

Assessing the impact of the invasive buff-tailed bumblebee (*Bombus terrestris*) on the pollination of the native Chilean herb *Mimulus luteus*

Gabriel Esterio · Roxana Cares-Suárez ·
Catalina González-Browne · Patricia Salinas ·
Gastón Carvallo · Rodrigo Medel

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Abstract The arrival of exotic pollinators to new habitats may introduce new patterns of floral preference and foraging behavior that modify the structure of the resident plant–pollinator community. The aim of this paper is to examine the potential impact of the exotic bumblebee *Bombus terrestris* on the pollination service provided by the native pollinator assemblage of the herb *Mimulus luteus*. The study was performed in a high-elevation locality in the Chilean Andes during the summer seasons of 2010, 2011, and 2012. We recorded visitation rate, and the number of pollen grains transported on the body of *B. terrestris* and native pollinators and the pollen deposition on the stigmas of *M. luteus*. Pollinator effectiveness (pollen deposited × visitation rate) was compared among species. Results revealed that *B. terrestris* was an inefficient pollinator, due to the low amount of pollen delivered on stigmas and the low and intermittent visitation rate across years. The parallel inter-annual variation in the visitation rate of *B. terrestris* and the native bumblebee *Bombus dahlbomii* suggests that the integration of *B. terrestris* had no important consequences for the congeneric species. In general, *B. terrestris* accounted for a low proportion of the pollen transfer in *M. luteus*, reaching 4.6 % in 2010, absence of effect in 2011, and 0.01 % in 2012. These

results suggest that in spite of being a quickly spreading species in Chile, *B. terrestris* is still in the initial phase of invasion in this area.

Keywords Plant–pollinator · Exotic bee · Pollinator effectiveness · Pollinator efficiency · Invasion process

Introduction

The introduction of exotic species to natural ecosystems has been described as one of the most important threats to biodiversity (Sala et al. 2000; Courchamp et al. 2003). Exotic species may affect native plant and pollinator species through different interaction pathways, often usurping interactions and disrupting plant–animal mutualistic relationships (Traveset and Richardson 2006). The introduction of exotic pollinators may have a series of effects on native pollinators, such as competition for floral resources and nesting sites, pathogen transmission, and risk of hybridization with congeneric species (Goulson 2003; Morales 2007; Stout and Morales 2009, Goulson 2010). In addition, the introduction of exotic pollinators may modify the organization of the native pollinator assemblage (Stout and Morales 2009), which might change reproductive patterns of the native plant community (Bjerknes et al. 2007). The negative effect of exotic pollinators on the native flora may occur through at least two mechanisms. First, exotic pollinators may be less efficient than native pollinators due to morphological mismatches in the plant–pollinator interface (Goulson 2003). Second, the presence of exotic pollinators may reduce the visitation rate of native pollinators when the foraging strategies used by exotic species make flowers less attractive to native pollinators (e.g., nectar theft, Dohzono and Yokoyama 2010).

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G. Esterio · R. Cares-Suárez · C. González-Browne ·
P. Salinas · R. Medel (✉)
Departamento de Ciencias Ecológicas, Facultad de Ciencias,
Universidad de Chile, Casilla 653, Santiago, Chile
e-mail: rmedel@uchile.cl

G. Carvallo
Instituto de Biología, Facultad de Ciencias, Pontificia
Universidad Católica de Valparaíso, Valparaíso, Chile

Bombus terrestris (Hymenoptera: Apidae) is one of the pollinator species most widely used for commercial purposes (Dohzono and Yokoyama 2010). The species often escapes from confinement, becoming naturalized in the invaded ecosystems (Inari et al. 2005). For example, the species is naturalized in New Zealand (introduced in 1885), Israel (introduced in 1960), Japan (introduced in 1991), and Tasmania (introduced in 1992) (Goulson 2003; Inoue et al. 2008; Dohzono and Yokoyama 2010). This species is an efficient invader with a high capacity to establish and displace native pollinator species, apparently through interspecific competition (Ings et al. 2005, 2006; Williams and Osborne 2009). In Japan, the invasion by *B. terrestris* has displaced native bumblebees mostly through competition for nesting sites rather than by competition for floral resources (Inoue et al. 2008). In Tasmania, where the species expands at a rate of 25 km per year in typical environmental conditions (Hingston et al. 2002), *B. terrestris* has a high potential to impact ecosystem functioning because of the high diversity of plants it visits, the ample overlap in floral resource use with native pollinators and the occurrence of visitation on flowering species not previously visited by other invasive pollinator species (Hingston and McQuillan 1998). For instance, introduced *B. terrestris* reduced the number of flowers visited and the time spent by two native megachilid bees on flowers of *Gompholobium huegelii* (Fabaceae) in Tasmania (Hingston and McQuillan 1999). In Israel, *B. terrestris* increased in abundance during the 80s and 90s, and at the same time, the honeybee *Apis mellifera* and solitary bee species decreased in abundance (Dafni and Shmida 1996).

In Chile, several bee species have been introduced for commercial purposes such as *Apis mellifera*, *Megachile rotundata*, *Bombus impatiens* and *Bombus ruderatus* (Arretz and Macfarlane 1986; Ruz 2002; Rebolledo et al. 2004). The introduction of *B. terrestris* to Chile occurred in 1997–1998 (Montalva et al. 2008). Several colonies from Israel and Belgium were established in different localities of north-central Chile, and their pollinator efficiency in tomato and avocado plantations was monitored (Fried 1999; Ruz 2002). The current distribution of *B. terrestris* in Chile ranges from ca. 30 to 38° S and from the sea level to 3,400 m elevation (Montalva et al. 2008). In spite of the increasing expansion of the area invaded by *B. terrestris* in southern South America, evidence on its ecological impact on native pollinators is still scarce, fragmented, and preliminary. It has been suggested that the bumblebee *Bombus dahlbomii* is under threat by the arrival of the exotic *B. terrestris* and *B. ruderatus*, in part because of competition for shared flower resources and nesting sites. This threat can be due to the ability of *Bombus terrestris* to visit a larger number of flowers per minute in comparison with *B.*

dahlbomii and other native bee species (Ruz and Herrera 2001).

In this report, we present data on the pollination service of native pollinators and the exotic *B. terrestris* on the herbaceous *Mimulus luteus* (Phrymaceae) during three consecutive years. More specifically, we attempt to answer the following two questions: 1) What is the contribution of the exotic *B. terrestris* to the overall pollination service received by *M. luteus*? 2) Does *B. terrestris* impact on the pollination service provided by native pollinators to *M. luteus*? To answer these questions, we estimated the pollination efficiency and effectiveness of each pollinator species by recording the number of pollen grains attached to their bodies, the number of pollen grains deposited on stigmas, and visitation rates in three consecutive years in an Andean locality of central Chile.

Materials and methods

Study site and natural history

Mimulus luteus is a hydrophilic perennial herb that grows from 0 to 3,650 m elevation and between 29° and 45°S (von Bohlen 1995). The species is hermaphroditic, slightly protogynous, and self-compatible. It presents solitary hermaphrodite flowers with yellow corollas of 721 mm² and a conspicuous red spot in the lower lobe, which acts as nectar guide (Medel et al. 2003; Botto-Mahan et al. 2004). Flowers are zygomorphic, 34 mm height (along the symmetry plane) and 26 mm width. Flowers remain open among 3.6–5.9 days, depending whether pollinated or not (Medel et al. 2003) and have a stigma exerted above anthers, which has been named “approach herkogamy” by Barrett (2002) (Fig. 1a). Previous studies performed in different populations of *M. luteus* indicate that even in the face of complete self-compatibility, autonomous self-pollination produces low fruit set and seed production, and pollen vectors are required to assure effective pollination (Medel et al. 2003; Pohl et al. 2006; Carvallo and Medel 2010). The flowering season of *M. luteus* extends from mid-December to late February with a pick of flowering during mid-January–mid-February. Because of its wide distribution in Chile, *M. luteus* is a useful model to examine the impact of invasive pollinator species on the composition and efficiency of native pollinator assemblages in replicated populations. For instance, current information indicates that the invasive *Bombus terrestris* is already present in four out of seven populations of *M. luteus* reported in Medel et al. (2007).

We focused on a population located in Juncal (32°51'S, 70°08'W, 2,398 m elevation), a high Andean locality about

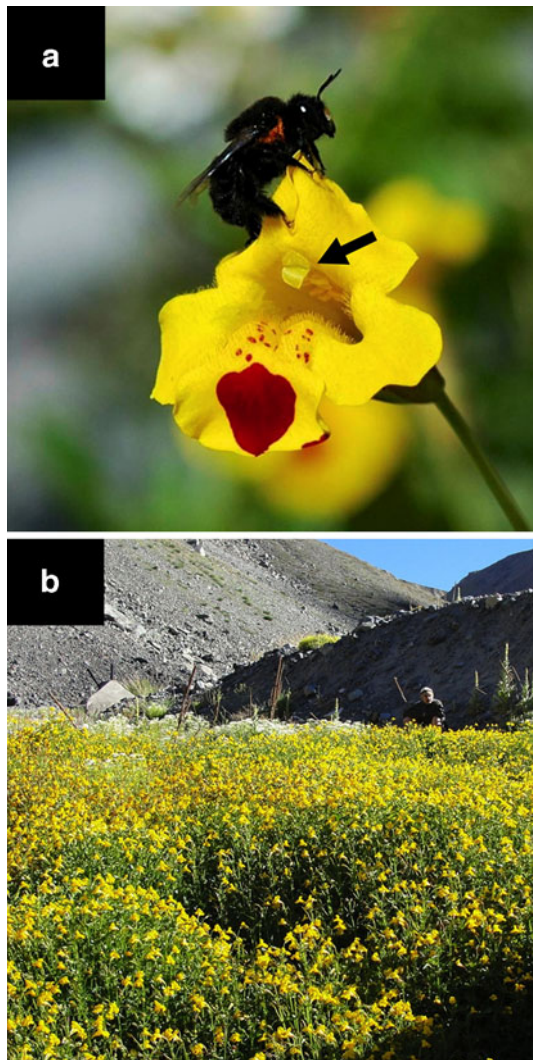


Fig. 1 **a** *Centris chilensis* (Apidae) in a flower of *Mimulus luteus*. Arrow indicates the bilabiate stigma that closes after mechanical contact. **b** Patch of *Mimulus luteus* in the study site. Patches are relatively discrete units of thousands of flowers located in well-drained areas of the Chilean Andes

80 km northwest of Santiago. The site is a well-drained substrate that is entirely covered by snow during at least 3 months a year. The vegetation type corresponds to associations of the subalpine belt (Hoffmann et al. 1998). The most represented plant species at the site are *Minulus luteus* (Phrymaceae) (relative abundance from five 10 m transects: 67.8 %), *Brassica campestris* (Brassicaceae) (11.7 %), *Chrysanthemum parthenium* (Asteraceae) (9.5 %), *Veronica anagallis-aquatica* (Scrophulariaceae) (6.4 %), *Verbascum densiflorum* (Scrophulariaceae) (1.9 %), *Solidago chilensis* (Asteraceae) (1.5 %), and *Lactuca serriola* (Asteraceae) (1.1 %). All these species co-flower with *M. luteus* but are less frequently visited by insects. As almost 70 % of the plants in the patch corresponded to *M. luteus* (Fig. 1b), it is unlikely that potential changes in the relative abundance of

the accompanying flora had influenced the visitation rate on *M. luteus* among years.

Field and laboratory procedures

We recorded the visitation rate of every pollinator species on flowers of *M. luteus* during 157 h in 2010 (February 5 to February 24), 77 h 45 min in 2011 (January 22–January 30), and 31 h 40 min in 2012 (January 7–January 17). A variable number of observers recorded the identity and frequency of visits during 30 min in flower patches in an area of ca. 20 m × 10 m (Fig. 1a). Each observer recorded the insect activity in 222 flowers on the average (range: 110–420 flowers). The same patches were surveyed each year, which ensured a comparable flower density among years (ca. 137.5 flowers/m² on the average). Observations were performed on sunny days only and between 8:00 and 12:30. Visitation rate was calculated as the average of the number of visits flower⁻¹ h⁻¹ using time intervals as replicates.

To estimate the pollen grains carried by insect species, a sample of each insect species was collected in the morning, only in 2010 (the sample sizes are shown in the results), and the following procedure was performed for all the species. Insects were deposited in killing jars containing a layer of paper tissue soaked in ethyl acetate to act as a rapid killing agent. The third pair of legs of each insect species was removed because pollen from these structures (corbiculae) is used by insects and not available for pollination (Inouye et al. 1994, Thorp 2000). All insects were stored in Eppendorf vials with 1 ml of 70 % ethanol and transported to the laboratory for pollen counting. Pollen was removed from the surface of the insect's body by shaking tubes in a vortex for 1 min. The resulting solution was stained with methylene blue and shaken again for 1 min, after which three samples of 10 µl were taken. The pollen grains from each sample were spread on a grid set in cover slips and counted using a compound microscope. Each count was extrapolated to 1 ml to get an average number of pollen grains per individual (Hingston 2006). The pollen transported by a pollinator species was calculated as the average value from all the individuals measured. In addition to pollen counting and to relate morphometric variables with pollinator efficiency, we recorded the body length (mm), thorax width (mm), and head width (mm) of the six pollinator species (five specimens per species).

Pollination intensity (PI), that is, the mean number of pollen grains deposited by pollinator species (Silander and Primack 1978), was assessed by removing stigmas of virgin flowers after being visited by the first time by a pollinator. To ensure that stigmas were not previously visited, we removed the corolla of all flowers in the patch at dusk (1,858 flowers on the average during 8 days) leaving only

flower buds ready to open next morning. Since nocturnal pollination is absent in this species, all the flowers in anthesis in the next morning were virgin. Observations of visits began before the arrival of the diurnal pollinators. Assessment of pollination intensity was performed only in the summer of 2012. The visited stigmas were immediately deposited in Eppendorf vials with 70 % ethanol. This method fixed the pollen grains on the stigma surface, which permitted subsequent counting of pollen grains in the laboratory. Because stigmas were fixed immediately after being visited by a pollinator, no formation of pollen tubes was expected. The tissues were softened with 2 M NaOH for 14 h and washed with distilled water during other 14 h (range 12–16 h). We checked the solution where stigmas were immersed in the search for pollen grains potentially lost during the washing process. As no pollen grain was detected in three stigmas analyzed, we did not repeat this procedure for the remaining samples. Stigmas were treated with aniline blue 0.1 % in a 0.07 M phosphate buffer (Na_2HPO_4) solution at pH 9 for 48 h (Kearns and Inouye 1993).

To quantify the contribution of each pollinator species to pollination service, we calculated two indexes: (a) Pollination efficiency (PE) (Inouye et al. 1994) calculated as PI/TG , where PI = number of pollen grains delivered on a virgin stigma after a single visit, and TG = number of pollen grains transported by the vector (i.e., total pollen load). This index was estimated from the mean PI and TG values per pollinator species; and (b) the index of pollen transfer effectiveness (PTE) (Herrera 1990) measured as $\text{PI} \times \text{visitation rate}$. This index is species-specific and independent of population size. It permits characterization and comparison of pollinator effectiveness on the same plant species (Gross 2005). Additionally, we calculated an overall PTE per year, measured as the addition of the PTE of each species per year.

Results

The native pollinator assemblage for *M. luteus* at this site consists of the solitary bees *Centris chilensis* and *Centris nigerrima* (Anthophoridae), *Megachile saulcyi* and *Megachile semirufa* (Megachilidae), and the bumblebee *B. dahlbomii* (Apidae). *Bombus terrestris* (Apidae) was the only exotic pollinator species in the study site. The body size of each species is shown in Table 1. There was significant heterogeneity in the body length of species (one-way ANOVA on log-transformed data, $F_{5,24} = 23.4$, $P < 0.001$). Similarly, the species differed globally in thorax width ($F_{5,24} = 17.2$, $P < 0.001$) and head width ($F_{5,24} = 7.3$, $P < 0.001$). A posteriori Tukey's comparisons revealed that *B. terrestris* tend to differ mainly with *C.*

chilensis and *M. saulcyi*, being largely undistinguishable from the native *B. dahlbomii* and *C. nigerrima* (Table 1). The visitation rates in 2010 differed among species (one-way ANOVA, $F_{5,228} = 11.6$, $P < 0.001$). *Centris chilensis* and *B. terrestris* were the species that most visited flowers of *M. luteus* (Table 2); their visitation rate was higher than that of the native *C. chilensis*, *M. saulcyi*, and *M. semirufa* (Tukey's tests, $P < 0.05$). A different situation was observed in 2011, when the two *Bombus* species were absent in the study site. There was significant heterogeneity in the visitation rates of the remaining species (one-way ANOVA, $F_{3,84} = 7.0$, $P < 0.001$). In this year, *Megachile saulcyi* had a higher visitation rate than *M. semirufa* and *C. chilensis* (Table 2). The visitation rates in 2012 differed among species (one-way ANOVA, $F_{5,48} = 15.3$, $P < 0.001$). *Centris chilensis* and *C. nigerrima* were the species with the highest visitation rate of the pollinator assemblage (Table 2). In general, the visitation rate of *B. terrestris* was highly variable over years. Unlike the situation observed in 2010, when *B. terrestris* showed the highest visitation rate, this species did not visit the study site in 2011 and had the lowest visitation rate of the pollinator assemblage in 2012 (Table 2). Interestingly, the pattern of temporal change of *B. terrestris* was similar to *B. dahlbomii*, suggesting that the exotic and native bumblebees responded in a similar way to environmental variation.

All the insect species carried a higher proportion of pollen of *M. luteus* (70–90 %) on their bodies than pollen from the accompanying flora. There was significant heterogeneity in the amount of pollen transported by pollinator species (one-way ANOVA, $F_{5,93} = 3.43$, $P = 0.007$). The introduced *B. terrestris* was the species with the greatest number of pollen grains on the average (Table 2). *Bombus terrestris* and *C. nigerrima* transported more pollen than *M. semirufa* (Tukey's test, $P < 0.05$). Albeit not significant, the exotic *B. terrestris* transported a twofold greater amount of pollen than its congeneric native bumblebee *B. dahlbomii* and almost 25 % more than *C. nigerrima*. These differences were not paralleled by the number of pollen grains deposited on stigmas per visit (pollination intensity). Even though pollination intensity differed among species (Kruskal–Wallis test, $\chi^2 = 25.0$, $df = 5$, $P < 0.001$), the two *Bombus* species tended to deposit a lower number of pollen grains than *Centris* and *Megachile* species (Table 2). Notwithstanding, the only significant effects at $P < 0.05$ were observed in comparisons with the native species: *B. dahlbomii*–*M. saulcyi* and *B. dahlbomii*–*C. nigerrima*. It is likely that the absence of significance in the contrast of *B. terrestris* with the native species results from the low sample size for this species, reflecting its low relative abundance in the study site. However, even if not significant, the mean number of pollen grains deposited on stigmas by *B. terrestris* is sufficiently low to assume this result has biological significance.

Table 1 Size descriptors of the insect pollinators visiting *M. luteus* in the study site

Species	Body length (mm)	Thorax width (mm)	Head width (mm)
<i>B. terrestris</i>	16.78 ± 1.08 ^{ab}	7.31 ± 0.33 ^a	5.12 ± 0.41 ^a
<i>B. dahlbomii</i>	18.35 ± 1.46 ^a	7.06 ± 0.64 ^a	5.13 ± 0.40 ^a
<i>C. chilensis</i>	14.10 ± 0.55 ^{cd}	5.63 ± 0.35 ^{bc}	5.13 ± 0.09 ^a
<i>C. nigerrima</i>	15.26 ± 0.73 ^{bc}	6.43 ± 0.33 ^{ab}	5.16 ± 0.22 ^a
<i>M. saulcyi</i>	12.71 ± 0.99 ^d	5.00 ± 0.65 ^c	4.29 ± 0.32 ^b
<i>M. semirufa</i>	14.94 ± 0.45 ^{bc}	5.79 ± 0.32 ^{bc}	5.05 ± 0.14 ^a

Figures are mean ± SE. Within columns, values that do not share letters are significantly different ($P < 0.05$, one-way ANOVA followed by Tukey's HSD test). All measurements were taken on five individuals per species

The pollination efficiency of *B. terrestris*, that is, the number of pollen grains delivered onto stigmas relative to the amount of pollen on the body, was the lowest of the pollinator assemblage, only comparable to the native bumblebee *B. dahlbomii* (Table 2). The overall effectiveness of the pollinator assemblage, corresponding to the sum of the PTE values per year, varied substantially across years (1.82 in 2010, 0.64 in 2011, and 6.98 in 2012), indicating that the overall contribution of the pollinator assemblage to the reproductive success of *M. luteus* is variable among years. The solitary bee *C. nigerrima* was the species that consistently contributed most to pollen transfer, reaching 64.8 % in 2010, 45.9 % in 2011, and 88.8 % in 2012 (Fig. 2). Regarding *B. terrestris*, PTE values indicate that this species had an overall low importance, reaching 4.6 % in 2010, absence in 2011, and 0.01 % in 2012 (Fig. 2).

Discussion

The aim of this study was to evaluate the potential impact of the exotic bumblebee *B. terrestris* on the pollination

service received by the Andean monkeyflower, *M. luteus*. Our results indicate that the native bee *C. nigerrima* provided consistently most pollination service (Fig. 2). Even though *B. terrestris* was the species with the greatest amount of pollen of *M. luteus* on the body, the low number of pollen grains deposited on stigmas, and the medium–low visitation rate exhibited by the exotic pollinator in comparison with native pollinators in 2010 and 2012 make this species one of low effectiveness (Table 2; Fig. 2). Why did *B. terrestris* have no impact on the pollination service of *M. luteus*? We suggest two non-mutually exclusive explanations. First, *B. terrestris* is one of the largest bee species currently inhabiting Chile, only equivalent to the exotic *B. ruderatus* and the native *B. dahlbomii* in size. As such, it does not match the flower phenotype of most native flowering plants, leading to pollen removal and subsequent delivery in wrong places, especially in species with narrow and small floral tubes. Kenta et al. (2007) suggested that limitation in accessibility to nectar makes the short-tongued *B. terrestris* more prone to nectar robbing than native pollinators. We did not observe nectar robbing of *B. terrestris* on *M. luteus*, which allows us to reject this pathway of detrimental impact. Second, it is likely that the importance of *B. terrestris* in pollen transfer depends on the plant species involved. *Mimulus luteus* has the peculiarity of having a mobile stigma with lobes that close quickly (10 s on the average) after mechanical contact and remain closed for 13 min on the average (Carvallo and Medel 2010) (Fig. 1a). As *B. terrestris* and *B. dahlbomii* have a large size relative to *Centris* and *Megachile* species (Table 1), they have a high chance of accidentally touch and close stigmas while manipulating flowers. This phenomenon may be increased by the foraging behavior of *B. terrestris*. Unlike *Centris* and *Megachile* bees that access easily to the nectar concealed at the end of the corolla tube, *B. terrestris* often bend downward and manipulate extensively the flowers of *M. luteus* increasing the chance of accidental contact with bilabiate stigmas.

Table 2 Summary of results of pollination variables per insect species

Species	TG (SE, N)	PI (SE, N)	Efficiency	Visitation rate (SE)		
				2010	2011	2012
<i>B. terrestris</i>	22,076 (3,394, 22) ^a	8.5 (6.5, 2) ^{ab}	0.39	0.987 (0.267) ^a	0	0.009 (0.009) ^b
<i>B. dahlbomii</i>	9,502 (2,049, 30) ^{ab}	4.0 (3.0, 2) ^b	0.42	0.652 (0.132) ^{ab}	0	0.048 (0.045) ^b
<i>C. chilensis</i>	15,887 (6,523, 5) ^{ab}	28.1 (8.3, 22) ^{ab}	1.77	0.121 (0.040) ^c	0.055 (0.024) ^c	2.395 (0.627) ^a
<i>C. nigerrima</i>	17,165 (3,627, 24) ^a	117.1 (15.1, 92) ^a	6.82	1.179 (0.236) ^a	0.249 (0.065) ^{ab}	5.295 (2.385) ^a
<i>M. saulcyi</i>	13,133 (5,773, 7) ^{ab}	83.6 (8.5, 137) ^a	6.37	0.219 (0.040) ^{bc}	0.333 (0.057) ^a	0.111 (0.060) ^b
<i>M. semirufa</i>	8,062 (3,463, 11) ^b	37.6 (6.8, 57) ^{ab}	4.66	0.296 (0.115) ^{bc}	0.137 (0.035) ^{bc}	0.025 (0.013) ^b

TG = number of pollen grains transported by the vector, PI = number of pollen grains deposited on virgin stigmas after a single visit, pollinator efficiency (PI/TG × 1,000), and visitation rate (visits flower⁻¹ h⁻¹). Within columns TG and VR, values with no letters in common are significantly different ($P < 0.05$, one-way ANOVA followed by Tukey's HSD test). Nonparametric, a posteriori contrasts (Zar 1999) for PI-values were performed in R (R Core Team 2013). Parentheses in TG and PI indicate standard error (SE) and sample size (N)

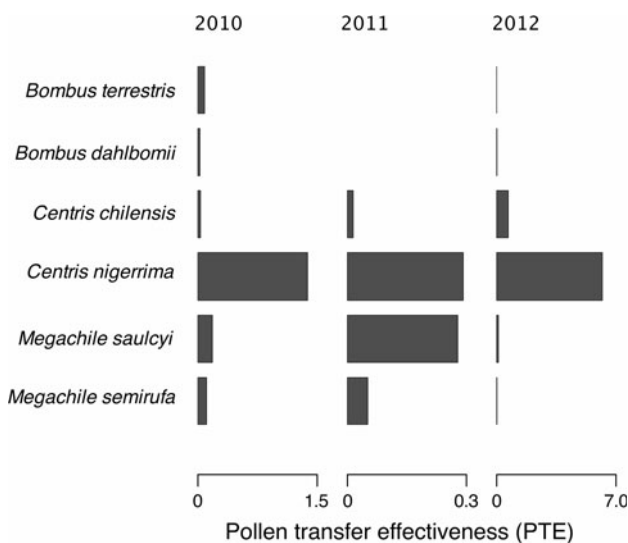


Fig. 2 Pollen transfer effectiveness (PTE) per year and pollinator species. PTE values were estimated from the product between visitation rate \times pollination intensity (see “Materials and methods” section). Note that PTE is not in the same scale among years

The low abundance of *B. terrestris* and *B. dahlbomii* in 2010 and 2012 and their absence in 2011 is intriguing and may relate to several factors. On the one hand, the occurrence of bumblebees may be restricted only to years with favorable environmental conditions, especially as the study site is located at the edge of their elevation range in the Andes, which may render bumblebees more sensitive to environmental change (see also Goulson et al. 2005). El Niño/La Niña events are the most important factors determining inter-annual variation in climate conditions in central Chile, especially in the Andean zone comprised between 30° and 35° S (Escobar and Aceituno 1988). It is known that many ecological processes in central Chile are strongly influenced by these large-scale climate processes (see reviews in Jaksic 1998, 2001). Winters with extremely low temperatures and extensive rainfall and snow in central Chile characteristic of El Niño events probably limit the successful emergence of queens from hibernation reducing colony initiation. On the contrary, La Niña events associated with hot and dry years may influence bumblebee colony development through environmental conditions that exceed the thermal and humidity range needed for colony homeostasis (e.g., Vogt 1986; Yoon et al. 2002). It is likely that the absence of *B. terrestris* and *B. dahlbomii* in the summer season of 2011 occurred as a consequence of the strong La Niña episode that occurred from autumn 2010–summer 2011 in central Chile. In spite of the importance of linking population-level pollination processes with El Niño/La Niña events, few studies have analyzed the consequences of large-scale climate processes on natural populations (e.g., Inouye et al. 2002).

Interestingly, the congeneric native bumblebee, *B. dahlbomii*, showed a parallel pattern of occurrence with *B.*

terrestris over years (Table 2), suggesting that similar thermal niche constraints account in part for this pattern in bumblebees as a whole (see also Goulson 2003; Wiggam and Ferguson 2005; Dohzono et al. 2008). Alternatively, bumblebees and other species may all be responding in the same way to inter-annual variation in floral density and nectar availability in the area rather than climate variation (e.g., Bowers 1985). Even though we cannot rule out this explanation at present, ecological reasons make this possibility unlikely. *Mimulus luteus* occurs in dense patches of ca. 137 flowers/m² (Fig. 1b) that do not change substantially across years, ensuring nectar availability for the limited demand imposed by pollinators as revealed by their extremely low visitation rates (see also Medel et al. 2003; Carvalho and Medel 2010).

In summary, we did not detect an important impact of the exotic bumblebee *B. terrestris* on the pollinator assemblage of *M. luteus*. Even though information of the time of arrival of this species to the study site is lacking, judging from the time of introduction to Chile, it should not exceed 15 years. It is known that as the population size of invasive species changes with environmental variation, their impact in new habitats does not necessarily occur at the beginning of the invasion process (Strayer et al. 2006). In general, the invasion process occurs through a series of events that include: (a) transport, (b) introduction, (c) establishment, (d) propagation, and (e) impact (Lockwood et al. 2007). The observation that *B. terrestris* has not yet become the dominant species in the area but occurred intermittently in the study site suggests that the invasion process is still in the establishment stage. It is likely that the impact of *B. terrestris* on the native pollinator assemblage occurs in more developed stages of the invasion process, once colonies have established definitively. In spite of being a quickly spreading species in Chile, *B. terrestris* currently has a low impact on the pollination service of the Andean monkeyflower *M. luteus*. More research into the effects of *B. terrestris* on pollination of other species of native plants seems necessary to extract legitimate conclusions of its impact on Chilean plant–pollinator communities.

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