

Alejandro A. Muñoz · Mary T. K. Arroyo

Negative impacts of a vertebrate predator on insect pollinator visitation and seed output in *Chuquiraga oppositifolia*, a high Andean shrub

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Abstract Studies on plant-pollinator interactions have largely neglected the potential negative effects of the predators of pollinators on seed output, even though anti-predatory behaviour of pollinators may affect visitation patterns, pollen transfer, and therefore potentially, plant reproductive output. We tested the hypothesis that the presence of lizards and insectivorous birds, by reducing pollinator visitation, can have significant negative effects on seed output in the insect-pollinated, genetically self-incompatible lower alpine Andean shrub, *Chuquiraga oppositifolia* (Asteraceae). The lower alpine belt supports a high density of territorial *Liolaemus* (Tropiduridae) lizards and low shrubs interspersed among rocks of varying sizes, the latter inhabited by lizards and commonly used by flycatchers *Muscisaxicola* (Tyrannidae) as perching sites. In a 2×2 factorial predator-exclusion experiment, visitation rates of the most frequent pollinators of *C. oppositifolia* (the satyrid butterfly *Cosmosatyrus chilensis* and the syrphid fly *Scaeva melanostoma*), the duration of pollinator visits, and seed output, were 2–4 times greater when lizards were excluded, while birds had no effect. In a natural experiment, visits by *S. melanostoma* were 9 times shorter, and pollinator visitation rates of *C. chilensis* and *S. melanostoma*, and *C. oppositifolia* seed output were 2–3 times lower on shrubs growing adjacent to lizard-occupied rocks compared to those growing distant from rocks. Our results, verified for additional Andean sites, suggest that lizard predators can alter the behaviour of pollinators and elicit strong top-down indirect negative effects on seed output. Such effects may be especially

important in high alpine plant communities, where pollinator activity can be low and erratic, and pollen limitation has been reported.

Keywords Central Chilean Andes · Anti-predatory behaviour · Vertebrate predators · Plant-pollinator interactions · Plant reproductive output

Introduction

Carnivores can influence terrestrial, aquatic, and marine community structure. Top-down trophic cascades, which describe the indirect effects of carnivores on plants through herbivore control, have been the focus of much attention (e.g. Estes et al. 1978; Power 1990; Spiller and Schoener 1990; McIntosh and Townsend 1996; see reviews by Schmitz et al. 2000 and Halaj and Wise 2001). In three-trophic-level systems, plants often benefit from the control of herbivores by carnivores as a result of concomitant reduced leaf damage, enhanced growth or higher seed set (e.g. Vasconcelos 1991; Marquis and Whelan 1994; Moran et al. 1996; Chase 1998; Schmitz et al. 2000; Dawes-Gromadzki 2002). Positive effects for plants can occur when predators consume herbivores (e.g. Atlegrim 1989; Spiller and Schoener 1990; Dial and Roughgarden 1995; Moran et al. 1996; Sipura 1999). In addition, predators may elicit positive effects on plants by provoking herbivore behavioural responses to predation risk, i.e. behaviour-mediated (non-lethal) effects (e.g. Abrams et al. 1996; Beckerman et al. 1997; Letourneau 1998; Lima 1998; de la Fuente and Marquis 1999; Schmitz and Suttle 2001).

Insect pollinators, like herbivores, are known to fall prey to carnivorous predatory invertebrates (e.g. Louda 1982; Kevan and Baker 1983; Morse 1986; Caron 1990; Greco and Kevan 1995; Craig et al. 2001; Dukas 2001a, 2001b; Dukas and Morse 2003) and birds, particularly insectivorous flycatchers (Tyrannidae) (Ambrose 1990). Nevertheless, research on plant-pollinator interactions has been conducted almost exclusively within the framework

A. A. Muñoz (✉) · M. T. K. Arroyo
CMEB, Laboratorio de Sistemática y Ecología Vegetal,
Departamento de Biología, Facultad de Ciencias, Universidad
de Chile,
Casilla 653,
Santiago, Chile
e-mail: amunoz@icaro.dic.uchile.cl
Tel.: +56-2-6787345
Fax: +56-2-2712983

of two trophic levels (but see Louda 1982; Willmer and Stone 1997; Altshuler 1999), with carnivores being neglected in spite of potential negative effects on pollinator visitation patterns (but see Dukas 2001a, 2001b; Dukas and Morse 2003; Suttle 2003). Some authors have argued that pollinator predation events may occur too infrequently to influence pollinator behaviour and pollinator visitation rates (PVR) (e.g. Miller and Gass 1985; Morse 1986; Schmalhofer 2001).

Behavioural responses per se on the part of insect pollinators to perceived predation danger by carnivores could lead to reduced pollinator visitation. Even when predation levels are low, insects and vertebrates are known to alter diurnal activity patterns, diet, and habitat in response to predation risk, all of which may affect fitness, life history, and among-species interactions (e.g. Nonacs and Dill 1990; Scrimgeour and Culp 1994; Beckerman et al. 1997; Schmitz and Suttle 2001). Ultimately, carnivores could be responsible for negative indirect effects (sensu Wootton 1994) on plant fitness given that fewer and/or shorter pollinator visits to plants could lead to reduced pollen transfer and lower seed output. Numerous studies have shown reduced pollinator visitation to result in lower seed output (e.g. Johnson et al. 1995; Bosch and Waser 2001; Brown et al. 2002). Thus, the presence of carnivores may not only have positive (e.g., Vasconcelos 1991; Letourneau 1998), but also negative effects on plant reproduction.

Here, we tested the hypothesis that the presence of predatory lizards and insectivorous birds produces negative effects on insect pollinator visitation and on plant seed output in shrubs of *Chuquiraga oppositifolia* in the high Andes of central Chile. Pollinator visitation patterns and seed output were quantified for plants of the target species growing alongside rocks in a manipulative predator-exclusion experiment in which lizards, insectivorous birds, both predators, or neither were excluded from plots. Further, in a natural experiment, we compared pollinator visitation patterns and seed output on shrubs located adjacent to and away from rocks harbouring lizards.

Materials and methods

Study site and system

Research was conducted between late October 2001 and early May 2002 on an 18-ha site at 2,600 m altitude in the lower alpine belt, Valle Nevado ski complex area (33°21'S, 70°16'W) in the Andes of central Chile, 33°S. The climate is Mediterranean, with a 5–8 month snow-free growing season, commonly extending from mid-October to mid-May (Arroyo et al. 1981). The study site is south-facing with gentle (<15°) slopes. The vegetation is dominated by low (<45 cm) spiny shrubs of *Chuquiraga oppositifolia* (Asteraceae), *Anarthrophyllum cumingii* (Papilionaceae), and *Berberis empetrifolia* (Berberidaceae). Rocks of varying size (0.001–2.5 m³), with an estimated cover of 15%, are interspersed among the shrubs.

The study site is characterized by a high density of large (10–12 cm snout-vent length) territorial lizards belonging to *Liolaemus bellii* (Tropiduridae) (>95%) and *L. leopardinus* (A. A. M., personal observations) which use rocks as permanent territories in the spring–

autumn snowless period. Most rocks (>80%) are occupied by one or two lizards throughout the entire snowless period, when lizards can be commonly observed basking in the sun on rocks and awaiting potential insect prey. Based on mark-recapture methods conducted in January 2002, we estimated mean lizard density in five 10×10-m plots as 20±5 (1 SE) individuals/100 m². Thirty-minute filming events (Sony Handycam video camera) of 40 lizard-inhabited rocks showed that individuals remained on top of rocks for a mean (±1 SE) of 18±6 min, alternating with retreats to the shade provided by shrubs growing adjacent to the rocks. Gut content analysis of 20 individuals of *L. bellii* captured between November 2000 and February 2001 revealed small quantities of bee and fly remains in 70 and 50% of the specimens, respectively. Medium-sized flycatchers (*Muscisaxicola* spp., Tyrannidae), are also commonly observed, often visiting rocks. Based on point count data taken in January 2002, estimated flycatcher density is low in comparison with that of lizards, with a mean of 2.6±0.3 (1 SE) birds/2,000 m² (25 fixed observation points within a radius of 25 m).

C. oppositifolia (Asteraceae) is a dominant (Arroyo et al. 1981; Cavieres et al. 2000), insect-pollinated (Arroyo et al. 1982), fully self-incompatible (Rozzi 1990) and hence outcrossed shrub of the lower alpine belt (16% cover) in the central Chilean Andes. Based on transects, 25% of shrubs grow adjacent to rocks. *C. oppositifolia* has small (<1 cm) pubescent spiny thick-cuticle leaves and spiny branches. Leaf damage is minimal; the only herbivores observed are grasshoppers in low densities. *C. oppositifolia* flowers late in the season (January to April) (Arroyo et al. 1981). The shrubs become replete with large, golden-yellow capitula (12 florets on average) containing abundant pollen shed as an exposed layer over the protruding stigmas. Reported pollinators of *C. oppositifolia* are bumblebees and solitary bees (Apidae, Anthophoridae), flies (Syrphidae, Bombyllidae), and butterflies (Satyridae) (Arroyo et al. 1982; Rozzi 1990). Further, Rozzi (1990) showed that open pollination of *C. oppositifolia* by insects resulted in seed set, whereas bagging (given that this species is self-incompatible) only did so following cross-pollination. Thus, without doubt, insect pollinators effect pollination. Detailed pollen-load analysis demonstrated that the main pollinators of *C. oppositifolia* carry copious pollen (Rozzi 1990). Indeed, any insect alighting on the capitula, no matter how small, becomes covered with pollen. However, not all pollen-carrying insects are equally efficient pollinators. Butterflies, followed by bees, syrphid flies and finally tiny andrenid bees travel decreasing distances between shrubs, to the extent that andrenid bees and other rare visitors tend to remain mostly on the same shrub. As *C. oppositifolia* is self-incompatible, visits by andrenid bees, in particular, tend to be largely superfluous insofar as cross-pollination is concerned. A supplemental hand-pollination experiment conducted concurrently at the study site resulted in a two- to three-fold increase in seed output, indicating that seed output in *C. oppositifolia* is strongly pollen-limited (A. A. M. and M. T. K. A., unpublished data).

Predator-exclusion experiment

To assess the effects of lizards and birds on *C. oppositifolia*'s pollinators and seed output, we conducted a 2×2 factorial predator-exclusion experiment that allowed for selective exclusion of lizards and/or insectivorous birds. Forty-eight shrubs of comparable size (ca. 0.2–0.3 m³) growing adjacent to rocks (ca. 0.4–0.5 m³) that were visually verified to contain a territorial lizard at the beginning of the experiment, were selected. Neither shrub size, nor floral display (number of open capitula per shrub) differed among treatments (mean±1 SE=117±22 capitula/shrub; one-way ANOVA, $df=1,44$, $F=0.46$, $P=0.71$). Shrub-rock pairs were used as the central points of 4-m-diameter circular plots that were randomly assigned to one of four treatments each with 12 replicates: (1) control (all predators allowed access), (2) lizard exclusion (allowing access to birds but not to lizards), (3) bird exclusion (lizards, but not insectivorous birds allowed access), and (4) total exclusion (access to both predators denied). Lizard exclusion was achieved by placing

smooth 30-cm-high vertical aluminium sheets (which lizards could not climb) around the circumference of plots, dug 5 cm into the ground. The complete absence of lizards was ensured by relocating all individuals to nearby rocks during the installation process. Bird exclusion was achieved with enclosures made of 5×5-cm-aperture wire mesh on the top and sides mounted on frames of six 1.5-m vertical wooden poles. Such enclosures allowed uninhibited access to all types of pollinators, lizards, and smaller (ca. 8 cm tall) granivorous birds such as *Diuca diuca* (Fringillidae), but not to the larger insectivorous *Muscisaxicola* flycatchers. Total exclusion plots contained aluminium sheeting and wire mesh, which together denied access to both lizards and flycatchers.

Potential caging artefacts were assessed by assigning: (1) three additional shrubs to an aluminium control treatment, allowing access to lizards via 20-cm-high triangular perforations cut every 1.5 m along sheet circumferences; and (2) another three to a wire mesh control treatment, lacking mesh on the top, thus allowing access to birds from above. We commonly observed lizards on top of rocks inside the aluminium control plots and flycatchers fly onto rocks within wire mesh control plots from above. Hence, any differences in insect pollinator visitation between aluminium control, wire mesh control, and control plots could only be ascribed to the exclusion structures themselves. PVR and duration of pollinator visits (DPV) were unaffected by the aluminium or wire mesh (Kruskal-Wallis, $H_{PVR}=0.06$, $P=0.97$; and $H_{DPV}=0.03$, $P=0.98$). Potential artefacts of the aluminium or wire mesh on ambient temperature which could affect pollinator visitation and/or shrub seed output within plots were assessed by monitoring maximum and minimum daily temperatures at the shrub level over two periods: December 2001–January 2002 (15 days) and February–March 2002 (15 days). No differences were detected across exclusion treatments for maximum (overall mean±1 SE=27.8±0.5°C, one-way ANOVA, $F=0.13$, $df=3,56$, $P=0.95$) and minimum temperature (4.4±0.3°C, $F=0.28$, $P=0.84$) in the first period, or for maximum (28.0±0.4°C, $F=2.47$, $P=0.07$) or minimum temperature (5.0±0.2°C, $F=0.23$, $P=0.88$) in the second period.

Natural experiment

Pollinator visitation patterns and seed output were assessed for shrubs growing adjacent to and distant from lizard-inhabited rocks. Twenty shrubs of comparable size (ca. 0.2–0.3 m³), each growing next to a lizard-inhabited rock (ca. 0.4–0.5 m³), and 20 shrubs located at least 5 m away from rocks were selected. Shrub-rock pairs were designated following visual verification of the presence of lizards on the rocks at the start of the experiment. During the course of the experiment, we daily verified that the lizards remained associated with the rocks. Floral display of shrubs did not differ between shrubs adjacent to rocks (mean±1 SE=123±17 capitula/shrub) vs. away from rocks (141±18 capitula/shrub) (Student $t=0.779$, $df=38$, $P=0.44$). Using a needle thermometer, we regularly measured the surface temperature of capitula on shrubs adjacent (mean±1 SE=18.7±1.9°C) and away from rocks (18.1±1.5°C), with no differences being detected (Mann-Whitney $U=764$, $Z=-0.35$, $P=0.73$).

Pollinator visitation

Pollinator taxa identity, PVR, and duration of individual visits to shrubs were determined for each exclusion treatment and control, as well as for shrubs adjacent to and distant from rocks, following Arroyo et al. (1982, 1985) and Rozzi (1990). On each day, previous to taking pollination data, we visually verified that the lizards remained associated with the rocks in the natural experiment and in the control and bird-exclusion treatment plots in the predator-exclusion experiment. Lizard presence was recorded on ca. 90% of the days. Pollinators were defined as insects that made contact with the anthers and/or stigmas of the florets and that actually carried pollen. Three 10-min observation periods per hour per shrub were

made between 9.00 a.m. and 5.00 p.m. on 30 sunny days from late January to early March 2002 (austral summer). We defined a “visit” to a shrub as one in which the insect landed on at least one open capitulum. Observations were made some 6–8 m away from the target shrubs using binoculars, so as to avoid disturbing pollinator and predator activity. We accumulated 397 and 351 ten-minute observations in the predator-exclusion and natural experiments, respectively. Response variables were expressed as mean values for each shrub (plot) for 10-min observation periods. PVR and DPV under different predator-exclusion treatments were analysed by two-way ANOVA with lizards and birds as factors (Zar 1996; StatSoft 1998), after testing for normality and homogeneity of variances using the Shapiro-Wilks and Bartlett tests, respectively. Data were transformed appropriately when the normality condition was not met. A posteriori multiple comparisons were conducted using the Tukey honestly significant difference test. PVR and DPV to shrubs adjacent to and distant from rocks were compared using Student t -tests, or Mann-Whitney U -tests when normality assumptions were not met by the transformed data.

Seed output

From late February 2001 until early March 2002, as flowering ceased and the open capitula had withered, 50 randomly selected capitula per shrub per predator-exclusion treatment and 30 randomly selected capitula per shrub adjacent and distant from rocks were covered with 8×6-cm yellow mesh bags so as to assess mature achene production (later in April–May). Seed output was expressed as: (1) percentage of capitula per shrub that set one or more achenes (%CA), and (2) mean number of achenes per capitulum per shrub (NAC). Seed output in shrubs subjected to different exclusion treatments and adjacent vs. distant from rocks were compared as above.

Results

Predator-exclusion experiment

Chusqueira oppositifolia was visited by 18 different pollinator taxa that made a total of 454 observed visits. The most frequent pollinators were the satyrid butterfly *Cosmosatyrus chilensis* (28.0% of all visits), the syrphid fly *Scaeva melanostoma* (22.7%), and the andrenid bee *Heterosarus* sp. (22.2%). Less frequent visitors were the andrenid bee *Liphanthus* sp. (5.3%), bombyliid flies *Villa* spp. (4.8%), other bombyliid flies (4.6%), tachinid flies (3.5%), and the bumblebee *Bombus dahlbomii* (2.4%).

Considering all pollinator groups combined, exclusion of lizards and birds individually and collectively did not affect overall PVR (Fig. 1a; two-way ANOVA, $df=1,44$, $F_{lizards}=3.07$, $P=0.09$; $F_{birds}=0.03$, $P=0.90$). However, when the most important and efficient pollinators are analysed individually, lizard exclusion lead to significantly higher PVR in the case of the butterfly, *C. chilensis* (Fig. 2, two-way ANOVA, $df=1,44$, $F_{lizards}=4.13$, $P<0.05$; $F_{birds}=0.32$, $P=0.58$) and the fly *S. melanostoma* (Fig. 2, $F_{lizards}=4.06$, $P=0.05$; $F_{birds}=2.67$, $P=0.11$). No increase in PVR occurred for the fairly common, but far less efficient, small andrenid bee, *Heterosarus* sp. (Fig. 2, $F_{lizards}=0.99$, $P=0.33$; $F_{birds}=0.22$, $P=0.64$).

DPV increased from 12 to 30 s for all pollinators combined in the control vs. lizard exclusion plots,

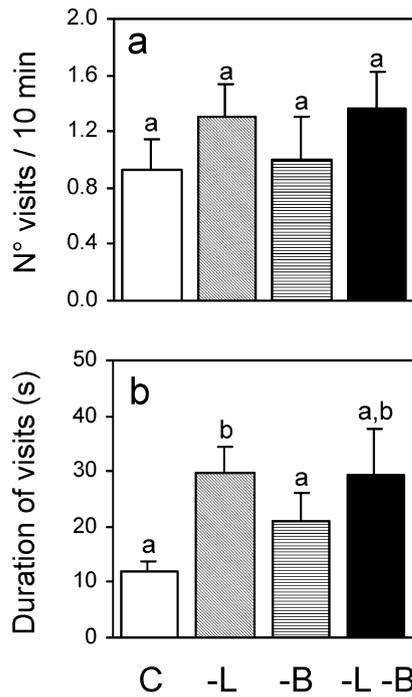


Fig. 1a, b Pollinator visitation in the predator-exclusion experiment. Effect of lizards and insectivorous birds on pollinator visitation rate (number of pollinator individuals visiting shrubs/10 min) (a) and on the duration of visits (s) (b) of all pollinator groups combined. Each predator-exclusion treatment had 12 replicates [$n = 12$ shrubs (plots)]. Treatments sharing the same letter do not differ significantly ($P > 0.05$). Bars are means ± 1 SE. C Control, -L lizard exclusion, -B bird exclusion, -L -B total exclusion

respectively (Fig. 1b, two-way ANOVA $df = 1,44$, $F_{\text{lizards}} = 5.73$, $P < 0.05$; $F_{\text{birds}} = 0.64$, $P = 0.43$). Considering individual pollinators, neither lizards nor birds affected DPV in the case of *C. chilensis* (Fig. 2, two-way ANOVA $df = 1,44$, $F_{\text{lizards}} = 1.75$, $P = 0.19$; $F_{\text{birds}} = 1.88$, $P = 0.18$) and *Heterosarus* sp. (Fig. 2, $df = 1,44$, $F_{\text{lizards}} = 0.02$, $P = 0.88$; $F_{\text{birds}} = 0.01$, $P = 0.94$). In contrast a four-fold increase in DPV for *S. melanostoma*, from 7 to 27 s in control vs. lizard-exclusion plots (Fig. 2, $F_{\text{lizards}} = 9.06$, $P < 0.01$; $F_{\text{birds}} = 3.45$, $P = 0.07$), was observed.

The %CA increased two-fold from 17 to 38% (control vs. lizard exclusions) (Fig. 3a, two-way ANOVA $F_{\text{lizards}} = 17.90$, $df = 1,44$, $P < 0.01$), while the NAC increased significantly from 0.25 to 0.62 (Fig. 3b, $F_{\text{lizards}} = 16.24$, $df = 1,44$, $P < 0.01$). In contrast, neither %CA nor NAC were affected by the exclusion of birds (Fig. 3a, b, $df = 1,44$, F_{birds} for %CA = 1.39, $P = 0.25$, and F_{birds} for NAC = 0.65, $P = 0.43$).

Natural experiment

Chuquiraga oppositifolia was visited by 21 pollinator taxa which made a total of 477 observed visits. As in the exclusion experiment, the most important pollinators were *Cosmosatyrus chilensis* (18.2% of visits), *S. melanostoma* (17.6%), and *Heterosarus* sp. (14.0%). Less frequent

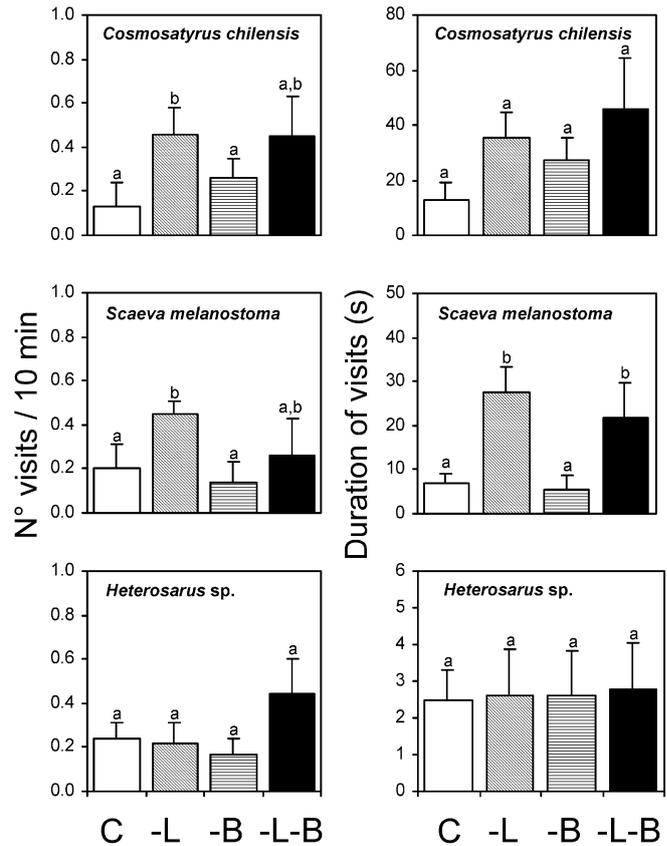


Fig. 2 Pollinator visitation in the predator-exclusion experiment. Effect of lizards and insectivorous birds on pollinator visitation rate (number of pollinator individuals visiting shrubs/10 min) and on the duration of visits (s) of the satyrid butterfly *Cosmosatyrus chilensis*, the syrphid fly *Scaeva melanostoma*, and the andrenid bee *Heterosarus* sp. Bars are means ± 1 SE [$n = 20$ shrubs (plots)]. Treatments sharing the same letter do not differ significantly ($P > 0.05$). Abbreviations as in Fig. 1

visitors were *Liphanthus* sp. (9.0%), *Villa* spp. (7.1%), other bombyliids (7.6%), tachinids (5.2%), and other satyrid butterflies (3.8%).

PVR, when considering all species, was significantly lower on shrubs adjacent to rocks compared to those distant from rocks (0.90 vs. 1.84 visits/10 min) (Fig. 4, Student $t = 3.18$, $df = 38$, $P < 0.01$), as was that of *C. chilensis* (Fig. 4, $t = 2.05$, $df = 38$, $P < 0.05$), and *S. melanostoma* (Fig. 4, $t = 2.13$, $df = 38$, $P < 0.05$) alone. In contrast, PVR for *Heterosarus* sp. did not differ (Fig. 4, Mann-Whitney $U = 194$, $Z = 0.18$, $P = 0.85$). For all pollinator groups combined, as well as for *C. chilensis* and *Heterosarus* sp. alone, DPV did not differ significantly for shrubs adjacent to and away from rocks (Fig. 4, $t_{\text{overall}} = 0.81$, $df = 38$, $P = 0.43$; Mann-Whitney $U_{\text{C. chilensis}} = 149$, $Z = 1.45$, $P = 0.15$; $U_{\text{sp.}} = 189$, $Z = 0.33$, $P = 0.74$). However, *S. melanostoma* visits were 9 times briefer on shrubs adjacent to rocks (3 s) compared to those distant from rocks (29 s) (Fig. 4, $U_{\text{S. melanostoma}} = 116$, $Z = 2.43$, $P < 0.01$).

Seed output, expressed as %CA and NAC, was significantly lower (>two-fold) on shrubs growing next to rocks (24% and 0.37 achenes per capitulum, respec-

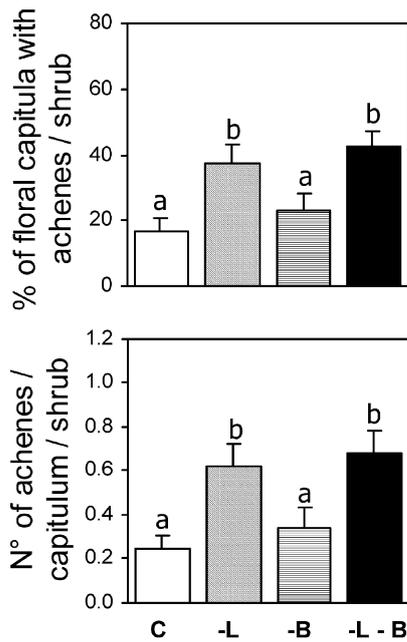


Fig. 3 Seed output in the predator-exclusion experiment. Effect of lizards and insectivorous birds on seed output expressed as the percentage of capitula per shrub that produced achenes (*upper graph*), and as the mean number of achenes per capitulum per shrub (*lower graph*). Bars are means+1 SE [$n = 12$ shrubs (plots)]. Treatments sharing the *same letter* do not differ significantly ($P > 0.05$). Abbreviations as in Fig. 1

tively) in comparison with shrubs growing away from rocks (67% and 0.94 achenes) (Fig. 5a, t for %CA=9.37, $df = 38$, $P < 0.01$; Fig. 5b, U for NAC=21.5, $Z = 4.85$, $P < 0.01$).

Discussion

Results of the predator-exclusion and natural experiments strongly suggest that lizards (but not insectivorous birds) can produce strong negative effects on the most frequent and efficient pollinators (the butterfly *Cosmosatyrus chilensis* and the fly *Scaeva melanostoma*) of *Chuquiraga oppositifolia*. The combined effect of diminished visitation frequency and reduced DPV lead to an outstanding two- to three-fold reduction in seed output. As all treatments in the manipulative experiment involved shrubs alongside rocks, differences among treatments cannot be attributed to a “rock effect” on pollinator behaviour per se, or for that matter, on seed production. Theoretically, a “rock effect” could explain the results obtained in the natural experiment. However, given the unequivocal results of the manipulative experiment, together with the fact that rocks in the natural experiment were always inhabited by lizards, it stands to reason that lizards were responsible for the reduced pollinator visitation and seed set in shrubs growing alongside rocks in the natural experiment.

Lower PVR in shrubs growing adjacent to lizard-inhabited rocks has three non-mutually exclusive explanations: (1) pollinators use visual cues to avoid plants when lizards are present on a rock, (2) pollinators avoid

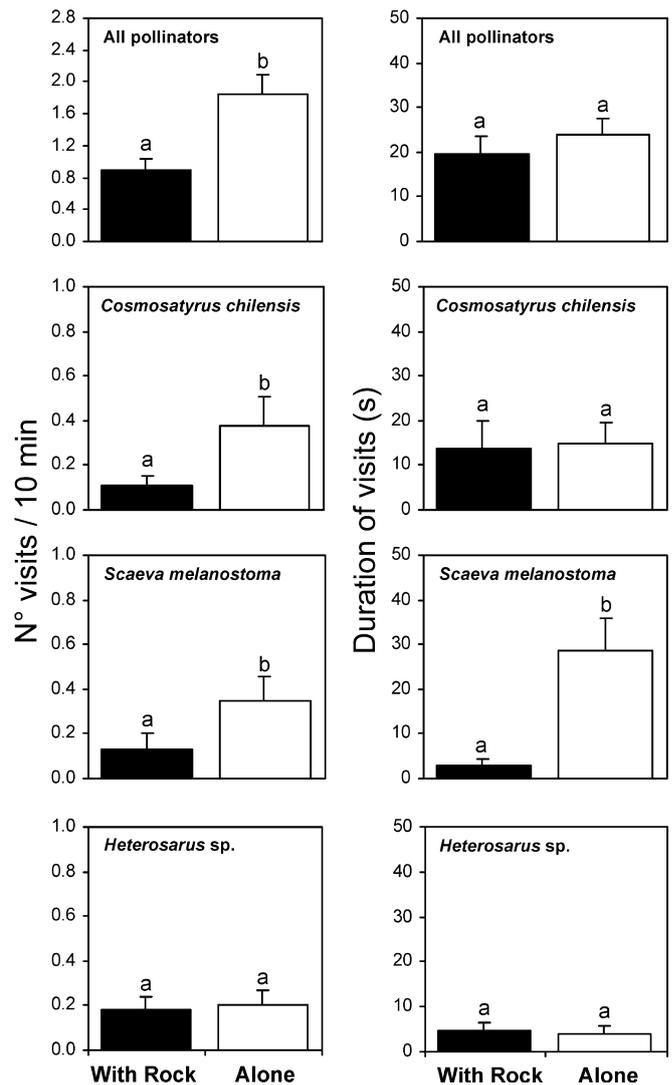


Fig. 4 Pollinator visitation in the natural experiment. Pollinator visitation rate (number of pollinator individuals visiting shrubs/10 min) and duration of pollinator visits (s) to shrubs growing adjacent to rocks (*With Rock*) vs. those growing away from rocks (*Alone*), of all pollinator groups combined, the satyrid butterfly *C. chilensis*, the syrphid fly *S. melanostoma*, and the andrenid bee *Heterosarus sp.* Bars are means+1 SE [$n = 20$ shrubs (plots)]. Treatments sharing the *same letter* do not differ significantly ($P > 0.05$)

revisiting plants following an event of escape from predation, and (3) fewer pollinators are locally available as a result of consumption by lizards. In other words the effects of lizards on pollinators could be due to: (1) pollinators taking anti-predatory measures to avoid predators, i.e. behaviour-mediated effects (previous points 1 and 2), (2) consumption of pollinators by lizards (previous point 3), i.e. density-mediated effects, or (3) both. Our predator-exclusion and natural experiments do not allow us to determine the relative importance of each mechanism. However, at least two lines of evidence suggest that lizard effects stem principally from the mere presence of lizards on rocks, with little actual predation involved. Between the 10-min observation periods on pollinators,

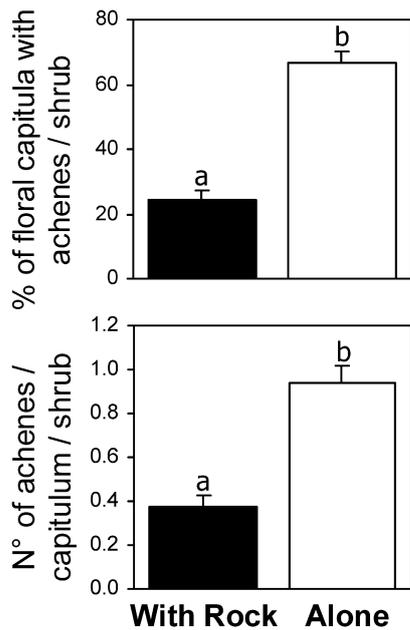


Fig. 5 Plant seed output in the natural experiment. Seed output of shrubs growing adjacent to rocks (*With Rock*) vs. those growing away from rocks (*Alone*), expressed as the percentage of capitula that produced achenes (*upper graph*), and as the mean number of achenes per capitulum per shrub (*lower graph*). Bars are means ± 1 SE [$n = 20$ shrubs (plots)]. Treatments sharing the *same* letter do not differ significantly ($P > 0.05$)

we documented over 1,200 predation attempts by lizards in the general study area, of which only six (0.5%) were successful. Usually, the movement of a lizard was enough to scare pollinators away from the shrub. Secondly, visits by *S. melanostoma* were 4 times longer on shrubs in lizard-exclusion plots compared to controls, and up to 9 times longer on those growing away from rocks compared to those adjacent to lizard-inhabited rocks. Greater DPV in the absence of lizards also argues for a behaviour-mediated reason. It is worthwhile examining whether the negative effects of lizards on seed output in our study are produced via an alternative four-trophic-level trophic chain. If lizards were to control the density or activity of spiders, herbivore density could be enhanced to the extent of the plant suffering reductions in resources for seed production. This possibility is unlikely in our system given that levels of leaf damage on *C. oppositifolia* are practically inexistent, and the associated insect herbivore fauna is scarce, consisting mostly of grasshoppers at very low densities. Our results strongly suggest that carnivores, through an entirely different interaction (i.e. alterations of pollinator behaviour), can produce strong top-down indirect negative effects on plant reproduction.

The results of the manipulative and natural experiments were consistent when the two most important and efficient pollinators were considered (*C. chilensis*, *S. melanostoma*). However, some differences in the results that arose when all pollinators were considered, beg explanation. Although PVR increased in the absence of lizards for the main pollinators in both experiments, there was no significant difference in PVR in the manipulative exper-

iment when all pollinators were considered together. This could be a result of interactions among the large and small pollinators. For example, the tiny and less efficient andrenid bees tend to be scared off by the larger pollinators. Thus although PVR increased for the large and efficient pollinators, the total number of visits would not necessarily increase statistically. Although hard to quantify, there can be no doubt that the quality of the pollinator visits increased in both experiments. In the absence of lizards, DPV considering all pollinators only increased statistically in the predator-exclusion experiment. In the latter, both of the large and more efficient pollinators tended to make longer visits, which when added to the significant difference for *Scaeva*, impinged positively on the overall mean (Fig. 2). Much smaller increases in the DPV in the absence of lizards in the case of the main pollinators in the natural experiment, added to the greater variation in the data, lead to a non-significant difference in DPV, in spite of the fact that *Scaeva*, as in the manipulative experiment, made significantly longer visits. Again local interactions among the pollinators groups are probably behind this difference.

Responses of insect pollinators to predation risk by spiders have been reported previously and include reduced visitation of flowers and patches with spiders nearby and avoidance of revisiting sites where pollinators had escaped predation attempts (Dukas 2001a, 2001b; Dukas and Morse 2003; Suttle 2003), and bypassing of spider webs (Craig 1994). Although she did not study pollinator visitation patterns, Louda (1982) reported reduced seed set in the presence of spiders, which was attributed to both direct predation and spider interference on pollinator visitation. However, the net effect of spiders on reproductive output in *Haplopappus venetus* was positive, given that spiders also interfered with seed predators, leading to a net higher numbers of viable, undamaged seeds. Positive effects of predaceous ants on fruit set were documented in *Psychotria limonensis* by Altshuler (1999). Here, although exclusion of ants resulted in increased within-shrub visitation by winged insect pollinators, fruit set was also reduced. These seemingly contradictory results were explained by hypothesising that predatory ants aided fruit production by forcing winged pollinators to make shorter within-plant foraging trips and more among-plant trips (Altshuler 1999). On the other hand, negative effects of ants on reproductive output were suggested by Willmer and Stone (1997) who showed that aggressive ant-guarders normally protecting the species against herbivorous insects, are deterred from patrolling flowers at the time of anthesis. These authors speculated that *Acacia* emits chemicals, so as to avoid producing potential negative effects on pollinator visitation and plant seed set. Further, in a recent study, Dukas and Morse (2003) experimentally manipulated the density of spiders on milkweed (*Asclepias syriaca*). Bumblebees visited patches harbouring spiders at a lower frequency than spider-free plants, allowing these authors to conclude that pollinator responses to predation risk may influence pollinator-plant interactions. Finally, Suttle (2003) recently demonstrated that the presence of

crab spiders on flowers of the ox-eye daisy *Leucanthemum vulgare*, by reducing the frequency and duration of floral visits by pollinating insects, resulted in a decrease in seed production. However, ours is the first experimental study demonstrating that vertebrate carnivores (lizards), by altering pollinator behaviour, produce indirect negative effects on plant reproductive output.

Both spiders and lizards are central-place foragers (Stephens and Krebs 1986; Chase 1998), i.e. predators that concentrate activities around their refuge sites, always returning to these sites. In contrast, flycatchers, *Muscisaxicola* spp., frequently fly from one rock to the next, spending little time on any one rock, and forage mostly on the ground (A. A. M., personal observations). These differences, together with the lower abundance of birds compared to lizards, may explain the lack of effects of flycatchers on pollinator visitation and seed output. In our view central-place foragers and sit-and-wait predators such as lizards, some spiders, and inflorescence-inhabiting predatory insects (e.g. Morse 1986; Greco and Kevan 1995; Chase 1998; Schmalhofer 2001; Suttle 2003), are more likely to cause significant behaviour-mediated indirect effects on plants than predators that move continuously. Support for this prediction was documented by Schmitz and Suttle (2001), who compared the direct and indirect effects of sit-and-wait, sit-and-pursue, and actively hunting spiders on grasshoppers and plants. Here, only the sit-and-wait species caused indirect effects on plants via changes in grasshopper foraging behaviour, while the other two spiders produced indirect effects on plants by reducing grasshopper density.

Lizards constitute a species-rich and abundant predator group (e.g. Marquet et al. 1989; Carothers et al. 2001) in the central Chile Andes; the striking negative indirect effects documented at the Valle Nevado study site have subsequently been demonstrated elsewhere. During the 2002–2003 growing season, we carried observations at three additional high Andean localities (Farellones, Lagunillas, and Portillo) at a distance of ca. 5, 30, and 60 km, respectively, from the Valle Nevado area. At Farellones, seed output was 2 times lower in shrubs growing adjacent to lizard-inhabited rocks compared to that of shrubs growing away from rocks, with non-significant trends in the same direction at the other two sites (A. A. M. and M. T. K. A., unpublished data). Predator-mediated reductions in pollinator visits on the other hand, could be especially critical in alpine environments where entomophily is common (e.g. Arroyo et al. 1982; Körner 1999), pollinator abundance and activity levels are often low (e.g. Arroyo et al. 1985; Rozzi 1990; Bingham and Orthner 1998), seed set can be pollen limited (e.g. Totland 2001), and outbreeding is the dominant breeding system in long-lived species (e.g. Arroyo and Squeo 1990; Gugerli 1998). It thus would be of great interest to learn whether lizard effects are found in other high mountain areas in the world, and study its repercussions at the level of community structure.

We conclude that lizards, notwithstanding occasional successful predation attempts, by altering pollinator

behaviour, can produce negative top-down indirect effects on plant reproductive output. Our findings provide further support for the growing body of evidence that predators can induce effects on plants, not only via consumptive (lethal) mechanisms, but also through behavioural (non-lethal) mechanisms (e.g. Abrams et al. 1996; Beckerman et al. 1997; Schmitz et al. 1997; Lima 1998; Schmitz and Suttle 2001). Along with results for spiders (Louda 1982; Suttle 2003) and ants (Willmer and Stone 1997; Altshuler 1999), they suggest that predator effects on seed output via the alteration of pollinator visitation may be more widespread than previously recognized.

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