

## CONDITIONAL OUTCOMES OF INTERACTIONS: THE POLLINATOR–PREY CONFLICT OF AN INSECTIVOROUS PLANT

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**Abstract.** Insectivorous plants benefit from trapped, dead insects as a source of nutrients. On the other hand, most insectivorous plants depend on free-moving, live insects as pollen vectors for sexual reproduction. In *Pinguicula vallisneriifolia* Webb, the spatial and temporal coincidence between flowers and the first distal leaves presents a potential conflict between the plant–pollinator and plant–prey systems. To examine this possibility, I combined an extensive regional survey to identify the pollinator assemblages and to analyze prey capture with a local intensive study to investigate the mechanisms of interference between the two systems. I analyzed the possible conflicting functions of pollination vs. insectivory under contrasting abiotic scenarios. Field observations and experiments confirmed the pollinator role of small flower visitors and the interference between the plant–pollinator and plant–prey systems. The degree of this interference is determined by the size and foraging behavior of pollinators, given that only small pollinators become entangled on leaves. Plants in bloom had a greater probability of capturing thrips than did plants without flowers. Because *P. vallisneriifolia* is pollen limited, the capture of small pollinators depleted the availability of the resource that limited plant reproduction. The pollinator–prey conflict is, however, environment specific, because the shadiness of the microsite where the plant grows determines both the distribution and abundance of different-sized insect species and the retention capacity of the mucilage. The present results indicate that plant–pollinator and plant–prey interactions can be strongly determined by species-specific eco-physiological responses of flying insects and sessile plants to abiotic characteristics of the environment. The physical environment affects the outcomes of plant–animal interactions and, ultimately, the balance between mutualistic and antagonistic systems.

**Key words:** *carnivorous plant; Lentibulariaceae; Mediterranean ecosystem; microclimate; mucilage retention; Pinguicula vallisneriifolia; plant reproductive ecology; pollen limitation; pollinator–prey conflict; pollinator size; pollinators and sunlight; prey capture.*

### INTRODUCTION

The outcomes of interspecific interactions are affected in predictable ways by current ecological conditions (Bronstein 1994). Several biotic factors, such as age and size of individuals, density and spatiotemporal structure of populations, and the general community context, have been outlined as determining the nature of interactions (Thompson 1988, 1994). Moreover, abiotic conditions can also play a major role in determining the outcome of interactions (Dunson and Travis 1991). In this respect, site-specific effects in plant–insect relationships may be particularly important, because the physical environment governs the ecophysiology of both plants, due to their sessile lifestyle, and insects, due to their small size. Therefore, the ecological and evolutionary consequences of plant–insect interactions can vary between contrasting environments, even at a restricted spatial scale. Evidence supporting this possibility has recently been provided by Herrera (1995*b*, 1997) for plant–pollinator systems,

and by Zamora (1995) for plant–prey interactions of insectivorous plants. A further possibility is that the full ranges of outcomes of these plant–animal relationships are conditional, and that the degree of interference between mutualistic and antagonistic systems depends on the abiotic characteristics surrounding the interaction. I have tested this hypothesis for an insectivorous plant, which faces possible antagonism in the diverse relationships between plant and pollinators, plant and prey, plant and herbivore and/or plant and kleptoparasite.

Despite the fact that plants experience, simultaneously or sequentially, mutualistic and antagonistic interactions with animals, few studies have examined the combined effects of these opposing interactions on plant evolutionary ecology (see Strauss and Armbruster 1997 for a recent review). For example, studies commonly assume that pollinators behave exclusively as plant mutualists. However, pollinators may act also as floral herbivores (Faegri and van der Pijl 1979), seed predators (Pellmyr 1989), or as vectors of plant disease (Jennersten 1988). In this context, the interplay of plant–pollinator and plant–prey interactions has sel-

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dom been explored in the ecological literature, and only anecdotal references are available (Juniper et al. 1989). Here, I analyze the effect of local physical conditions on the possible conflict between plant-pollinator and plant-prey systems.

Ideally, an insectivorous plant would, independently, attract prey to traps and pollinators to flowers. However, many traps use traits that also attract pollinators, such as color, scent, nectar, and UV guides (Joel et al. 1985), thereby blurring the distinction between prey and pollinator attraction. This conflict of interests could be alleviated by spatial or temporal separation of traps and flowers. For example, the widespread aquatic genus *Utricularia* sets traps underwater, but flowers above the water surface. In most terrestrial carnivorous plants, the flowering stalk extends high above the trapping devices, separating flowers from traps (Givnish 1989, Juniper et al. 1989). This solution applies to most species of *Pinguicula* (Lentibulariaceae), in which flowers stand well above the rosette of leaves. However, in *P. vallisneriifolia* Webb, an endemic insectivorous plant of wet rocky habitats and cliffs in southeastern Spain, the flowers not only open near the adhesive leaves, but emerge as the leaves sprout. Thus, the possible pollinators of *P. vallisneriifolia* are also its potential prey.

*Pinguicula vallisneriifolia* produces two types of glandular leaves. The first 5–7 leaves form a rosette tight against the rock wall; these are followed by erect, distal leaves. A typical reproductive plant produces 1–4 spurred flowers at the top of leafless, 8–18 cm stalks (Zamora et al. 1996). The 10–30 cm long distal leaves occupy the same spatial zone as the flowers, both overhanging the rock wall on which the plant grows. The blooming period (May–June) precedes the period of maximum prey capture (July). However, a typical flowering individual has 2–4 fully functional distal leaves. Thus, the spatial and temporal coincidence of flowers and the first distal leaves creates the potential for interference between the plant's pollination and insectivory systems. This carnivorous Mediterranean species inhabits a broad range of irradiance regimes, so that the variability of interactions can be assessed in contrasting abiotic scenarios. Indeed, local abiotic conditions govern prey capture of *P. vallisneriifolia*, because both the abundance of flying insects and the retention capacity of leaf mucilage varies between habitats (Zamora 1995, Zamora et al. 1998).

In this paper, I assess possible conflicts between pollination and insectivory for *P. vallisneriifolia* under contrasting scenarios, and their consequences for the plant's reproductive success. To explore these issues, I first carried out a large-scale study to identify the pollinator and prey assemblages over the entire geographical range of *P. vallisneriifolia*. Later, I performed an intensive local study, combining observations and field experiments, to investigate the possible mechanisms of interference between the plant-pollinator and plant-prey systems. Specifically, I analyzed: (1) the

plant breeding system and pollination ecology of this carnivorous plant; (2) the spatial distribution of pollinators and prey in relation to microclimatic conditions; (3) the identity and capture rate of insects trapped on leaves during the flowering period, in relation to the retention capacity of the mucilage; and (4) whether flowering plants captured more or different prey than did nonflowering ones.

#### METHODS

I sampled pollinators in 11 populations distributed broadly throughout the range of *Pinguicula vallisneriifolia* in the Sierras de Cazorla, Segura y Las Villas, Spain, an orographically complex area with mountainous escarpments and narrow valleys. All populations grew on cliffs (5–80 m in height) with varying sun exposure. For each population, I recorded floral visitors for 15-min periods evenly distributed over the day (8 h of observation per population) during the peak of blooming (May–June) of 1992. These observations focused on a sector of the cliff representative of the predominant irradiance conditions in the population. Population size ranged from 150 to 600 flowering plants. All observations were conducted on cloudless days. Any insect seen moving among the flowers and making contact with the anthers and stigma was considered a likely pollinator. Some insects (e.g., Diptera) that landed and rested on the lower lip of the corolla were not considered pollinators because they did not touch the plant's sexual organs. In addition, I noted the presence/absence of small flower visitors (e.g., thrips) in the perianth of 40–60 flowers (individuals) per population. I later visited the 11 populations sampled for flower visitors during fruiting to record the percentage of flowers setting fruit, and to identify and count prey on a sample of 40–60 distal leaves per population (one leaf per reproductive plant), noting especially any floral visitors trapped on the leaves.

After collecting this broad-scale information, I made an intensive study in a population situated in the center of the geographical range of *P. vallisneriifolia*. This study site lies at the headwater of a spring surrounded by 50-m high cliffs, and harbors one of the largest populations of this endemic species (see Zamora 1995). To quantify fruit set and seed set under natural pollination conditions, I tagged flowering buds on different reproductive individuals in three sections of the vertical cliff. Although these sites were very close to each other, they received different irradiance levels and constituted three distinct habitats: (1) *sunlight habitat*, a northeast-facing section of the cliff receiving 2 h of direct morning sunlight; (2) *shade habitat*, a north-facing sector of the cliff that receives no direct sunlight and is intermediate in the irradiance gradient; and (3) *deep shade habitat*, a small cave situated at the bottom of the north-facing wall, the shadiest environment. The three habitats were located at the same altitude, with the shade and deep-shade habitats ~10 m from each

other and both ~30 m from the sunlight habitat. I quantified several abiotic variables to characterize the environment of the three habitats. Total radiation was measured using a Li-Cor LI-200 sz pyranometer sensor connected to a Li-1000 data logger (Li-Cor, Lincoln, Nebraska, USA). Air temperature and air humidity were taken using a Rotronic YA-100 (Rotronic ag, Zürich, Switzerland) combined sensor. Readings were collected simultaneously in the three habitats, consistently using the same recording point (the center of the sample zone in each habitat). Sensors were placed near the flowers of *P. vallisneriifolia*.

To test pollen limitation under natural pollination conditions, I tagged flowering buds in the three habitats. After anthesis, I hand-pollinated each flower twice, with pollen from three different donors. To determine self-fertility, I covered flower buds with cellophane bags attached to the peduncle, excluding all pollinators. I then assigned these flowers to one of three pollination treatments: (1) spontaneous autogamy in the absence of insects; (2) self-pollination, and (3) cross-pollination with pollen from other plants in the same population. Each treatment involved 30 flowers (30 plants) in the shade habitat during 1994. I harvested mature capsules prior to dehiscence, counted the seeds and aborted ovules, and weighed all seeds produced by a capsule as a group.

As with the geographic survey, I sampled flower visitors during 15-min periods distributed evenly during the day over the entire flowering period (15 May–15 June 1992) in the three habitats, where plants flowered simultaneously. In the sunlight habitat, I noted whether plants received direct sunlight during the observation period. In each period, I noted all floral visitors to plants growing in 10 m<sup>2</sup> of cliff per habitat, and with similar flowering plant density. In the same quadrats, I also periodically counted small insects in flowers (for a total of 50 flowers on 50 plants per habitat) from the beginning to the end of blooming. Nocturnal and crepuscular observations of flower visitors were also made over the flowering period.

To test experimentally the potential role of thrips as pollinators, in 1994 I caged two groups of plants, with 1.5-mm mesh tents attached to the rock wall. This mesh size excluded all flying floral visitors except thrips. I tagged 30 caged buds belonging to 30 different reproductive individuals in the sunlight habitat, another 20 different flowering reproductive individuals in the sunlight habitat, and another 20 flowering buds in the shade habitat. Additionally, 20 caged floral buds were emasculated in the sunlight habitat to test cross-pollination by thrips. Further checks confirmed that the thrips were able to pass through the mesh, whereas the other larger species of floral visitors, including small beetles, were excluded.

To quantify insect captures by the leaves, I sampled 30 distal leaves per habitat belonging to different flowering plants at the end of blooming in 1994 and again

in 1995. Later, in the laboratory, I measured leaf area and identified and counted any trapped insects.

To test specifically whether plants in bloom captured more prey than did nonflowering plants, I cut all flowering buds from 30 tagged reproductive plants in the sunlight habitat at the onset of blooming in 1995. After the end of blooming, I harvested the longest functional distal leaf from each of these tagged plants for later prey identification and determination of leaf area. In addition, I harvested other functional leaves from 30 nonreproductive plants (one leaf per plant) in the same habitat. The capture rate of distal leaves of flowering plants in the sunlight habitat was used as a control for comparing prey capture.

Statistical analyses were performed using the computer software JMP 3.1.5 (SAS Institute 1995) for Macintosh. ANOVAs were performed using type III sums of squares. Prior to all parametric analyses, I arcsine-transformed the ratio variables and log-transformed the remaining variables (Zar 1996). Throughout this paper, data are expressed as means  $\pm$  1 SE.

## RESULTS

### *Large-scale study*

During 88 h of observation, 36 floral visitors were observed at flowers of *Pinguicula vallisneriifolia* in 11 populations. The presence of medium-to-large flower visitors varied significantly with insolation, being almost absent from the shadiest populations ( $r_s = 0.697$ ,  $P < 0.05$ ,  $df = 10$ ; Fig. 1A). Furthermore, even in the sunniest populations, the presence of flower visitors was restricted to the period of sunlight on the plants. In fact, censuses grouped by insolation regime instead of by population (sunlight on flowers, presence vs. absence), revealed that 32 (89%) floral visitors were observed during the 15 h (17% of the total time) of observation when the plants were in sunflecks (12 *Bombus* sp., eight *Lasioglossum* sp., five *Anthophora* sp., three *Halictus* sp., three *Bombus terrestris*, and one *Macroglossum stellarum*). By contrast, during the remaining 65 h of observation, when no direct sunlight reached the plants, I observed only three *Bombus terrestris* and one unidentified small bee visiting the flowers. Fruiting success was also positively associated with the amount of insolation ( $r_s = 0.783$ ,  $P < 0.025$ ,  $df = 10$ ); that is, the shadiest populations had the lowest fruiting success, probably because of the scarcity of pollinators (Fig. 1B).

Unlike the medium-to-large floral visitors, thrips (*Taeniothrips meridionalis* Priesner, Thysanoptera) and beetles (*Eusphalerum scribae* Schläufuss, Staphylinidae) appeared at flowers in all but one population (Fig. 1C, D). *Taeniothrips meridionalis* is a common visitor of many other Mediterranean plant species (Bournier 1983). In some sunny populations, thrips appeared in most flowers (Fig. 1C). However, the presence of thrips

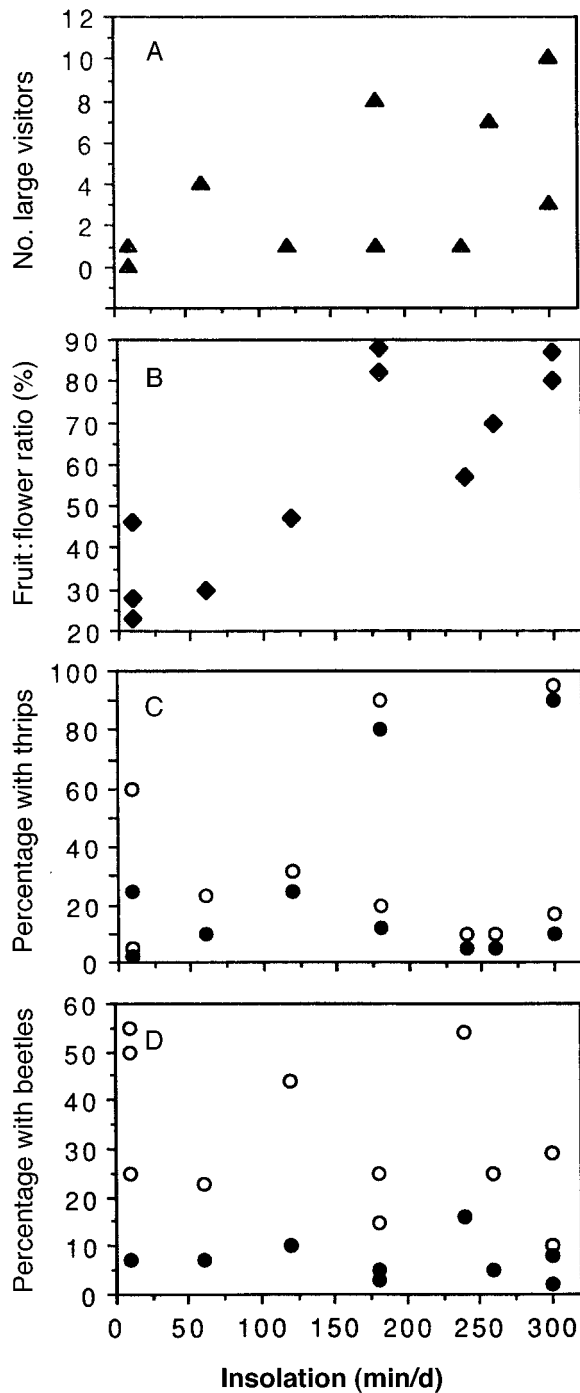


FIG. 1. Results of pollination censuses in 11 populations of *Pinguicula vallisneriifolia* broadly distributed throughout the geographic range (altitudinal range of populations: 1000–1400 m). (A) The number of medium-to-large flower visitors/8 h of observation. (B) the percentage of flowers setting fruit. (C) the percentage of flowers (●) and leaves (○) with thrips (floral visitors or prey, respectively), and (D) the percentage of flowers (●) and leaves (○) with beetles are shown in relation to total insolation time per day received by each population at flowering peak on a typical cloudless day.

and beetles did not vary significantly with insolation (thrips:  $r_s = 0.322$ , NS; beetles:  $r_s = -0.196$ , NS).

Prey counts indicated that the same species of thrips (*T. meridionalis*) and beetles (*E. scribae*) that visited *P. vallisneriifolia* flowers were also frequently trapped on the leaves. Furthermore, the abundance of these species on leaves correlated positively with their abundance in flowers (thrips:  $r_s = 0.98$ ,  $P < 0.005$ ,  $df = 10$ ; beetles:  $r_s = 0.74$ ,  $P < 0.05$ ,  $df = 10$ ; see Fig. 1C, D). In contrast, the most common medium-to-large floral visitors, *Bombylius* sp., *Lasioglossum* sp., and *Bombus terrestris*, were never found trapped on the leaves, although some rare floral visitors, such as hoverflies and butterflies, were sporadically found adhering to the leaves by their appendages. Thus, only thrips and beetles were both common flower visitors and common prey in most *P. vallisneriifolia* populations.

#### Intensive study

**Habitat differences.**—The three habitats differed markedly in mean irradiance because of the differences in exposure and degree of cliff coverage. Only the sunlight habitat received direct morning sunlight, for ~2 h, coinciding with the flowering peak of *P. vallisneriifolia* plants. Differences between habitats in mean irradiance (sunlight,  $128.2 \pm 26.5$  W/m<sup>2</sup>; shade,  $29.2 \pm 4.8$  W/m<sup>2</sup>, deep shade,  $6.5 \pm 0.6$  W/m<sup>2</sup>;  $F = 91.8$ ;  $df = 2, 32$ ;  $P < 0.0001$ ) were relatively greater than differences in air temperature (sunlight,  $19.8 \pm 1.6^\circ\text{C}$ ; shade,  $18.4 \pm 0.7^\circ\text{C}$ ; deep shade,  $17.1 \pm 0.5^\circ\text{C}$ ;  $F = 3.48$ ;  $df = 2, 32$ ;  $P < 0.05$ ) and relative air humidity (sunlight,  $50.0 \pm 4.4\%$ ; shade,  $56.3 \pm 3.7\%$ ; deep shade,  $63.6 \pm 3.4\%$ ;  $F = 3.72$ ,  $df = 2, 32$ ;  $P < 0.05$ ). These values are averages from sunrise to sunset collected at 1-h intervals in the three habitats on 8 June 1994 during a sunny day. Diurnal temperatures registered during observations of flower visitors ranged from a minimum of  $8^\circ\text{C}$  in the deep shade to a maximum of  $23^\circ\text{C}$  in the sunlight habitat, during direct sunlight. Thus, the sunlight site was the warmest and driest, the deep-shade habitat the coolest and wettest, and the shade habitat intermediate.

**Pollination and seed production.**—*Pinguicula vallisneriifolia* is self-compatible, but spontaneous autogamy does not occur due to herkogamy (30 bagged flowers set no fruit). All experimental hand-crosses resulted in a high percentage of flowers setting fruit (selfing treatment, 81.5% of flowers set fruits; cross-pollination treatment, 95.8% of flowers set fruit). Furthermore, self- and cross-pollinations resulted in similar numbers and mass of seeds (for selfing,  $145.43 \pm 18.12$  seeds per capsule,  $n = 24$ ; for xenogamy,  $165.43 \pm 16.31$  seeds per capsule,  $n = 27$ ;  $F = 0.417$ ;  $df = 1, 49$ ; NS; mean seed mass for selfing,  $12 \pm 0.1$   $\mu\text{g}$ ; for xenogamy,  $13 \pm 0.1$   $\mu\text{g}$ ;  $F = 2.104$ ;  $df = 1, 49$ ; NS). Thus, *P. vallisneriifolia* depends totally on insect pollinators for reproduction, but natural seed set could represent a mixture of selfing and cross-fertilization. Individual



TABLE 1. Effects of the pollen addition on *Pinguicula vallisneriifolia* reproductive success (values expressed as mean  $\pm$  1 SE) in sunlight, shade, and deep-shade habitats. Results of the experimental exclusions performed to test pollination by thrips (Thrips pollination) and cross-pollination with emasculated flowers (Thrips xenogamy) in the sunlight and shade habitats are also shown. Statistical results of the two-way ANOVAs comparing control vs. pollen addition treatments are presented in the footnotes.

Treatments by habitat <sup>†</sup>	Fruit: flower ratio (%)	Seed : ovule ratio (%) <sup>‡</sup>	No. seeds per capsule <sup>§</sup>	Mean seed mass ( $\mu$ g) <sup>  </sup>
<b>Sunlight</b>				
Naturally pollinated (20)	70	55.0 $\pm$ 7.1	107.2 $\pm$ 21.1	13.5 $\pm$ 0.6
Pollen addition (20)	100	72.9 $\pm$ 4.3	167.4 $\pm$ 16.1	16.0 $\pm$ 0.7
Thrips pollination (27)	22	58.2 $\pm$ 8.2	149.6 $\pm$ 28.8	12.0 $\pm$ 0.5
Thrips xenogamy (20)	20	48.2 $\pm$ 10.5	98.0 $\pm$ 9	13.3 $\pm$ 2.1
<b>Shade</b>				
Naturally pollinated (20)	35	34.1 $\pm$ 6.7	67.14 $\pm$ 16.0	12.6 $\pm$ 0.8
Pollen addition (20)	90	65.0 $\pm$ 5.2	136.7 $\pm$ 16.0	15.4 $\pm$ 0.8
Thrips pollination (20)	5	21	24	12.0
<b>Deep shade</b>				
Naturally pollinated (20)	60	52.0 $\pm$ 9.3	96.0 $\pm$ 21.4	14.9 $\pm$ 0.1
Pollen addition (20)	100	75.8 $\pm$ 4.2	183.7 $\pm$ 15.0	16.2 $\pm$ 0.6

Note: Percentages of flowers setting fruit in 1995 were 80% in sunlight, 45% in shade, and 60% in deep-shade habitat ( $n = 20$  flowers per habitat).

<sup>†</sup> The sample size (no. flowers per treatment) is given in parentheses.

<sup>‡</sup> Habitat:  $F = 3.32$ ;  $df = 2, 81$ ;  $P < 0.05$ . Treatment:  $F = 26.37$ ;  $df = 1, 81$ ;  $P < 0.0001$ . Treatment  $\times$  habitat:  $F = 0.64$ ;  $df = 2, 81$ ; NS.

<sup>§</sup> Habitat:  $F = 1.82$ ;  $df = 2, 81$ ; NS. Treatment:  $F = 28.79$ ;  $df = 1, 81$ ;  $P < 0.0001$ . Treatment  $\times$  habitat:  $F = 0.35$ ;  $df = 2, 81$ ; NS.

<sup>||</sup> Habitat:  $F = 1.80$ ;  $df = 2, 81$ ; NS. Treatment:  $F = 12.14$ ;  $df = 1, 81$ ;  $P < 0.001$ . Treatment  $\times$  habitat:  $F = 0.519$ ;  $df = 2, 81$ ; NS.

flowers bloomed for  $12.6 \pm 2.8$  d ( $n = 26$  flowers) under natural pollination conditions, and  $20.6 \pm 3.7$  d ( $n = 25$ ) for unpollinated bagged flowers.

Naturally pollinated plants in the sunlight habitat had a greater fruit set than did shade and deep-shade plants (Table 1). Nearly 100% of the flowers subjected to supplemental hand pollination set fruit in the three habitats. These flowers produced more and larger seeds per capsule than did naturally pollinated flowers. Thus, plant reproductive success was pollen limited in all habitats, regardless of the component of plant reproduction considered (Table 1).

The presence of medium-to-large flower visitors varied between habitats. In the shade habitat during 1992 and 1993, I observed only three *Bombus terrestris*, one *B. pascuorum*, and one unidentified small bee visiting flowers of *P. vallisneriifolia* during 34 h of diurnal observation. Crepuscular and nocturnal observations in the three habitats (over 10 h) revealed no floral visitor. During 1994, I observed two *B. terrestris* and one *B. pascuorum* in the shade habitat during a total of 14 h of observation, whereas no large pollinators were observed over 10 h in the deep shade. In contrast, during 8 h of observation when the sunlight habitat was exposed to sunlight (0900–1100), I counted 47 flower visitors (17 *Bombylius* sp., 12 *Lasioglossum* sp., four Calliphoridae, three *Halictus* sp., three *Pararge aegeria*, two *Osmia cornuta*, two Empididae, one *Macroglossum stellarum*, one *Pieris napi*, one *Andrena* sp., and one *B. terrestris*). In the same sector, but with-

out sunlight (1100–2000), the abundance of flower visitors fell drastically: during a similar period (8 h), I observed only seven visitors (three *B. terrestris*, two *Bombylius* sp., one *Macroglossum stellarum*, and one *Lasioglossum* sp.). Thus, even in the sunlight habitat, large insects visited flowers during the brief period when sunlight fell directly on the flowers. Medium-to-large pollinators were scarce and visited few flowers (normally 1–5 flowers) per foraging trip, probably because the flower of *P. vallisneriifolia* produces only traces of nectar (0.03  $\mu$ L per bagged flower per day,  $n = 30$  flowers). These pollinator species are common flower visitors to other plants in the same study area (*personal observation*).

Thrips and small beetles also visited *P. vallisneriifolia* flowers. Thrips (*T. meridionalis*) were very common in flowers in the sunlight habitat (Table 2), less abundant in the shade, and almost absent from the deep-shade habitat. On the other hand, beetles (*Eusphalerum scribae*) were less abundant in the sunlight habitat than in the shade and deep-shade habitats (Table 2). The abundance of thrips and beetles did not vary temporally. Thrips and beetles colonized recently opened flowers of *P. vallisneriifolia* at the beginning of blooming. Only adult thrips were seen at flowers. Observations indicated that thrips and beetles remained active at flowers of *P. vallisneriifolia* all day long, irrespective of the irradiance level. Thrips and beetles foraged actively on the anthers, thus carrying pollen grains of *P. vallisneriifolia* on their bodies ( $5.5 \pm 6.6$  grains per

TABLE 2. Abundance of thrips and beetles in *P. vallisneriifolia* flowers in the three habitats during 1994 and 1995, as indicated by the percentage of flowers with small flower visitors (thrips or beetles) and the mean ( $\pm 1$  SE) number of small flower visitors per flower at peak flowering. Statistical results of the two-way mixed-model ANOVAs (year as random effect) are presented in the footnotes.

Small pollinators	Sunlight		Shade		Deep shade	
	Percentage of flowers	No. per flower	Percentage of flowers	No. per flower	Percentage of flowers	No. per flower
1994						
Thrips†	97	4.48 $\pm$ 0.23	49	1.09 $\pm$ 0.10	3	0.03 $\pm$ 0.01
Beetles‡	5	0.04 $\pm$ 0.02	9	0.11 $\pm$ 0.03	13	0.16 $\pm$ 0.04
1995						
Thrips†	93	5.61 $\pm$ 0.34	38	1.05 $\pm$ 0.18	2	0.02 $\pm$ 0.01
Beetles‡	4	0.02 $\pm$ 0.01	17	0.19 $\pm$ 0.04	15	0.18 $\pm$ 0.04

† Habitat:  $F = 658.93$ ;  $df = 2, 744$ ;  $P < 0.0001$ . Year:  $F = 0.17$ ;  $df = 1, 744$ ; ns. Habitat  $\times$  year:  $F = 3.4$ ;  $df = 2, 744$ ;  $P < 0.05$ .

‡ Habitat:  $F = 10.36$ ;  $df = 2, 744$ ;  $P < 0.0001$ . Year:  $F = 1.56$ ;  $df = 1, 744$ ; ns. Habitat  $\times$  year:  $F = 1.40$ ;  $df = 2, 744$ ; ns.

thrips, up to 24,  $n = 20$ ;  $13.5 \pm 9.8$  grains per beetle, up to 30,  $n = 12$ ). Both species crawled repeatedly over the upper part of the corolla tube (site of anthers and the stigmatic surface), transferring pollen to the stigma. Furthermore, the exclusion experiments demonstrated that thrips provide pollination: 22% of flowers set fruit (Table 1) despite a cage effect (thrips abundance with the netting was clearly less than without). There were  $1.26 \pm 0.21$  thrips per caged flower in the sunlight habitats vs.  $0.36 \pm 0.14$  thrips per caged flower in the shade habitats, in comparison with 1994 data for the same habitat (Table 2; differences in thrips abundance between caged and uncaged flowers:  $F = 93.15$ ;  $df = 1, 393$ ;  $P < 0.0001$ , two-way ANOVA). The number and mass of seeds produced by thrips pollination resembled the results for naturally pollinated flowers (Table 1). Most thrips-mediated pollen movement was probably autogamous. However, some emasculated

flowers excluded from large pollinators also set seed, thus indicating outcrossing by thrips (Table 1).

*Insect capture.*—No medium-to-large pollinators (e.g., *Lasioglossum* sp., *Bombylius* sp., and *Bombus* sp.) were found adhering to the leaves of *P. vallisneriifolia*, although a few butterflies (*Pararge aegeria* and *Pieris napi*) were found trapped in the sunlight habitat. Captures of small flower visitors by the leaves reflected visitor abundance: many thrips were captured in the sunlight habitat, few thrips in the shade habitat, and almost none in the deep shade (Table 3). The capture of beetles and insects that did not visit flowers (mainly mosquitos, flies, and aphids) also differed between habitats, but not between years (Table 3).

In addition, I analyzed whether flowering plants captured more insects than did nonflowering individuals in the sunlight habitat. Plants with their flowers removed captured only  $1.7 \pm 0.24$  thrips/10 cm<sup>2</sup> of leaf

TABLE 3. Abundance of thrips and beetles trapped by *P. vallisneriifolia* leaves in the three habitats during 1994 and 1995, as indicated by the percentage of leaves with small flower visitors entangled and the mean ( $\pm 1$  SE) number of small flower visitors captured per 10 cm<sup>2</sup> of leaf. "Others" refers to other nonpollinator prey taxa (mainly mosquitos, small flies, and aphids). Statistical results of the two-way mixed-model ANOVAs (year as random effect) are given in the footnotes.

Prey taxa	Sunlight		Shade		Deep shade	
	Percentage of leaves	Capture rate	Percentage of leaves	Capture rate	Percentage of leaves	Capture rate
1994						
Thrips†	100	18.56 $\pm$ 2.40	100	3.22 $\pm$ 0.55	35	0.18 $\pm$ 0.07
Beetles‡	35	0.24 $\pm$ 0.08	60	0.9 $\pm$ 0.24	15	0.10 $\pm$ 0.09
Others§		6.14 $\pm$ 0.89		5.66 $\pm$ 0.59		10.52 $\pm$ 1.13
1995						
Thrips†	100	12.32 $\pm$ 1.75	95	2.38 $\pm$ 0.41	5	0.07 $\pm$ 0.07
Beetles‡	10	0.07 $\pm$ 0.04	50	1.0 $\pm$ 0.33	5	0.05 $\pm$ 0.05
Others§		3.29 $\pm$ 0.46		6.37 $\pm$ 0.55		7.15 $\pm$ 0.44

† Habitat:  $F = 305.58$ ;  $df = 2, 124$ ;  $P < 0.0001$ . Year:  $F = 1.64$ ;  $df = 1, 124$ ; ns. Habitat  $\times$  year:  $F = 1.84$ ;  $df = 2, 124$ ; ns.

‡ Habitat:  $F = 17.93$ ;  $df = 2, 124$ ;  $P < 0.0001$ . Year:  $F = 0.298$ ;  $df = 1, 124$ ; ns. Habitat  $\times$  year:  $F = 0.59$ ;  $df = 2, 124$ ; ns.

§ Habitat:  $F = 17.97$ ;  $df = 2, 124$ ;  $P < 0.0001$ . Year:  $F = 0.88$ ;  $df = 1, 124$ ; ns. Habitat  $\times$  year:  $F = 9.43$ ;  $df = 2, 124$ ;  $P < 0.0005$ .

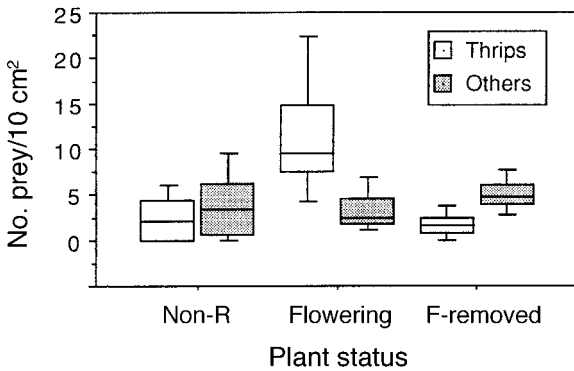


FIG. 2. Number of insects trapped per 10 cm<sup>2</sup> of leaf (thrips and other nonpollinator insects) by nonreproductive (non-R), reproductive (flowering), and flower-removed plants (F-removed) in the sunlight habitat. In the box plots, the top of a box represents the 75th percentile, and the bottom the 25th percentile. The line in the box represents the median. The top whisker ranges from the 25th to the 10th percentile.

surface, compared to  $12.3 \pm 1.75$  thrips/10 cm<sup>2</sup> captured by plants with intact flowers ( $F = 52.55$ ;  $df = 2, 78$ ;  $P < 0.0001$ ; see Fig. 2). Moreover, nonreproductive plants also captured significantly fewer ( $2.5 \pm 0.56$  thrips/10 cm<sup>2</sup>) than did flowering ones. Both flower-removed plants and nonreproductive plants captured equivalent numbers of insects that did not visit flowers (mainly mosquitos, flies, and aphids) as did flowering plants ( $F = 3.08$ ;  $df = 2, 78$ ; ns; Fig. 2).

The interference between plant–pollinator and plant–prey interactions depended on pollinator size. Thrips were the smallest floral visitors of *P. vallisneriifolia* ( $0.98 \pm 0.02$  mm body length,  $n = 75$ ), followed by the beetles ( $2.4 \pm 0.3$  mm,  $n = 32$ ). In contrast, *Lasioglossum* sp. (measuring  $7.3 \pm 1.3$  mm,  $n = 20$ ), *Bombylius* sp. ( $8.7 \pm 1.3$  mm,  $n = 15$ ), and *Bombus* sp. ( $>10$  mm) were strong enough to escape from the leaves in case of contact. However, entrapment also depended on the mode of locomotion between flowers. Medium-to-large pollinators, such as *Lasioglossum* sp. and *Bombylius* sp., flew between flowers, approaching the plant from the front of the corolla and rarely touching the leaves. Beetles usually left the flower by descending the peduncle, often touching a leaf and thus becoming trapped. Thrips became entangled on the leaves when leaving the senescing flowers by means of erratic jumps or short flights.

Although thrips remained immersed in the glandular surface immediately after being trapped because of their tiny body size, beetles sometimes escaped from plants growing in the deep-shade habitat. However, in the sunlight and shade habitats, any beetle that made contact with a leaf invariably become entangled. I tested the retention capacity of the mucilage by placing living beetles (*E. scribae*) on functional leaves. The experiment involved 10 plants per habitat (one beetle on one leaf per plant) in the sunlight, shade, and deep-shade habitats. Beetles were placed in a natural landing

position. All beetles placed on sunlight and shade plants remained fixed for  $>1$  h, whereas 80% of beetles escaped from the leaves of plants growing on the deep-shade habitat, because of the low retention capacity of the mucilage there. By escaping from the traps, beetles had a greater chance to visit more flowers in the deep-shade habitat than in the sunlight and shade habitats. In fact, beetles were more abundant as flower visitors than as prey in the deep-shade habitat, whereas the reverse occurred in the shade habitat (Tables 2 and 3).

## DISCUSSION

### Pollination and seed production

Seed set in *Pinguicula vallisneriifolia* is pollen limited in all habitats, both in terms of fruit production and in the percentage of ovules developing into seeds (Table 1). Results of the intensive study agree with fruit set data obtained from the 11 populations of the broad study (Fig. 1B). This suggests that failures to set fruit are due to the absence of pollinator visits, a fact corroborated by the results of pollinator censuses. Because flower longevity is shortened by pollination, the prolonged flower life also suggests a prevailing scarcity of pollinator visits. The scarcity of floral visitors of *P. vallisneriifolia* is also characteristic of other Mediterranean (*P. mundi* and *P. submediterranea*; Zamora et al. 1996) and boreal *Pinguicula* species (*P. vulgaris*, *P. alpina*, and *P. villosa*; Molau 1993), being thus a widespread phenomenon of the *Pinguicula* genus.

Despite specialized floral traits of *P. vallisneriifolia* (large, spurred flowers with zygomorphic shape, contrasting colors and presence of floral guides) that might indicate a bee pollination syndrome, the paucity of medium-to-large pollinators increases the importance of small insects as pollen vectors of *P. vallisneriifolia*. Given that a typical ovary contains between 150 and 360 ovules, repeated floral visits of small flower visitors are likely to be valuable to the fruit set (Silander and Primack 1978, Mcdade and Dadivar 1984). In this respect, the long-lasting flowers of *P. vallisneriifolia* both increase the chance of a visit by large pollinators (Primack 1985) and favor repeated pollen transfers by thrips and beetles over the blooming period. Because *P. vallisneriifolia* reproduces by both selfing and outcrossing, any pollen transported by small flower visitors would be potentially suitable for seed production.

The low individual efficiency of thrips in transporting pollen is somewhat counterbalanced by their abundance at flowers (up to 20 thrips per flower in the sunlight habitat) and their constant presence over the day (up to 100% of the flowers had thrips in the sunlight habitat; Table 2) from the beginning to the end of blooming, thus enabling repeated pollen transfer. In fact, the experimental exclusion demonstrates that thrips are responsible for a proportion of both the fruit set and seed set of flowers that are not visited by large pollinators (Table 1; see Baker and Cruden 1991). In

this respect, thrips are quantitatively important as pollinators, because thousands of thrips living in hundreds of flowers can transfer a considerable bulk of pollen, not only within flowers (self pollen), but also between flowers (outcross pollen; see also Ananthakrishnan 1993). Pollination by beetles also appears to be highly influential, because their abundance increased in the shaded habitats (Table 2). They are almost the only pollen vector present in the deep-shade habitat and, consequently, the only potential agent of fruit set and seed set in *P. vallisneriifolia* in this habitat (Table 1).

Even though only a few meters separate the three habitats, pollinator assemblages differ markedly in both taxonomic composition and abundance, spanning the variability evident over the entire geographical range of *P. vallisneriifolia*. The environmental patchiness appears to determine the main differences in pollination regimes. The cliffs where the plants grow cast shadows where sunlight barely reaches, and where temperatures remain low during blooming. This shading can strongly limit occupancy by many ectothermal insect species, which cannot remain active below a specific temperature threshold (Corbet 1990, Herrera 1995a). In fact, both the large-scale and the intensive studies indicate that most medium-sized pollinators, such as *Bombylius* sp. and *Lasioglossum* sp., forage only during the short period when sunlight falls directly on the flowers. On the other hand, large insects that maintain elevated body temperatures while they are active, such as bumble bees and sphingid moths (Heinrich 1993), forage indiscriminately across the thermal range (see also Herrera 1990, 1995a, b). In contrast to medium-sized pollinators, the small pollinators visit all habitats (thrips mainly in the sunlight, and beetles mainly in the shade and deep shade), and remain active all day long, indicating that their presence does not directly depend on scattered sunlight. These patterns of habitat distribution persist at both the spatial and temporal scale, for large pollinators and especially for thrips and beetles (Table 2). Therefore, although there is a within-population spatial mosaic in the pollinator assemblage of *P. vallisneriifolia*, there is site-specific predictability in the interaction between *P. vallisneriifolia* and its pollinators. As a result, pollen dispersal appears to be concentrated within habitats, being strongly restricted between patches a few meters apart that differ with respect to irradiance and thermal regimes (e.g., sunlight and deep-shade habitats) and, hence, pollinator assemblages. Furthermore, this spatial variability in pollinator assemblage translates into differential female reproductive success (Table 1), and can result in spatially variable selective pressures of pollinators upon the plants.

#### *Interference between pollinator and prey systems*

*Pinguicula vallisneriifolia* leaves have no obvious mechanism for differentially attracting prey, although the size, form, and spatial distribution of the leaves,

overhanging from the cliff wall, increase the probability of capturing flying insects (Zamora 1995). Because leaves do not mimic flower characteristics, as occurs in pitcher plants (Joel 1988, Moran 1996), the key traits favoring the capture of small flower visitors are the spatial proximity of flowers and traps, and the way in which the pollinators move between flowers. On the other hand, flowering and nonflowering plants appear to catch nonpollinating taxa, such as mosquitos, flies, and aphids, which are the most common prey of *P. vallisneriifolia* (Zamora 1995), with equal frequency. Captures of these insects during the blooming period are also dependent on habitat (Table 3). This spatial pattern of prey capture is reinforced during the summer, when rising temperatures encourage many flying insects to concentrate in the wet, shaded places, avoiding the generally warm and dry conditions (Zamora 1995).

The long-lived flowers of *P. vallisneriifolia*, which are produced sequentially, allow a typical flowering individual with 2–3 flowers to attract floral visitors and, hence, potential prey, for 20–40 d. Because plants in bloom capture some flower visitors (Fig. 2), flowers increase the prey capture of visitors smaller than ~5 mm long (Zamora 1995), creating a positive association between the percentage of flowers and leaves visited by thrips and small beetles (Fig. 1C, D). Thus, flowers attract insects that subsequently may become prey, increasing prey capture in reproductive plants (see also Karlsson et al. 1994 for *P. vulgaris*). This enhanced prey capture could further promote vegetative and reproductive success, because the performance of *P. vallisneriifolia* is prey limited under natural conditions (Zamora et al. 1997, 1998).

Because seed production by *P. vallisneriifolia* is pollen limited in all habitats (Table 1), the capture of thrips and beetles by the leaves depletes an already limiting resource for plant reproduction: the number of free (untrapped) pollen vectors. The value of such insects as pollinators may decrease, because trapped insects cannot transfer pollen loads. Thus, this conflict of interests can represent a greater negative effect on plant sexual reproduction, through reduced pollination, than a positive effect on plant growth, given the small size of beetles and, especially, thrips as nutrient packets.

Moreover, the prey-pollination conflict is environment specific, because a plant's habitat determines both the availability of floral visitors and the retention capacity of the mucilage. In the sunlight habitat, flowers attract thrips, resulting in pollen transfer. In addition, thrips also provide a substantial reward of nutrients, because the great number trapped by flowering plants (up to 100 thrips per plant) offsets their small size (mean dry mass 7  $\mu$ g). Flowers receiving exclusively thrip-borne pollen could have lower fitness than flowers cross-pollinated by medium-to-large pollinators available in this habitat. In this situation, trapping thrips would benefit reproduction directly (i.e., reducing the



number of this comparatively lower quality pollen vector), as well as indirectly through nutrition, because of the great number trapped. In contrast, the deep-shade environment filters out the presence of most large pollinators and thrips, and beetles become the only pollinators found on the flowers. Because leaves are less adhesive in the deep-shade habitat (Zamora 1995), beetles are less likely to remain fixed on a leaf at first contact and, consequently, they could escape and pollinate several flowers before becoming trapped (Tables 2 and 3). On the other hand, flowering plants in the shade suffer the greatest interference because there are very few large pollinators, and few thrips per flower. All beetles are liable to be trapped once they make contact with the leaves after exiting from the flower, as demonstrated with the experimental placements of beetles on leaves. Thus, they could visit fewer flowers, resulting in the lowest plant reproductive success in the shade habitat (Table 1). In this habitat, pollination proves to be more clearly affected by insectivory than in the sunny and deep-shade habitats.

In conclusion, both the survey of 11 populations and the more intensive field observations and experiments in a single population indicate that *P. vallisneriifolia* does not efficiently separate the mutualistic plant–pollinator interaction from the antagonistic plant–prey interaction. Flowers and traps of *P. vallisneriifolia* compete for the same limiting resource: the small flower visitors. Thus, carnivory imposes a cost to the mutualism. This cost, however, is dependent on the local environment.

In a more general context of plant–animal interactions, it should be emphasized that the plant–pollinator and plant–prey interactions reported here depend on species-specific ecophysiological responses of insects and sessile plants to the abiotic characteristics of the environment (see also Herrera 1995b, 1997, and Zamora 1995). When plants are locally distributed throughout contrasting microenvironments, plant and animal populations may not interact homogeneously, creating a site-specific mosaic of mutualistic and antagonistic outcomes. Because of the geographical coincidence between *P. vallisneriifolia* populations and their small and large pollinator species throughout the plant's range, there is a limited possibility for interspecific outcomes and their potential variation to be geographically structured (sensu Thompson 1994). This plant–insect relationship appears to be microclimatically structured, with the local environment acting as the driving force conditioning the nature, strength, and variability of interspecific interactions. These results are relevant in the larger context of plant–animal interactions, clearly indicating that the physical environment can predictably affect the outcomes of interactions and, ultimately, the balance between mutualistic and antagonistic systems.

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