

Assessing the effects of native plants on the pollination of an exotic herb, the blueweed *Echium vulgare* (Boraginaceae)

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Abstract The impacts of exotic plants on the pollination and reproductive success of natives have been widely reported; however, in spite of its importance for the invasive process, the role of native plants in the pollination and reproduction of exotic plants has been less explored. To fill this gap, we compared the patterns of pollination and reproductive success in the invasive herb *Echium vulgare* (Boraginaceae) between monospecific patches (only *E. vulgare*) and mixed patches (sympatry with native herbs *Schizanthus hookeri* and *Stachys albicaulis*) in central Chile. Using sample quadrats of 1 m × 2 m, we quantified the richness, diversity and visitation rate of flower visitors in 15-min observation intervals. We conducted an assay to assess the effect of the patch types (monospecific and mixed) and the isolation of flowers to visitors on both the fruit set and seed/ovule ratio. We showed that native plants favoured the richness of visitors of *E. vulgare*; however, they did not lead to increases in visitation rate. The reproductive success of *E. vulgare* did not show differences

between contrasted patches; however, the isolation of visitors decreased the fruit set, although seed production was maintained in the absence of pollinators, presumably by an autogamous mechanism. Complementary to our main research focus, we assessed changes in pollination variables and reproductive output in two coflowering native plants that occur with *E. vulgare*, *S. hookeri* and *S. albicaulis*. Despite the fact that our correlational study did not allow us to dissect the effects of mixed patches and relative plant abundances on variables measured for natives, we observed an increase in pollinator richness in mixed patches for the two plants studied. These results suggest a potential facilitation for visitor richness of the exotic plant in coexistence with native plants, although this facilitation does not result in changes in the visit rate or on the reproductive success of any of the studied species. This work underlines the need for additional research on community levels that assess reciprocal effects on pollination between coflowering natives and exotics.

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Introduction

Exotic plants are notorious for their effect on the biotic pollination and reproductive success of native plants, because these factors can negatively impact native plant fitness by changing the behaviour of flower visitors (Wilcock and Neiland 2002; Steffan-Dewenter et al. 2005; Chacoff and Aizen 2006; Mitchell et al. 2006; Bjerknes et al. 2007; Cariveau and Norton 2009; Morales and

Traveset 2009). The mechanisms underlying these effects seem to be related to factors such as native pollinator displacement (Traveset and Richardson 2006; Sargent and Ackerly 2008), the inefficiency of exotic pollinators to act as pollen carriers (Aigner 2004), the modification of animal behaviour (Bjerknes et al. 2007), changes in patterns of flower preference (Muñoz and Cavieres 2008; Pysek et al. 2011; Morales and Traveset 2009) and the contribution of heterospecific pollen from exotic plants that might interfere with the pollination of natives (Moragues and Traveset 2005; Yang et al. 2011). Overall, the indirect effects of exotic plants on natives through pollinator-mediated modifications have been summarised in three general consequences (reviewed by Bjerknes et al. 2007): (1) The exotic plant may monopolise the pollinator assemblage, hence reducing the pollinator diversity and visitation rate on native flowers (e.g. Brown and Mitchell 2001; Chittka and Schürkens 2001; Cariveau and Norton 2009; Pysek et al. 2011); (2) the exotic plant may increase the pollinator diversity and pollination rate on the surrounding native flora (e.g. Campbell and Motten 1985; Moragues and Traveset 2005; McKinney and Goodell 2011); and (3) the exotic plant may have a neutral effect on the pollination and reproductive output of natives (Aigner 2004), an effect much less reported to date. Under these scenarios, the arrival of an exotic plant might decrease or increase the reproductive success of native plants through changes in the quantitative component of pollination, namely the number and frequency of pollinator visits to the flowers (e.g. Chittka and Schürkens 2001; Brunet and Sweet 2006; Traveset and Richardson 2006). A recent meta-analysis showed that exotic plants adversely affect the visitation rate and reproductive success of native plants (Morales and Traveset 2009), hence underpinning the idea of pollinator monopolisation by exotic plants.

Although the impact of exotic plants on the pollination of native plants is relatively well documented (Bjerknes et al. 2007), studies that emphasise the impact of native plants on the pollination of exotic species are less frequent (but see Stout et al. 2002). This lack of emphasis is unfortunate because both the pollination and reproductive success of exotic plants may represent a key issue at the beginning of the invasion process (Pysek et al. 2011) in which exotic species would be facilitated by resident species (Simberloff and Von Holle 1999; Bruno et al. 2003; Morales and Aizen 2006). It is known that exotic plants may be either reproductively benefited or disadvantaged depending on the structure and composition of the native plant communities (Levine et al. 2004; Sax et al. 2007) and the abundance of native species (Muñoz and Cavieres 2008; Williams et al. 2011; Yang et al. 2011). In principle, the presence of native plant species may increase the pollination of exotic plants through synergistic effects that

mutually favour their pollination (Morales and Aizen 2002; Stout et al. 2002; Molina-Montenegro et al. 2008). In contrast, if native plants monopolise pollinators, the exotic species will experience a pollination cost from their coexistence with native species. In this study, we analysed the pollination process of an exotic plant species in the presence and absence of native species.

Our study focused on prairies along the Andean range in central Chile where the exotic plant *Echium vulgare* L. (Boraginaceae) forms monospecific patches and regularly coexists with the endemic plants *Schizanthus hookeri* Gill. ex Graham (Solanaceae) and *Stachys albicaulis* Lindl. (Lamiaceae). The three species overlap extensively in floral phenology in the region of study. The exotic plant has showy flowers and produces abundant nectar that might be an attractive resource for native pollinators (Carvallo 2011). The two native species, in turn, are the most abundant plants in the study site. As a way of assessing whether the exotic plant is favoured by native plants in terms of pollination and reproductive output, we compared the richness and diversity of flower visitors, the visitation rate and the reproductive success of *E. vulgare* in two contrasting situations: (1) when the exotic species coexists with native plants and (2) when it forms monospecific patches. Complementary to our main research focus, we explored the changes in pollination and reproductive output of the native plants *S. hookeri* and *S. albicaulis* when they form monospecific patches compared to when they coexist with *E. vulgare*. Specifically, our work was guided by an attempt to answer the following question: Does coflowering with native plants affect the pollination and fecundity of an exotic plant?

Methods

Study site

The study was carried out during the austral summer season of 2007 (January 5–February 12) in Termas de Chillán (36°54'34''S, 71°24'46''W, 1,834 m a.s.l.). The climate in the study area is temperate, and the vegetation consists of deciduous forests (Luebert and Pliscoff 2006) of the Fagaceae *Nothofagus pumilio* Krasser and *N. dombeyi* Oerst. We studied seven patches in a total area of approx. 267 ha (Table 1). We attempted to include as many patches as possible to obtain a good representation of the complete landscape and to avoid autocorrelation effects (Table 1). Two patches consisted of both native plants and *E. vulgare* (mixed patches, hereafter), and the remaining patches were chosen to represent the presence of only one species (monospecific patches, hereafter). Two patches had *E. vulgare* only, two patches had *S. hookeri* only, and one

patch had *S. albicaulis* only. We considered patches as monospecific whenever the relative abundance of the focal species was greater than 80 % within the patch, in spite of the fact that they hosted more than one species; the patch sizes was variable reflecting natural conditions in the landscape (Table 1). Although other plant species existed in the mixed patches and coflowered with the focal plants included in this study, we excluded them from the analyses because we concentrated on the study of the most abundant species in the sites.

Study species

This study focused on the most abundant exotic plant in the area, the blueweed *E. vulgare* L. (Boraginaceae). Of European origin, this plant was introduced in Chile in the late nineteenth century (Matthei 1995); it is currently naturalised in the country, with an ample latitudinal distribution range (from 25°17'S to 53°01'S). Several reports indicate that the introduction of *E. vulgare* to new habitats often causes reductions in the reproductive success of native plants (Kearns et al. 1998; Goulson 2003; Kenta et al. 2007; Dohzono et al. 2008). The large earth bumblebee *Bombus terrestris* has been described as a flower visitor of this plant in various parts of the world, such as New Zealand (Primack 1983) and the UK (Peat et al. 2005). In Chile, information on flower visitors to *E. vulgare* is scarce. Only two species have been reported as visitors: the native bee *Alloscirtetica tristigata* (Vivallo 2003) and the exotic bumblebee *B. terrestris* (Montalva et al. 2008).

Schizanthus hookeri Gill. ex Graham (Solanaceae) is a protandrous annual herb that requires pollinators for seed production (Pérez et al. 2009). It has zygomorphic flowers with petals fused to form a floral tube that protrudes from the calyx; the floral morphology of this species has been described as a bee pollination syndrome (Pérez et al. 2006,

2007). Visitors of *S. hookeri* have been largely described in other studies in this site (Pérez et al. 2006) and comprise mainly the bees *Svastrides melanura* (Apidae) and *Megachile semirufa* (Megachilidae), the native bumblebee *Bombus dahlbomii* (Apidae) and the Andean hummingbird *Oreotrochilus leucopleurus* (Trochilidae). The perennial herb *S. albicaulis* Lindl. (Lamiaceae) has flowers with a bilabiate corolla. Some visitors to *S. albicaulis* in Chile include the bees *Alloscirtetica tristigata* (Apidae) (Vivallo 2003) and *Megachile saulcyi* (Megachilidae) and the white-sided Hillstar *O. leucopleurus* (Trochilidae) (Herrera et al. 2004). Details of plant species studied are given in Table 2.

Pollination measurements

To quantify the pollination variables, we set 1 m × 2 m quadrats every 10 m along linear transects within patches. Within each quadrat, the number of individuals and flowers per individual at each focal plant species were recorded before pollination measurements. In the mixed patches, some quadrats contained only the focal species or a combination of species; because our work aimed to compare the pollination properties between patches, we assumed that the overall composition of patches (monospecific and mixed) exerted a greater effect on variables than did the variability within the quadrats for the measured pollination variables. Therefore, we quantified within each quadrat the number of individuals and flower of the focal species, in spite other species are within quadrats. The identity and number of flower visitors were recorded during 15-min observation intervals in each quadrat; quadrats without flowers were excluded from the analyses. Details of the sampling effort are shown in Table 3. Observations were carried out during January 16–February 10, always on sunny days between 9:30 and 19:30 h. To avoid bias in the sampling process, we rotated the time of the observation

Table 1 Characteristics of patches studied in Termas de Chillán, central Chile, 2007

Patch	Type	Area (m ²)	Plant species present	Number of 1 m × 2 m quadrats	Plant richness	Plant diversity
01	Mixed	2412	<i>E. vulgare</i> (0.43); <i>Schizanthus hookeri</i> (0.14); <i>Stachys albicaulis</i> (0.06)	53	8	2.44
02	Monospecific	855	<i>Schizanthus hookeri</i> (1.00)	36	1	0
03	Mixed	1922	<i>E. vulgare</i> (0.14); <i>Schizanthus hookeri</i> (0.18); <i>Stachys albicaulis</i> (0.23)	83	4	1.73
04	Monospecific	624	<i>Schizanthus hookeri</i> (0.93)	24	2	0.25
05	Monospecific	1080	<i>E. vulgare</i> (0.99)	22	2	0.06
06	Monospecific	918	<i>E. vulgare</i> (1.00)	15	1	0
07	Monospecific	962	<i>Stachys albicaulis</i> (0.99)	25	3	0

We measured the richness and diversity (Shannon index) of plant species in each patch; values in parenthesis after plant names are the relative abundance of focal species in patches

Table 2 Characteristics of plants studied in Termas de Chillán, Chile

Characteristics	<i>E. vulgare</i> (Boraginaceae)	<i>Schizanthus hookeri</i> (Solanaceae)	<i>Stachys albicaulis</i> (Lamiaceae)
Origin	Exotic, Eurasia	Endemic to Chile	Endemic to Chile
Frequency among mixed patches	52 %	39 %	47 %
Culm heights (range)	0.2–1.3 m	0.6–0.8 m (Hoffmann 1978)	0.4–0.8 m (Hoffmann 1978)
Number of flowers per individual (mean \pm SE, <i>N</i>)	39 \pm 1, 943 ind.	29 \pm 2, 335 ind.	36 \pm 2, 301 ind.
Nectar volume per flower per individual per day (mean \pm SE, <i>N</i>)	1.47 \pm 0.04, 90 fw. (Carvallo 2011)	0.87 \pm 0.15, 27 fw. (Carvallo 2011)	0.47 \pm 0.02, 105 fw. (Carvallo 2011)
Reproductive system	Self-compatible (Leiss et al. 2004)	Self-compatible (Pérez et al. 2007)	Self-compatible (Arroyo and Uslar 1993)

The frequency in the mixed patches depicts the percentage of quadrats where the focal species was observed in relation to all quadrats where the focal species would potentially be found ($n = 136$ quadrats); because a plant species could occur in more than one quadrat, these frequency values do not represent the relative abundances and sum more than 100 %. Nectar volume per flower is expressed in μL

fw. is the number of flowers; *ind.* is the number of individuals

period of different patches, alternating between a time before noon and a time after noon. In total, we performed 393 observation periods that were 15 min each (5,895 min of observation). The identity of visitors was determined to the species level. When animals were unknown, in situ specimens were captured and transported to the laboratory for subsequent identification. Because animals that contact both reproductive structures and enter floral tubes are not necessarily efficient pollinators (Alarcón 2010), we call them “flower visitors” hereafter.

The similarity of the flower visitors’ composition was compared for each plant species separately between monospecific and mixed patches based on the number of visits that each focal plant species received using quadrats as sampling units. Two similarity indexes were used: Sørensen (based on only presence–absence information) and Bray–Curtis (based on species abundance information) (Cassey et al. 2008). Both indices can take values ranging from 0 to 100, and these indices will take the value of 0

when no common species are shared between paired contrasting patches. The Bray–Curtis index will reach the value of 100 when all species are shared between the two patches and each species has exactly the same abundance in each (Cassey et al. 2008). For each focal plant, community matrices of the form quadrat \times visitor were built which allows to generate dissimilarity matrices. To evaluate whether the similarities observed are statistically significant between patch types, permutational analysis of variance (PERMANOVA; Anderson 2001) was performed for each plant species; PERMANOVA allows the partitioning of the sum of squares from a centroid in each source of variation (patch types in this study) in analogous way to MANOVA (Oksanen et al. 2011). These analyses were carried out using the *adonis* function (999 iterations) from the *vegan* package (Oksanen et al. 2011) in R.2.14.2 (R Development Core Team 2011).

The richness and diversity of flower visitors (hereafter *S* and *H*, respectively) were estimated for the mixed and

Table 3 Sampling effort and properties of the flower visitor assemblages for three plant species studied in Termas de Chillán, central Chile, in monospecific patches (MS) and mixed patches (MX) during 2007

Species	Patch types	Quadrats studied	Quadrats visited	Flowers visited	S_{OBS}	$S' \pm \text{CI}_{95} \%$	$H' \pm \text{CI}_{95} \%$	AVR \pm SE
<i>Echium vulgare</i>	MS	37	36	300	14	13 \pm 0	1.47 \pm 0.02	0.06 \pm 0.01
	MX	197	188	910	20	15 \pm 0	1.23 \pm 0.01	0.05 \pm 0.02
<i>Schizanthus hookeri</i>	MS	60	42	229	13	14 \pm 1	1.39 \pm 0.03	0.04 \pm 0.01
	MX	186	67	266	16	16 \pm 1	1.79 \pm 0.01	0.07 \pm 0.01
<i>Stachys albicaulis</i>	MS	25	24	136	10	8 \pm 0	1.67 \pm 0.08	0.09 \pm 0.02
	MX	220	125	559	20	15 \pm 0	1.68 \pm 0.01	0.12 \pm 0.03

The sampling effort is represented by the number of quadrats with the focal plant, number of quadrats with at least 1 visit for the focal plant and number of flowers visited. Properties of the flower visitor assemblage are represented by the observed richness (S_{OBS}), estimated richness and diversity for 100 samples (S' and H' , respectively) and the mean visit rate (AVR). The visit rate accounted for the number of visits \times number of conspecific flowers⁻¹ in 15-min observation periods

$\text{CI}_{95} \%$ is the 95 % confidence interval obtained with sample-based rarefaction curves, *SE* is the standard error

monospecific patches for each plant species using sample-based rarefaction curves with the quadrats as sample units. Rarefaction parameters were estimated with plot replacement (1,000 iterations) for both richness and diversity using the Mau Tau estimator (Colwell et al. 2012) and Shannon index, respectively, in EstimateS 9 (Colwell 2013). Significant differences in parameters (S' and H') between monospecific and mixed patches were assumed when the 95 % confidence intervals did not overlap 100 samples (Badano and Cavieres 2006); when the number of samples was less than 100, we used the extrapolation routine proposed by Colwell et al. (2012) to obtain S' and H' to 100 samples.

Finally, the animal visitation rate to flowers (AVR hereafter) was estimated for each plant species as the number of visits recorded in the 15-min observation intervals divided by the number of flowers of conspecific individuals per quadrat. A general linear model was used to assess the variation of AVR between patch types for each plant species; we used the *lm* function for R.2.14.2 (R Development Core Team 2011).

Fruit and seed production

We conducted assays to evaluate the effect of the patch type (mixed and monospecific) and the potential effect of flower visitors (presence and absence) on the fruit set and on the seed/ovule ratio (S:O ratio, hereafter) for each plant species. Because this work focused on the assessment of the changes in pollination of plants, we considered it is important to evaluate and compare the role of floral visitors on the reproductive success of the studied plants in the monospecific and mixed patches. For our assays, the number of individuals selected in monospecific and mixed patches (MS/MX) from each focal plant species was *E. vulgare* (80/120), *S. hookeri* (80/120) and *S. albicaulis* (40/120). To avoid any confounding effects on our estimations of the visitation rate (described in the previous section), plants from outside of the transects previously settled were chosen. 50 % of the individuals in each patch type were isolated from flower visitors using silk bags that covered the stem with flower buds; the remaining fraction was left uncovered to be used as the control. Five flower buds were tagged per plant and followed to the fruit stage. Fruits were removed from plants when ripe, 27, 23 and 21 days after flower anthesis in *S. hookeri*, *S. albicaulis* and *E. vulgare*, respectively. The fruits were kept in paper bags and transported to the laboratory for seed counting. We estimated the fruit set (fraction of flowers converted to fruits) and the S:O ratio (number of seeds \times number of fruits⁻¹ \times number of ovules⁻¹) as measures of reproductive success per individual and per fruit, respectively; specifically, fruit set measures pollination success (Blanche

et al. 2006), while S:O ratio per fruit assesses the quality of pollen deposited on stigmas (Griffin and Barrett 2002). The S:O ratio was estimated for *E. vulgare* and *S. albicaulis* (4 ovules per flower), but estimation was not feasible for *S. hookeri* because the number of ovules per flower was not quantified before fecundation. We used a two-way ANOVA test to assess the extent to which variation in reproductive output was attributable to patch type and flower visitor exclusion, independently for each plant species. Before the analyses, the variables were log₁₀-transformed; the analyses were performed using the *lm* function of R.2.14.2 software (R Development Core Team 2011).

Results

Pollination

We recorded 2,400 flower visitors that contacted flowers, with a total of 23 insect taxa belonging to the orders Hymenoptera (48 % of observed taxa), Lepidoptera (22 %), Diptera (17 %) and Coleoptera (14 %) (see Online Resource 1). The compositional similarity of flower visitors in *E. vulgare* between monospecific and mixed patches reached 45 and 35 % ($N = 232$ comparisons) for the Sørensen (presence/absence based) and Bray–Curtis (abundance based) indexes, respectively. The results of the PERMANOVA test showed that there were significant differences of similarity between compared groups for both indices (Sørensen: pseudo- $FI_{232} = 4.923$, $P = 0.001$; Bray–Curtis: pseudo- $FI_{232} = 1.989$, $P = 0.029$). For the natives, similarities reached values of 23 % (Sørensen: pseudo- $FI_{108} = 5.183$, $P = 0.010$) and 15 % (Bray–Curtis: pseudo- $FI_{108} = 13.995$, $P < 0.001$) in *S. hookeri*; *S. albicaulis* showed a compositional similarity of 33 % (Sørensen: pseudo- $FI_{148} = 10.283$, $P = 0.009$) and 26 % (Bray–Curtis: pseudo- $FI_{148} = 8.231$; $P = 0.002$). *Schizanthus hookeri* was the plant with the lowest similarity among the studied patches, while the exotic *E. vulgare* showed the greatest compositional similarities between compared patches.

The richness of flower visitors was greater in mixed patches than in monospecific patches for all studied plants, with an increase in richness of 43, 23 and 100 % for *E. vulgare*, *S. hookeri* and *S. albicaulis*, respectively (Table 3). A comparison of mean \pm confidence intervals (CI) between patches for each species showed that differences in richness have statistical significance in both *E. vulgare* and *S. albicaulis* but not in *S. hookeri* (Table 3). The diversity of flower visitors was greater in monospecific patches than in mixed patches for the exotic *E. vulgare*. The main visitors (accounting for at least 75 % of total

visits) of *E. vulgare* in the monospecific patches were the exotic bumblebee *B. terrestris* (61 % of visits), the native bee *Alloscirtetica gayi* (9 %) and the hoverfly *Syrphus octomaculatus* (6 %); in mixed patches, the main visitors of *E. vulgare* were *B. terrestris* (69 % of total visits) and *A. gayi* (10 %). These differences in relative abundance of visits between patches would explain, at least in part, the lower diversity of visitors observed in mixed patches for the exotic *E. vulgare*. The main visitors of *S. hookeri* were as follows (values for monospecific/mixed patches, respectively): the bees *A. gayi* (61 %/33 %), *B. dahlbomii* (6 %/6 %), *Chalepogenus* sp. (6 %/29 %), the dipterans *S. octomaculatus* (13 %/6 %) and *Lasia corvine* (6 %/13 %); for this plant, three flower visitors (*A. gayi*, *S. octomaculatus* and *B. dahlbomii*) and four flower visitors (*A. gayi*, *Chalepogenus* sp., *L. corvine* and *S. octomaculatus*) accounted for at least 75 % of visits in monospecific and mixed patches, respectively. For *S. albicaulis*, the main visitors were as follows (values for monospecific/mixed patches, respectively): *B. terrestris* (36 %/49 %), *B. dahlbomii* (30 %/9 %), *A. gayi* (8 %/5 %), *L. corvine* (7 %/4 %) and *Chalepogenus* sp. (4 %/19 %); For this plant, four and three species of flower visitors accounted for at least 75 % of visits in each patch type.

The AVR in the exotic *E. vulgare* showed decrease of 20 % in mixed patches compared to monospecific patches (Fig. 1), although this difference was not statistically significant (results of general linear model, $F_{1;158} = 0.409$, $P = 0.524$). For native plants, the AVR showed a tendency to increases in mixed patches (Fig. 1) by 43 % in *S. hookeri* ($F_{1;161} = 1.421$, $P = 0.235$) and 25 % in *S. albicaulis* ($F_{1;131} = 0.045$, $P = 0.832$), although the differences were neither statistically significant.

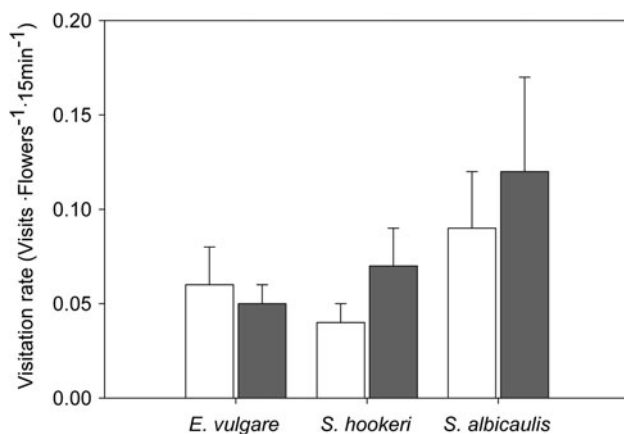


Fig. 1 Visitation rate (number of visits \times flower⁻¹ \times 15 min⁻¹) (mean \pm SE) received by the exotic plant *E. vulgare* and two natives, *S. hookeri* and *S. albicaulis*, in monospecific patches (white bars) and in mixed patches (grey bars)

Reproductive output

The patch type did not significantly affect the reproductive success (fruit set and S:O ratio) of any of the studied plant species (Table 4); however, the pollinator exclusion had a significant negative effect on the fruit set in the three studied plants (Table 4) by reducing it by 82, 77 and 55 % in *E. vulgare*, *S. hookeri* and *S. albicaulis*, respectively (Fig. 2). No significant effect of pollinator exclusion on S:O ratio was detected in *E. vulgare* and *S. albicaulis* (Table 4); for *E. vulgare*, the S:O ratio averaged 0.50 ± 0.02 ($N = 72$) and 0.53 ± 0.02 ($N = 118$) in excluded and non-excluded flowers, respectively. In *S. albicaulis*, the S:O ratio averaged 0.57 ± 0.1 ($N = 122$) and did not show differences among patch types (Table 4).

Discussion

The facilitation of pollination and reproductive success of exotic plants mediated by native plants is an expected, albeit scarcely observed phenomenon (Morales and Aizen 2006). Among the potential results that the introduction of an exotic plant on pollination of natives had (Bjerknes et al. 2007, detailed in the “Introduction”), our results suggest a facilitative effect of native flora on visitor richness for the exotic plant *E. vulgare*, but this increase in richness did not translate into significant increases in the diversity or in the visitation rate received by the flowers. The richness in mixed patches for *E. vulgare* could be improved by the existence of other coflowering species whose visitors might contribute sporadic visits to the flowers of *E. vulgare*, thus significantly increasing the visitor richness. At the same time, these sporadic visitors might discriminate among native and exotic species (Ghazoul 2006), causing only a subset of visitors, usually generalists in the use of resources, to exploit the exotic flowers of *E. vulgare* in mixed patches. Indeed, we observed that visits in mixed patches were due mainly to the exotic bumblebee *B. terrestris* (69 % of total visits), unlike the visits in monospecific patches where the same percentage of visits was reached by two visitors (*B. terrestris* and the native bee *A. gayi*). In spite of increase in richness, the visitation rate for *E. vulgare* did not show significant differences between the contrasted patches. This result could be explained by two, non-exclusive points of view. First, the presence of native plants in mixed patches could exert a neutral effect on visitors of *E. vulgare* because native flowers may not be attractive for visitors, in this case, *B. terrestris*; it has been recognised that *B. terrestris* prefer exotic flora to native flora in several regions, as reviewed in Hanley and Goulson 2003; Hingston 2005). Second, the relation between *E. vulgare* and *B. terrestris* would be maintained by the

Table 4 Results of the two-way ANOVA test that evaluated the effect of patch type and visitor exclusion on the (1) fruit set and (2) seed/ovule ratio of the exotic plant *E. vulgare*, and the natives *Schizanthus hookeri* and *Stachys albicaulis*

Sources of variation	<i>Echium vulgare</i>			<i>Schizanthus hookeri</i>			<i>Stachys albicaulis</i>		
	df	MS	F	df	MS	F	df	MS	F
(1) Fruit set									
Patch type	1	0.007	2.075	1	0.021	3.421	1	0.128	8.037
Exclusion	1	1.128	323.6***	1	0.362	130.6***	1	0.304	42.735***
Interaction	1	0.021	3.885	1	0.001	0.511	1	0.018	2.616
Error	197	0.003		188	0.003		155	0.007	
(2) Seed/ovule ratio									
Patch type	1	0.001	0.446				1	0.001	0.936
Exclusion	1	0.006	0.962				1	0.003	2.372
Interaction	1	0.000	0.023				1	0.003	1.831
Error	166	0.001					118	0.001	

The values of statistical significance were Bonferroni corrected and depicted as *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

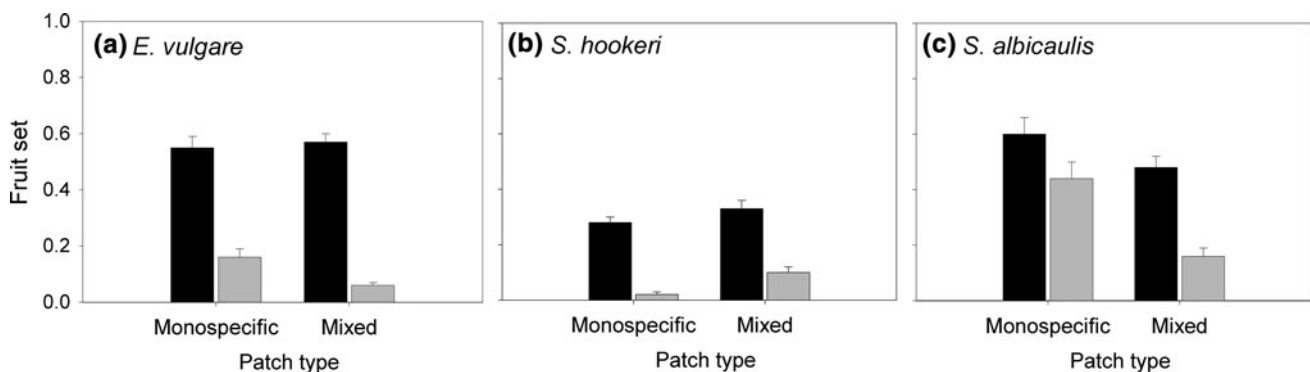


Fig. 2 Effect of patch type and visitor exclusion on the fruit set (mean \pm SE) of the exotic plant *E. vulgare* (a), and the natives *S. hookeri* (b) and *S. albicaulis* (c) studied in central Chile, 2007. For the

greater abundance of *E. vulgare* in studied patches generating that the number of visits of *B. terrestris* is kept more or less invariable, driving a fast naturalisation process of both species.

In relation to the fecundity of *E. vulgare*, we did not detect differences between the contrasted patches for the fruit set or the S:O ratio. The most straightforward explanation for this absence of differences in the reproductive output is that the similar visitation rates that *E. vulgare* receives in both monospecific and mixed patches create comparable levels of reproductive success. Interestingly, our results show the importance of visitors as pollen vectors and their role in the increase in fruit set. The similarity in the S:O ratio between patch types indicates that *E. vulgare* received comparable pollen qualities, which allowed to reach a fecundation of ca. 50 % of ovules per individual. Since pollen loads carried by *Bombus* show greater presence of *E. vulgare* pollen in mixed patches (Carvalho 2011), the limits to reach a proportion of 1:1 for S:O ratio could be more related to the transfer of pollen

exclusion treatment, control (no exclusion from visitors) is represented by black bars, and exclusion is represented by grey bars

from visitor to stigmas than to the differences in pollen quality between contrasted patches; in spite that the bumblebee *B. terrestris* is the main visitor of *E. vulgare*, other studies have shown the same tendency, with a 0.15 % of pollen collected by *B. terrestris* that made contact with the stigmas of *E. vulgare* (Rademaker et al. 1997).

Our study suggests certain level of autogamy in flowers of the exotic *E. vulgare* (and the two natives), which is an expected result since these species show self-compatibility levels (Leiss et al. 2004) and because the quality of pollen carried by visitors would be similar in a same patch (Carvalho 2011). One of the most common arguments used to account for the success of invasive plants is that they have high self-compatibility levels and some degree of autogamous reproduction (Baker 1974). Little quantitative information is available on the breeding biology and pollination requirements of most invasive plants (Pysek et al. 2011), but studies have shown that entirely autogamous and outcrossing exotic plant species are highly successful (Richardson et al. 2000). A promising field of research

regarding invasions focuses on the reproductive biology of exotics, specifically, the potential of a species to become invasive and show a rapid adaptive genetic change (Sax et al. 2007) favouring the “rapid evolutionary” process (Buswell et al. 2010). Regarding the exotic species in our study, the individuals of *E. vulgare* that are autogamous and support the genetic disadvantages of endogamy would be favoured by natural selection and permit the spread of this species in new ranges. Of course, future studies are necessary to elucidate whether this situation occurs in populations of *E. vulgare*.

Even though the correlational nature of our study prevents us from determining whether changes in pollination for native flowers are attributable to the presence of *E. vulgare* or to an overall effect of the mixed flora (e.g. abundances; Ebeling et al. 2008), it is likely that the presence of *E. vulgare* enhances the overall reward level in mixed patches through the increased availability of pollen and nectar. Exotic plants have been reported as the main factor in producing changes in these variables (Lopezariza-Mikel et al. 2007). For instance, the nectar production of *E. vulgare* (1.47 μL per flower, on average) largely exceeds that of *S. hookeri* (0.87 μL) and *S. albicaulis* (0.47 μL), which suggests that the variation in resource availability among patches is a parsimonious explanation for the greater richness of visitors observed in mixed patches for native plants; the nectar production differences also explain the tendency of natives to have a higher AVR in mixed patches than monospecific patches. Unlike *E. vulgare* and *S. albicaulis*, we did not detect significant differences in flower visitor richness for *S. hookeri* between monospecific and mixed patches, although the diversity of visitors was greater in mixed patches than in monospecific patches. It is likely that the low diversity of flower visitors observed in the monospecific patches of *S. hookeri* relates to the specialised floral design of this species compared to *E. vulgare* and *S. albicaulis*. *Schizanthus* has a long and narrow floral tube associated with bee pollination (Pérez et al. 2006) that may restrict the diversity of flower visitors to a relatively low fraction of the total assemblage. For instance, the native bee *Alloscirtetica gayi* accounted for 61 % of all visits in monospecific patches for *S. hookeri*; the remaining visitors consisted of species with specialised mouthparts, such as dipterans with long stylets (families Acroceridae and Nemestrinidae) and lepidopterans with uncoiled proboscises (families Hesperidae and Pieridae). The greater diversity of the flower visitors to *S. hookeri* in mixed patches may relate to the lesser prevalence of *A. gayi* (33 %). Our results also show that visitors increase the reproductive success of natives, although the fact that these plants are self-compatible could favour a mechanism of reproductive assurance, especially when pollinators are absent (Kalisz et al. 2004; Moeller and Geber 2005). Exotic

plants stand as an interesting study model that could be used to assess evolutionary changes in reproductive biology.

Although exotic species have been considered one of the most important factors that influence biodiversity loss in temperate ecosystems (Sala et al. 2000), only a limited number of studies have evaluated the effects of exotic species on pollination of native plants from the temperate biome of South America (see Morales and Aizen 2002, 2006; Aizen et al. 2008; Molina-Montenegro et al. 2008; Valdovinos et al. 2009). This study stresses the need to increase research on reciprocal exotic-native effects; pollination ecologists have focused their research mostly on the effect of invasive species on population-level variables of particular native plant species, leaving aside community-level effects. This population-centred perspective should be complemented with analyses of broad groups of mutual invasive and native species to have a more complete picture of the effects of exotic species on pollination processes.

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