = 0.88$) inside the flowers did not alter the abundance of flower visitors arriving to *C. alpina*, number of flowers observed ($t = 4.94$, $p < 0.0001$), time of the day ($t = 2.22$, $p = 0.028$), and current temperature ($t = 2.49$, $p = 0.014$) had a significant effect on relative abundance of flower visitors. In particular, more flower visitors were observed in larger flower patches, early during the day (peak at 12:00–1:00 pm), and mostly under lower than higher air temperatures (peak at 10° to 15 °C). Finally, there was no temporal variation (seasonally or daily) nor difference in the behavior between flower visitors and effective pollinators for any of these biotic and abiotic variables measured (data not shown).

3.3. Crab-spider abundance and diet

We observed a total of 240 individuals of *M. pallidus* during the 2001 flowering season. The occupation rate of *M. pallidus* reached 25% of the sampled plants, with at least one spider during the peak of the flowering period; while the lowest value was 2%, observed during both the first and last days of the flowering season. The average occupation value over the orchid flowering season was 10.5% of all the sampled plants, 8.2% of the inflorescences and 2.2% of the flowers. Finally, we estimated that individual spiders remained on an inflorescence, on average, 3.5 ± 3.0 d (mean \pm SD) while dead prey remained inside flowers on average 7.6 ± 4.4 d (mean \pm SD).

The spider's diet reflected a mixed foraging strategy but was predominantly insectivorous (Table 1). During the 38 days of the flowering season, the spiders captured 91 insects and one individual (a male) of their own species. Insect prey belonged to three orders: Hymenoptera (79.1%), Diptera (18.7%), and Lepidoptera (2.2%). The most common prey item was *R. mutabilis* (Halictidae), which comprised 65.2% of dead preys left by spiders in the flowers. Members of other taxonomic groups did not exceed 7%, and the most abundant families after the Halictidae (67.4%) were the Syrphidae and Formicidae family with 6.5% each. *M. pallidus* did not show a preference for any prey type, except for ants which seem to be preferred based on their occurrence as flower visitors (Table 1). Yet, overall, the results suggest that the spiders catch their prey as a function of their availability on flowers.

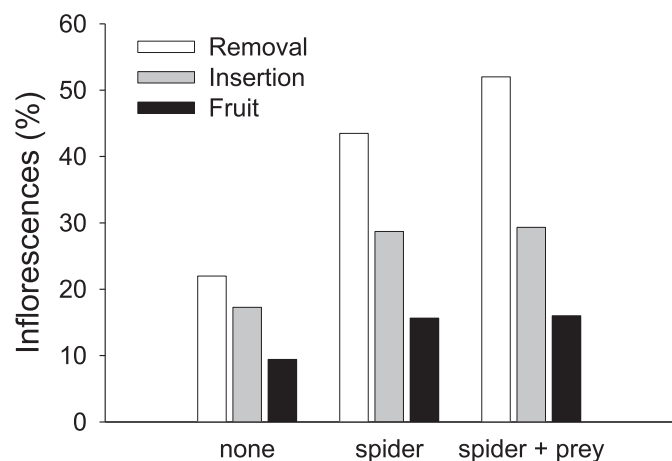


Fig. 2. Pollination and reproductive success of *C. alpina* (Orchidaceae) inflorescences that harbored no spiders (none), at least one crab spider (spider), or at least one crab spider plus dead prey (spider + prey) during the 2001 flowering season. Bars represent the percentage of inflorescences that recorded the presence of at least one pollinia removal (male pollination event), pollinia insertion (female pollination event), or developed fruit(s) (reproductive event) at the end of the flowering season per category.

3.4. Orchid reproductive success and crab-spiders

Contrary to our expectations, inflorescences that harbored spiders and/or spiders + dead prey during the flowering season had a higher pollination success and equivalent reproductive success than those inflorescences with neither spider nor dead prey (Fig. 2). Surprisingly, the probability that an inflorescence had male (removal) and female (insertion) pollination events increased, on average, twice as much on inflorescences that harbored spiders and/or dead prey than inflorescences that were never selected as hunting sites by spiders (Fig. 2). In the case of removal events, only inflorescences that harbored spiders + prey were significantly different from control ones (i.e. none) but inflorescences with just spiders were not, whereas for insertion events both 'treatments' were significantly different from inflorescences that lack both spiders and prey (Table 2). However, the probability that an inflorescence developed at least one fruit was not different among inflorescences housing or not housing crab spiders and/or dead prey during the flowering season (Table 2, Fig. 2). Number of flowers per inflorescence did not interact with the treatment factor in almost all models and therefore was excluded (see Table 2). The only exception was in the case of pollinia removal, where we detected an interaction between number of flowers per inflorescence and the treatment "spider + prey" (Table 2), indicating that spiders might be colonizing inflorescences that could be particularly attractive to pollinators.

Seed set did not differ between inflorescences with spiders and spider-free inflorescences: seeds/fruit $19,376.14 \pm 1,713.51$ and $17,102.61 \pm 2,436.71$ (mean \pm SE), respectively ($t = -0.78$, $d.f. = 46$, $p = 0.63$). Similarly, multiple regression analyses indicated that fruit and seed set were not significantly modified by the minimum number of days with presence of spiders (fruit set: $t = 0.79$, $p = 0.43$; seed set: $t = 1.89$, $p = 0.06$), nor by the minimum number of days with presence of dead prey (fruit set: $t = 0.04$, $p = 0.96$; seed set: $t = 0.79$, $p = 0.43$) or total number of prey per inflorescence (fruit set: $t = -0.28$, $p = 0.78$; seed set: $t = -0.63$, $p = 0.53$).

4. Discussion

Strong top-down effects of flower ambush predators should be expected only when predators alter the density or behavior of effective pollinators via consumptive and non-consumptive effects. In our study system, we observed a relatively diverse (i.e. >15 spp) and abundant (i.e. ~8 individuals/h) flower visitor assemblage throughout the flowering season of the rewardless orchid *C. alpina*. Yet, most of the flower visitors do not behave as effective pollinators and most effective pollinators were not observed as prey for the crab-spider *M. pallidus*. Furthermore, the non-consumptive effects of this predator, mediated by the dead corpses of flower visitors found inside flowers, were also weak to non-existent

Table 2

Result of the generalized linear model (GLM), with binary distribution, assessing the effect of treatment (none, spider, spider + prey) and number of flowers per inflorescence on presence/absence of removal, insertion, or fruit events per inflorescence.

	Removal		Insertion		Fruit	
	z-value	P-value	z-value	P-value	z-value	P-value
None-spider	1.22	0.2234	2.33	0.0197	1.51	0.130
None-spider + prey	3.71	0.0002	2.16	0.0306	1.31	0.190
Flowers	1.92	0.0550			1.71	0.088
Flowers*spider	-0.03	0.9791				
Flowers*spider + prey	-2.52	0.0011				

Significant results ($P < 0.05$) are highlighted in bold.

probably due to the highly erratic behavior of effective pollinators. Hence, in contrast to our expectations, we found a neutral effect of the spider on *C. alpina* fruit and seed set; and thus, in this system, the ambush predator can be considered mostly as a commensalist towards the orchid (Fig. 3).

The low reproductive success documented for *C. alpina* in this study (~6%) is not only consistent with previous estimations for this species (Clayton and Aizen, 1996) but also with the expected reproductive success predicted for rewardless orchids in general (Nilsson, 1992; Neiland and Wilcock, 1998; Tremblay et al., 2005). Yet, it is the lowest reported so far among other rewardless *Chloraea* spp. and *Gavilea* spp. (Orchideaceae, Chloraeinae), ranging between 12 and 90% (Lehnebach and Riveros, 2003; Humaña et al., 2008; Valdivia et al., 2010; Sanguinetti et al., 2012; Sanguinetti and Singer, 2014). As previously concluded, we believe the difference in reproductive success among these orchid species directly relates not only to the availability and abundance of insect pollinators but also to flower morphology (Sanguinetti et al., 2012; Sanguinetti and Singer, 2014). Low fruiting success has been associated with species with more “open” flowers (i.e., flowers with a larger distance between the column and the labellum) such as the case of *Chloraea crispa* and *Chloraea lamellata* (12–15.6%) versus *Chloraea galeata* and *Chloraea chrysantha* (>80%) (Humaña et al., 2008). A smaller flower cavity allows even smaller size bees, flies and beetles to act as effective pollinators, increasing the probability of a high fruiting success (e.g. Humaña et al., 2008; Sanguinetti et al., 2012). In the case of the open flowers of *C. alpina*, the most common flower visitors were too small to dislodge a pollinium, even when present in larger numbers (i.e. >40 individuals), such as noted for the halictid bee *R. mutabilis*, during their aggregated mating behavior. On the other hand, flower visitors that are large enough to detach and carry pollinia such as bumblebees (both the exotic *B. ruderatus* and originally the native *Bombus dahlbomii*, locally rare in the past decade, Morales et al., 2013) were infrequent, quickly learning to avoid deceptive flowers. Hence, we propose that the low fruiting success observed in *C. alpina* should be directly associated with the scarcity of pollinators combined with its flower morphology, which excludes many flower visitors to act as effective pollinators.

Besides the net outcome of plant–predator associations, it may be expected that ambush predators should alter some behavioral aspects of their prey. However, we did not detect any direct effect of predators on pollinator or flower visitor behavior. Non-significant

effects of ambush predators on their prey is often correlated with three main factors: (i) low natural densities of predators in the host plant population (e.g. Elliot and Elliot, 1991; Ott et al., 1998), (ii) large body size of pollinators (e.g. Dukas and Morse, 2003, 2005; Reader et al., 2006; Gonçalves-Souza et al., 2008), and (iii) high flower rewards that could overcome predation risk (Morse, 1986; Jones, 2010; Llandres et al., 2012). In our study system, these three factors might have worked together decreasing the relative importance of crab-spider predation risk on pollinator and flower visitor behavior. First, the density of *M. pallidus* in the studied population was always low, so that few individual pollinators actually suffer an attack that might deter future visits. This may explain why even the choices of the small halictid bees, *R. mutabilis*, were not affected by spider presence. Second, as suggested by Beckerman et al. (1997), spiders can actively hunt prey that are 10–30% larger than their body mass; yet, the large body mass of *B. ruderatus* (61.4 mg) and eventually *B. dahlbomii* (84.8 mg), which exceeds the maximum body weight that *M. pallidus* was able to capture during our observations (24.5 mg), make them unsuitable prey items, decreasing the importance of potential antipredatory behavior associated with risk of predation in these bumblebees. Nonetheless, at least one species of insect observed carrying a pollinium was also a prey (see Table 1), indicating that *M. pallidus* may have a minimal direct effect in increasing pollen limitation in *C. alpina*. Finally pollinators, especially large bees and bumblebees, are known to have high learning capabilities and be able to discriminate against and avoid deceptive flowers (Renner, 2006). Thus, the rewardless condition of *C. alpina* related to pollinators' learning abilities provides a more plausible explanation for the observed decrease in reproductive events during the length of the flowering season, than any possible antipredator behavior. After all, *C. alpina*, as other rewardless orchids from temperate regions, flowers at the onset of the spring when emerging bees and bumblebee queens just begin their learning process (Smithson and Gigord, 2003; Internicola et al., 2008).

While the larger size of effective pollinators may have been a limitation for the observed lack of direct effects on pollinator foraging behavior, still, visitation by effective pollinators could had been deterred by the presence of dead corpses remaining on the flowers. Previous studies have reported that honeybees, as well as bumblebees, are able to use indirect cues of predation risk such as dead corpses in an attempt to avoid predation (Dukas, 2001b;

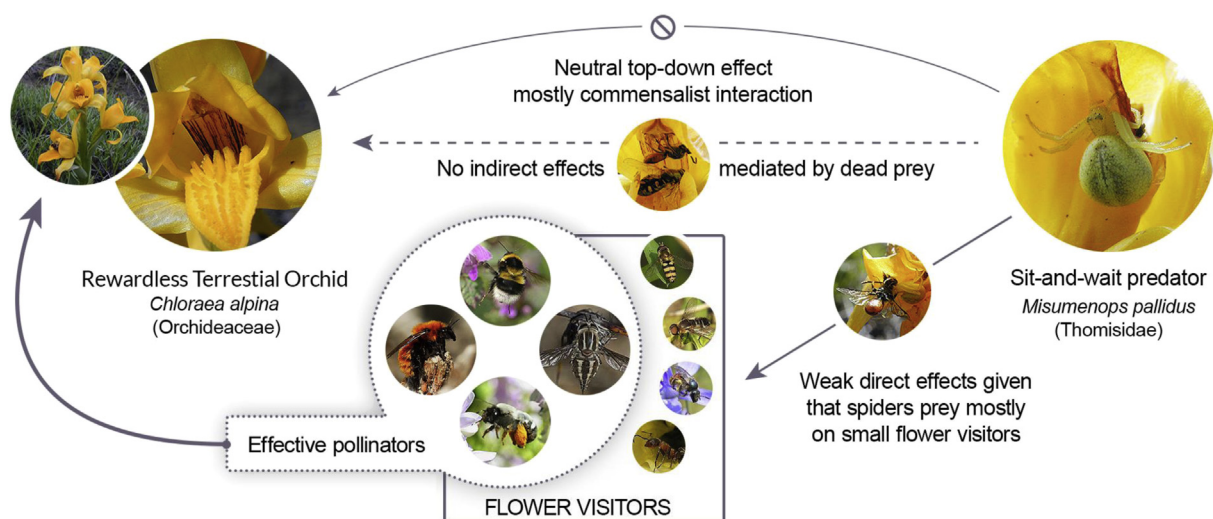


Fig. 3. Summary of the direct and indirect effects of predation risk driven by the crab-spider *Misumenops pallidus* (Thomisidae) on the reproductive success of the terrestrial rewardless orchid *Chloraea alpina* (Orchideaceae).

Abbott, 2006). In contrast, our results revealed no evidence for this potential indirect effect of spiders on pollinator behavior. Instead, contrary to our expectations, we found a positive relationship between pollinia removal and insertion and the presence of spiders and prey (Fig. 2). We believe that the absence of such indirect effects can be related to the erratic visitation frequency of these effective pollinators as well as to the low density of spiders and dead prey in the orchid population we studied. Moreover, we believe this positive association can be interpreted as spiders using similar cues as those used by the effective pollinators when choosing an inflorescence as a hunting site, as previously suggested by Wilkinson et al. (1991).

Given that these results come from an observational study, manipulative experiments increasing the frequency of direct and indirect predation risk in flowers could be used to further test their influence on pollinator and flower visitor behavior. Particularly, experiments assessing the effect of predator presence and/or indirect cues (i.e. dead prey or any object that leads to neophobia) on each species of prey or few size classes (e.g. small and large), separately, would be needed to confirm whether the lack of interaction between effective pollinators and spiders (Fig. 3) is mainly due to insect body size or other traits. Furthermore, because pollinators and their predators often share innate and learned preferences for high quality flowers, an experimental approach whereby spiders are added or removed from inflorescences to control for effects due to choice of inflorescences by spiders is needed.

All in all, in this system, the three trophic levels scarcely interact with each other in a way that could impair plant fitness. Besides the combination of low density of small ambush predators and large body size of effective pollinators, the lack of rewards offered by this plant species may also contribute to diminishing the frequency and magnitude of the pollinator–predator interaction. To date, all empirical studies assessing the effect of ambush predators on plant fitness have been focused on flowering plants that provide high rewards to flower visitors. Thus, it is not possible to assess the relative contribution of deceptive flowers and the high contrast between large effective pollinators and small predators towards the neutral response observed here. However, we propose that in rewardless flowering systems, because the magnitude of the plant–pollinator interaction is low and unpredictable, a mutualism exploiter has a small chance to play a strong selective role. Thus, we suggest that for pollination systems where the flowering plants are deceptive or else offer minimal rewards, the neutral effect of predation risk on plant fitness may be a common phenomenon. Future descriptive as well as manipulative experiments varying levels of floral rewards and predation risk will elucidate the relative role of top-down versus bottom-up factors on pollinator behavior and overall plant fitness.

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