


# Impact of Invasive Bees on Plant-Pollinator Interactions and Reproductive Success of Plant Species in Mixed *Nothofagus antarctica* Forests

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## Keywords

Honey bees, Bumblebees, Exotic pollinators, Pollination

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## Abstract

Invasive social bees can alter plant-pollinator interactions with detrimental effects on both partners. However, most studies have focused on one invasive bee species, while the interactions among two or more species remain poorly understood. Also, many study sites had a history of invasive bees, being hard to find sites with historical low abundances. In Patagonia, *Bombus ruderatus* (F.) invasion begun in 1993 and *B. terrestris* (L.) in 2006. Though honey bees (*Apis mellifera* L.) introduction started in 1859, their density is still low in some parts. By experimentally increasing honey bee densities, we evaluated the effect of honey bees and bumblebees floral visitation on native pollinator floral visitation, pollen deposition, and reproductive success of three plant species in mixed *Nothofagus antarctica* forests of northern Patagonia: *Oxalis valdiviensis*, *Mutisia spinosa* and *Cirsium vulgare*. Our results show that exotic bees became the main floral visitors. No negative association was found between invasive bee and native pollinator visitation rates, but there was evidence of potential competition between honey bees and bumblebees. Floral neighborhood diversity played an important role in pollinator behavior. Conspecific pollen deposition was high for all species, while deposition of heterospecific pollen was very high in *M. spinosa* and *C. vulgare*. Not as expected, honey bees visitation rate had a negative effect on heterospecific pollen deposition in *C. vulgare*. For *O. valdiviensis*, exotic visitation rates increased conspecific pollen deposition, which was positively related to reproductive success. Although exotic bees became main floral visitors, their contribution to reproductive success was only clear for one species.

## Introduction

Invasive bee species can alter native plant-pollinator interactions, with effects on both partners. As they can reach high

numbers, exotic bees can compete with native pollinators for floral resources and nesting sites (Goulson 2003, Mallinger et al 2017, Agüero et al 2018). As a consequence, native pollinator fitness and population dynamics can be

compromised (Thomson 2004, Paini & Roberts 2005). Moreover, the displacement of native pollinators might affect plant species partners thus the entire communities of both plants and pollinators are subject to modifications (Aizen et al 2008). Yet most studies have focused on the impact of one single invasive species on pollination patterns while the interaction between two or more invasive species remains unexplored.

Invasive bees can affect pollination outcomes in both direct and indirect ways. Directly, they can trigger pervasive negative effects on pollen flow if they behave as pollen thieves or if they promote selfing (Mendes do Carmo et al 2004, Traveset & Richardson 2006, Dohzono et al 2008). Because some invasive bees (e.g. honey bees and bumblebees) are generalist species (i.e., forage on a wide range of plant species) they are associated with an increase in heterospecific pollen deposition (Traveset & Richardson 2006, Marrero et al 2016). Indirectly, they can affect pollination by modifying the behavior of more efficient pollinators or by reducing pollen availability (Dohzono et al 2008). These modifications to pollen quantity/quality balance are expected to negatively affect plant reproductive success (Dohzono et al 2008, Magrach et al 2017, Valido et al 2019). It is thus necessary to assess the impact of invaders on plant-pollinator interaction patterns and plant reproductive success to better understand and forecast the response of plant communities to the introduction of novel invasive pollinator species.

Worldwide, honey bees (*Apis mellifera* L.) and bumblebees (*Bombus* spp.) are recognized as some of the most important invasive pollinator species (Stout & Morales 2009). Honey bees have been introduced for honey production and crop pollination all over the world except Antarctica (Moritz et al 2005, Abrol 2012). The spread of some bumblebee species is more recent and is related to crop pollination (Stout & Morales 2009). In the last decades, two species of bumblebees began to invade Argentinean Patagonia, after being introduced into Chile. It is thought that *Bombus ruderatus* (F.) invasion begun in 1993 and *Bombus terrestris* (L.) in 2006 (Roig-Alsina & Aizen 1996, Torretta et al 2006, Morales et al 2013). *Apis mellifera* introduction started in 1859 (Pérez Rosales 1859, Sanguinetti & Singer 2014).

Although numerous studies show that these social bees can disrupt plant-pollinator interactions with negative effects on both partners (Morales & Aizen 2006, Aizen et al 2008, de M Santos et al 2012, Magrach et al 2017, Valido et al 2019), no one has assessed the combined effect of the two groups of pollinators (i.e. honey bees and bumblebees) on plant-pollinator interaction and reproductive success of plant biota in Argentinean Patagonia.

Mixed *Nothofagus antarctica* forests of northern Patagonia represent one of the main forestal community of the Argentinean Patagonia. This community is one of the

most diverse ecosystems in the region and is characterized by many plant species that rely on animal-mediated pollination (Aizen and Ezcurra 1998, Speziale et al 2010). The relative recent invasion of *B. terrestris* and *B. ruderatus* to mixed *N. antarctica* forests of northern Patagonia and the low density of honey bees in some sites provide a good scenario for experimental sites that are relatively new to the presence of invasive bees. This also allows us to study their potential novel effects when they coexist. Therefore, by experimentally increasing honey bee density in a locality where invasive bumblebees (*B. ruderatus* and *B. terrestris*) are common flower visitors, we studied the effect of both groups of pollinators on plant-pollinator interaction in three different plant species of the mixed *N. antarctica* forests. To do this, we evaluated four different aspects: i) behavioral response of pollinators to the floral neighborhood, ii) the relationship between native and invasive pollinator visitation rates (i.e. potential competition), iii) changes in pollen deposition patterns and iv) changes in reproductive success of the three plant species. First, we expect that an increase in floral neighborhood diversity will lead to an increase in the visitation rate of invasive bees on focal plant individuals. Second, that an increase in invasive bee visits will negatively relate to native pollinators visits. Third, we expect that the increase in exotic pollinator visitation rates will increase both conspecific and heterospecific pollen deposition. Finally, we hypothesize that this increase in pollen deposition will not necessarily lead to an increase in reproductive success because of a decrease in pollen quality.

## Materials and Methods

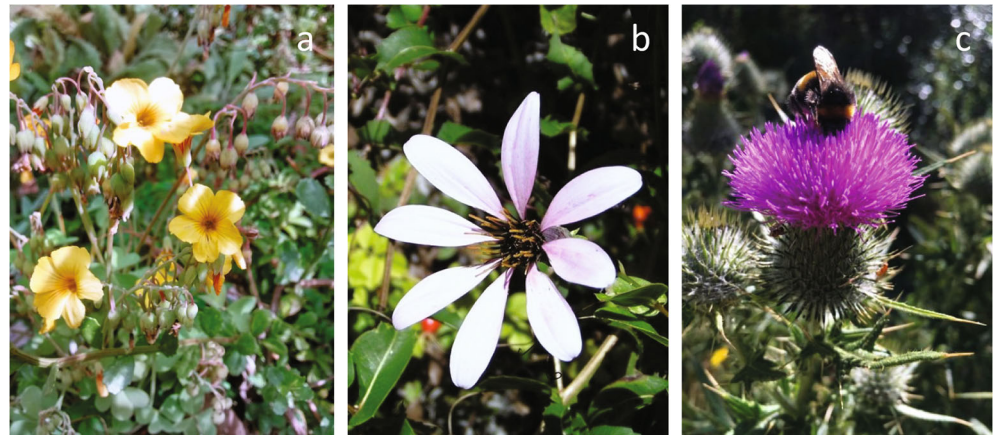
### Study site

The study was performed at the proximity of the locality El Foyel, Río Negro, Argentina (S 41°38'48.44"; W 71°29'59.06"). This ecosystem represents a forest/steppe ecotone, dominated by *N. antarctica* (Forst.) Oerst., accompanied with other woody species such as *Lomatia hirsuta* (Lam.) Diels ex J.F. Macbr., *Schinus patagonicus* (Phil.) I. M. Johnst. ex Cabrera and *Diostea juncea* (Gillies ex Hook.) Miers (Gyenge et al 2009). The climate is characterized by a mean annual temperature of 9.7°C (mean range 3.1°C–16.7°C) and an average annual rainfall of 920 mm, with a probability of frost throughout the year (Reque et al 2007).

### Studied species

*Oxalis valdiviensis* Barnéoud (Oxalidaceae) is a perennial herb, 5–25 cm tall (Zuloaga and Belgrano 2017) with cymose inflorescences bearing 9–31 flowers. Flowers are yellow and

**Fig 1** Flowers and inflorescences of the three studied species (a) *Oxalis valdiviensis*, (b) *Mutisia spinosa* and (c) *Cirsium vulgare* and one of the invasive bumblebee species: *Bombus terrestris*



up to 25 mm in diameter (Fig 1A) and the fruit is a capsule. It is an endemic species to Argentinean and Chilean Andes.

*Mutisia spinosa* Ruiz & Pav. (Asteraceae) is a semi-woody climber plant (Zuloaga & Belgrano 2017). Flowers are disposed of in solitary heterogamy capitula. Ray flowers (8–10) with ligulate light pink corollas (Fig 1B). The tube is ca. 15 mm long and the ligule is elliptic, ca. 30 × 8 mm. Numerous disk flowers with tubular yellow corollas, tube ca. 20 mm long. The fruit is a glabrous achene 15 mm long. It is an endemic species to southern Argentina and Chile.

*Cirsium vulgare* (Savi) Ten. (Asteraceae) is an annual or biennial herb that grows 0.5–2 m tall (Zuloaga and Belgrano 2017). The inflorescence consists of big capitula (mean = 15.7 mm, Fenner *et al* 2002) with purple bisexual flowers (Fig 1C). The fruit is an achene 3.5–4.8 mm long, slightly asymmetrical. Although native to Europe, West, Central and South Asia, North Africa and the Azores Islands; it is naturalized in most part of Argentina.

All three plant species whose blooming period overlap and have entomophilous pollination (Coulin personal observations, Madjidian *et al* 2008, Morales & Aizen 2002). Particularly, previous studies in this region demonstrated that *M. spinosa* main pollinator used to be the native giant bumblebee *Bombus dahlbomii* (Guer.) whose population is declining currently (Madjidian *et al* 2008).

#### Experimental design

In the study site, exotic bumblebees (*B. terrestris* and *B. ruderatus*) density (0.0097 individuals/m<sup>2</sup>) is higher than honey bee density (0.00065 individuals/m<sup>2</sup>) (Coulin *et al* 2019). In order to increase honey bee abundance in relation to exotic bumblebees, we brought 10 beehives to the site on January 12, 2017, and taken care of during the experiment in the site.

During the flowering season, we selected 16 individuals for each plant species. Plant individuals were separated more than 2 m from each other and marked with a code number. All individuals were located no more than 1 km from the hives so that they were included in the normal honey bee foraging range, which is 2–3 km (Abrol 2012). In each analyzed individual, we marked closed buds to identify which flowers were only exposed to an increase in honey bee abundance for pollen deposition and reproductive success analysis.

#### Visitation rates and floral neighborhood characterization

We measured visitation rates on all plant individuals from January 26, 2017 to February 20, 2017. To estimate visitation rates on each focal plant, we registered the total number of floral visitors during a specific period of time on a known (previously counted) number of flowers. It is worth to note that only those floral visitors that touched the reproductive parts of the flower were taken into account as a legitimate visit. Census started in the morning (ca. 10:00 h) up to afternoon (ca. 16:30 h). To avoid variation due to differences in the time of the day, we changed the order of individual census in every repetition. Mean total visit time for each plant individual is ca. 54 min.

We also characterized the flowering neighborhood in a circular plot (1 m of radius) around each studied individual. We registered in each plot the number of flowering individuals for all plant species and the number of open flowers.

#### Pollen deposition

We collected at least 10–20 flowers for each individual in post-anthesis from the previously marked buds and store them in 70% alcohol. In the lab, we randomly selected three flowers from each individual from each species. First, we separated the stigmatic area of each flower and macerated for 24 h in NaOH: water 1:10 (w:v). Then, we added a drop of melted glycerol-gelatine containing safranin on a slide, place one stigma and squashed it carefully with a coverslip

(Zarlavsky 2014). The safranin is used to stain the pollen grains and allowed better identification. Later, using a microscope, we counted the number of conspecific and heterospecific pollen grains on the stigma. For the identification, we analyzed the pollen morphology of each studied species by applying the same method to pollen grains directly collected from the anthers. For further information, we also consulted the corresponding bibliography.

### Reproductive success

In each individual, we also marked at least three buds or pre-anthesis capitula that were exposed to an increase in honey bee abundance. When the fruits of each plant individual were matured, we collected them (capsules of *Oxalis* were removed pre-dehiscence). Each capitulum of both Asteraceae species was collected and stored in paper bags, while *O. valdiviensis* fruits were stored in 70% alcohol for later analysis.

For each *O. valdiviensis* fruit, we counted the number of well-formed seeds (viable) in each locule. Seeds that were disintegrated or too small with a different color were considered as atrophied. We also counted the number of ovules per locule which we observed varied between two and three among individuals. Therefore, we estimated the correct value for each individual by counting the number of ovules per locule in three flowers per individual. Differences in ovule production have also been observed in other *Oxalis* species: *O. alpina* (Weller 1981), *O. magnifica* (Guth & Weller 1986), *O. corniculata* (Abid 2010). For Asteraceae species, we distinguished for each capitulum the number of well-formed achenes (healthy) from those that were hollow or shrunken. Achenes that are hollow or shrunken may indicate that they are infertile (Michaux 1989).

In the case of *O. valdiviensis*, we could not analyze 5 of the 16 individuals for reproductive success. For *M. spinosa*, for three individuals we could only analyze two capitulum, for two one capitulum and for one individual we did not have data. Those individuals for which we did not have data were not included in the model. Finally, for 6 individuals of *C. vulgare* we analyzed two capitulum and for one, only one capitulum.

### Data analysis

For describing floral neighborhood diversity we calculated Simpson's diversity (1-D) (Simpson 1949, Lande 1996). We grouped the floral visitors into three different categories: honey bees (*A. mellifera*), exotic bumblebees (*B. terrestris* and *B. ruderatus*) and native pollinators. Native pollinators include all other species visiting the flowers.

We calculated the visitation rate (measured as individuals/min\*flower or capitulum) per pollinator category and

both the number of conspecific and heterospecific pollen grains on stigma for each plant individual for each plant species. For *C. vulgare* and *M. spinosa*, we calculated the reproductive success as the number of healthy achenes divided by the total number of achenes. For *O. valdiviensis*, we calculated the reproductive success as the number of viable seeds divided by the total number of ovules.

We used structural equation modeling (SEM) to fit a path analysis to test our multiple hypotheses. SEM allows testing hypothesis related to causal relationships even in complex models (Mitchell 1992). We used the "SEM" function in the R package Lavaan (Rosseel 2012) for fitting all of our structural equations. Path analysis assumes normality so we transformed the variables to improve the normality of distribution: square root transformation for honey bees, bumblebees and native pollinators visitation rate, conspecific pollen grains and heterospecific pollen grains values and arcsin square root transformation for Simpson diversity index and for reproductive success values. We evaluated models to fit with a chi-squared test and Comparative Fit Index (CFI). We incorporated CFI because is less sensitive to sample size (Ainur et al 2017).

We constructed an initial theoretical model for the three species (Appendix Fig 4). Our model proposed causal links for our six variables of interest: Simpson's diversity (1-D), honey bees visitation rate, native pollinators visitation rate, bumblebees (*B. terrestris* and *B. ruderatus*) visitation rate, conspecific pollen grains (mean number of conspecific pollen grains deposited on stigma), heterospecific pollen grains (mean number of heterospecific pollen grains deposited on stigma) and reproductive success (number of viable seeds/total number of ovules for *O. valdiviensis* or healthy achenes/ total number of achenes for *M. spinosa* and *C. vulgare*) (Appendix Fig 4).

Initially, the Chi-square value  $>0.05$  and CFI  $<< 0.95$ , which indicates that goodness of fit of the models is not good (Hu and Bentler 1999). In order to improve the models, we included paths suggested by the analysis of the modification indices (MIs) (Mitchell 1992, Rosseel 2012). We only included parameters that have a biological interest. After this, the goodness of fit of each model improved considerably (chi-square value  $>0.05$  and CFI  $\geq 0.95$ ). This is why the final structural equations differ from our initial theoretical model for the Asteraceae species. Additionally, we also analyzed the correlation among the transformed variables by using Pearson's  $r$  ('record' function of the 'Hmisc' R-package) (Appendix Table 2).

## Results

For all studied species, data fitted properly our proposed model after incorporating influential relationships based on modifications indices: *O. valdiviensis*:  $P$  value (Chi-square) = 0.319; CFI = 0.93; *M. spinosa*:  $P$  value (Chi-square) = 0.466;

CFI = 1.00; *C. vulgare*: *P* value (Chi-square) = 0.361; CFI > 0.97. In general, there is a good correlation between path analysis results and Pearson correlation values, although the level of significance may differ for some relations between variables (Appendix Table 2).

#### Pollinator Categories Visitation Rate and Floral Neighborhood Diversity

Mean visitation rates were higher for honey bees and native pollinators in *O. valdiviensis* and for bumblebees follow by honey bees in both *M. spinosa* and *C. vulgare* (Fig 2). Path analysis shows that floral neighborhood had more frequently a significant positive effect on at least a pollinator category visitation rate for all plant species. In this sense, plant neighborhood diversity had a significant positive effect on native pollinators in *O. valdiviensis*, a significant positive effect on honey bees in *M. spinosa* and on honey bees in *C. vulgare* (Fig 3). Contrary, it had a negative effect on bumblebees visitation rate on *C. vulgare*.

#### Potential Competition with Native Pollinators

We found no significant negative effect of exotic pollinators, honey bees and bumblebees, on native visitation rate in any plant species (Fig 3). Instead, we found a significant positive relation between exotic bumblebees and native pollinators visitation rate in *M. spinosa* (Fig 3). We also found a significant positive relation between honey bee and bumblebees visitation rate in *C. vulgare*. We did not observe that any encounter between invasive bees and native floral visitors disrupted the foraging behavior of the last ones.

#### Pollen Deposition

Mean conspecific pollen grains deposited on stigma was one order of magnitude higher for *O. valdiviensis* ( $339.2 \pm 55.12$ ) than *M. spinosa* ( $92.11 \pm 11.15$ ) and *C. vulgare* ( $30.96 \pm 4.56$ ) (Table 1). Contrarily, mean heterospecific pollen grains deposited on stigma were highest for *C. vulgare* ( $35.25 \pm 7.20$ ), followed by *M. spinosa* ( $26.38 \pm 4.54$ ) and *O. valdiviensis* ( $11.53 \pm 4.18$ ) (Table 1). For *O. valdiviensis*, we found a significant effect of honey bees and native pollinators visitation rate on mean conspecific pollen deposition and a marginal significant relation between bumblebees and conspecific pollen grains (Fig 3). Moreover, native pollinators visitation rate had an also positive effect on heterospecific pollen deposition in *O. valdiviensis* (Fig 3). Bumblebees visitation rate had a marginally significant positive effect on heterospecific pollen grains in *C. vulgare*. While, contrarily to our hypothesis, we found that honey bees visitation rate had a significant negative effect on heterospecific pollen deposition in *C. vulgare* (Fig 3). We found no effect between pollinator categories and pollen deposition in *M. spinosa* (Fig 3).

#### Reproductive Success

Mean reproductive success was extremely high for *C. vulgare* ( $0.92 \pm 0.03$ ), while *O. valdiviensis* and *M. spinosa* had similar values ( $0.63 \pm 0.05$  and  $0.64 \pm 0.03$ , respectively) (Table 1). Based on our path analysis, we found a marginal significant relation between conspecific pollen deposition and reproductive success of *O. valdiviensis* (Fig 3). As expected we found a negative but marginal significant relation between heterospecific pollen deposition and reproductive success also for *O. valdiviensis* (Fig 3).

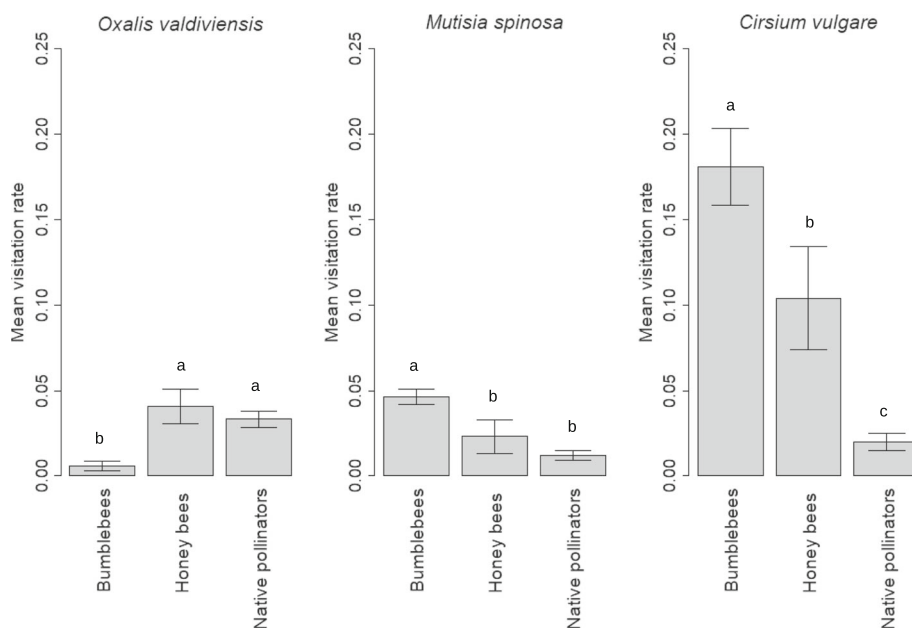


Fig 2 Barplot showing visitation rates of each defined pollinator categories (bumblebees, honey bees and native pollinators) for the three studied plant species (*Oxalis valdiviensis*, *Mutisia spinosa*, and *Cirsium vulgare*). Error lines indicate standard errors (SE). For each plant species, different letters indicate values that differ significantly (first ANOVA, then Tukey's test: *P* value < 0.05)

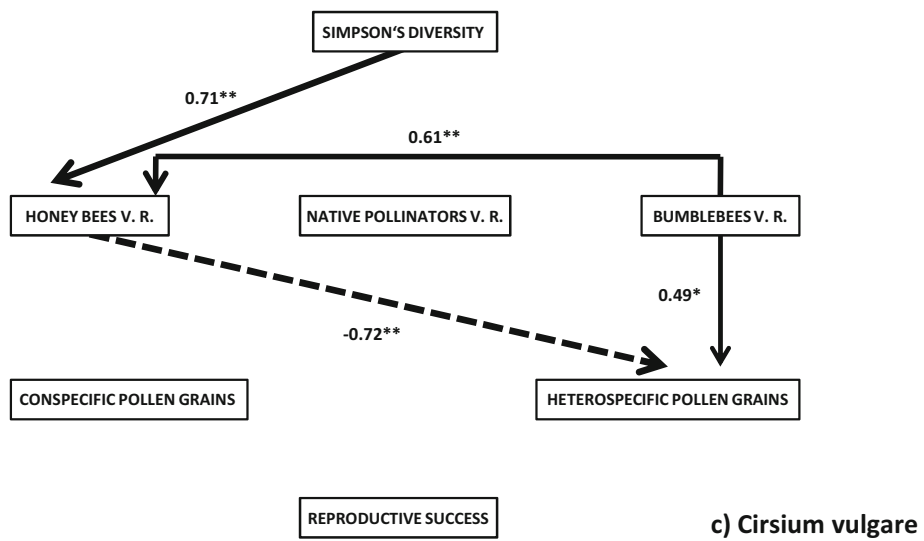
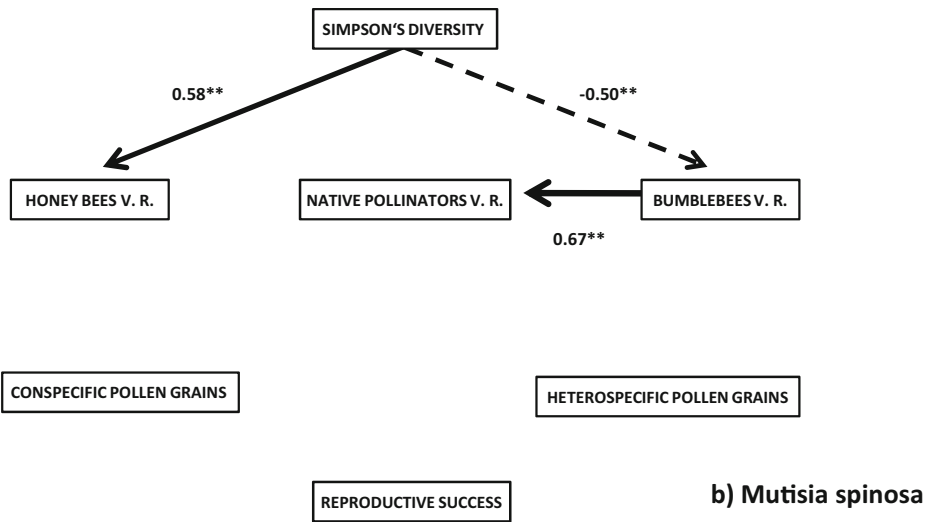
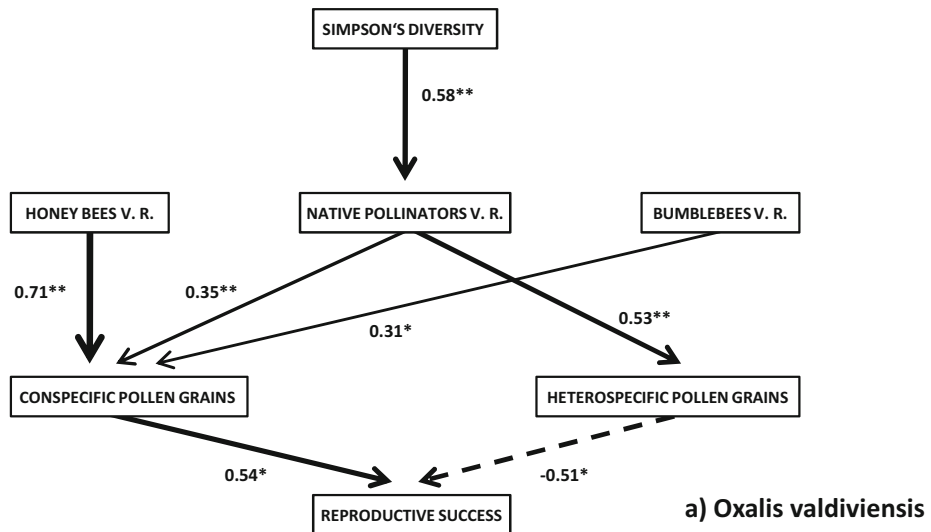


Fig 3 Path analysis showing the most important causal relationships between the variables of interest for each of the studied plant species (a) *Oxalis valdiviensis*, (b) *Mutisia spinosa* and (c) *Cirsium vulgare*. Variables: Simpson's diversity (1-D), honey bees v. r. (honey bees visitation rate), native pollinators v. r. (mean native pollinators visitation rate), bumblebees v. r. (mean *Bombus terrestris* and *B. ruderatus* visitation rate), conspecific pollen grains, heterospecific pollen grains and reproductive success (number of viable seeds/ total number of ovules for *O. valdiviensis* or healthy achenes/ total number of achenes for *M. spinosa* and *C. vulgare*). The models show the magnitude of the standardized coefficients of each path (arrows width) and their significance (\*\*:  $P$  value  $< 0.05$ ) or marginal significance (\*:  $P$  value  $0.05-0.1$ ). Full lines represent positive effects and dotted lines negative effects.

## Discussion

Our study shows that invasive bees became main floral visitors for the three studied plant species, with the diversity of floral neighborhood playing a considerable role in their behavior. Honey bees and native visitors were the main pollinators of *O. valdiviensis* contributing to conspecific pollen deposition, which marginally related to an increase in reproductive success. *Cirsium vulgare*, an exotic species mainly pollinated by exotic bees, had a high reproductive success. *Mutisia spinosa*, a native species whose local main pollinator is in decline and for which we did not record any visit (e.i., *B. dahlbomii*), had surprisingly an intermediate reproductive success. Invasive bumblebees may be contributing, at least partially, to the pollination of this species.

Flower neighborhood diversity was an important component shaping the foraging behavior of pollinators (i.e. visitation rate), especially for honey bees (Fig 3). We found a predominantly positive effect of flower diversity on pollinator visitation rates, a pattern that has been highlighted in several studies (Sih & Baltus 1987, Molina-Montenegro *et al* 2008, Muñoz & Cavieres 2008). Mechanisms such as the combined attraction by convergent floral syndromes or plants providing complementary resources can increase generalist pollinator visits (Ghazoul 2006). Solitary bees with small foraging ranges can also tend to nest in areas with high floral diversity and resource availability (Bruckman and Campbell 2014). However, competition rather than facilitation can also

occur between plants, the result depending on factors such as the composition and density of floral neighborhoods (Ghazoul 2006, Morales & Traveset 2009, Waters *et al* 2014). In our study, *C. vulgare* co-flowering around *M. spinosa* may be more attractive for bumblebees. This exotic thistle was the most common species present in *M. spinosa* floral neighborhood and bumblebees have innate preferences for violet and blue colors (Giurfa *et al* 1995).

Contrary to our hypothesis, we did not found a negative relation between invasive bees and native pollinators visitation rate that could suggest a potential competition or displacement. Instead, we found a positive relation in *M. spinosa*. A positive relation between invasive bees and native pollinators has already been observed (Mallinger *et al* 2017). However, the absence of potential competition cannot be ruled out. The positive relationship can be by the preference of both pollinators for the same plant individual or by competition between them. The second hypothesis is based on the idea that if the competition is reducing the resource availability per flower, animals could be visiting more flowers for collecting the quantity they require (Maloo & Inouye 2000). For *O. valdiviensis* and *C. vulgare*, the absence of a negative interaction may be because the actual density of exotic bees is not sufficient to reduce resource availability.

The hypothesis related to resource reduction could also explain the positive relation between honey bee and bumblebee visitation rates in *C. vulgare*. These generalist bees have a high preference for this exotic plant species (Morales & Aizen 2006). If visits by honey bees and bumblebees reduce the amount of nectar and/or pollen per capitulum, both can increase the number of visits to obtain the quantity they require. The fact that we observed honey bees and bumblebees fighting for foraging in capitulum also supports this. Interspecific scent marks could also explain this behavior: bumblebees and honey bees tend to avoid or depart quickly from flowers that were previously visited by an individual of the different species (Stout & Goulson 2001).

Table 1 The mean and standard error (SE) of the number of conspecific and heterospecific pollen grains deposited on stigma and the reproductive success for the three plant species (*Oxalis valdiviensis*, *Mutisia spinosa* and *Cirsium vulgare*). For each column, different letters indicate values that differ significantly (first ANOVA, then Tukey's test:  $P$  value  $< 0.05$ )

Plant species	Conspecific pollen grains	Heterospecific pollen grains	Reproductive success
<i>Oxalis valdiviensis</i>	339.21 ( $\pm$ 55.12) a	11.53 ( $\pm$ 4.18) b	0.63 ( $\pm$ 0.05) b
<i>Mutisia spinosa</i>	92.11 ( $\pm$ 11.15) b	26.38 ( $\pm$ 4.54) ab	0.64 ( $\pm$ 0.03) b
<i>Cirsium vulgare</i>	30.96 ( $\pm$ 4.56) b	35.25 ( $\pm$ 7.20) a	0.92 ( $\pm$ 0.03) a

As expected, we found that honey bee and bumblebee visitation rates related positively to conspecific pollen deposition but only in *O. valdiviensis*. Honey bees can be efficient pollinators outside their native range because they are generalists, and their pollination effectiveness can be similar to that of native pollinators (Hung *et al* 2018). Even if they are less efficient, their high abundance may compensate it (Agüero *et al* 2018). In the studied region, exotic bumblebees have demonstrated to be efficient pollinators, for example, of some orchids species (Sanguinetti & Singer 2014). Even though we did not found the same pattern for *M. spinosa* and *C. vulgare*, the mean number of conspecific pollen grains was very high as Asteraceae flowers only have one ovule per flower. This shows that these species could not be experiencing pollen limitation, at least at the quantity level (Aizen & Harder 2007). This also suggests that the lack of a clear increase in conspecific pollen deposition with an increase in pollinator visitation rates could be due to pollen saturation (Aizen & Harder 2007).

Contrarily to our hypothesis, we did not find a relevant positive effect of invasive bees visitation rates on heterospecific pollen deposition. Only bumblebees visitation rate had a marginally positive effect in *C. vulgare*. Interestingly, honey bees visitation rate had a negative relation. Although honey bees and bumblebees are both generalist species, they are both also characterized by their flower constancy (Goulson 2010, Grüter & Ratnieks 2011). Flower constancy is relatively higher for honey bees (Goulson 2010) and this characteristic may reduce the chances of deposition of heterospecific pollen on stigmas. However, it is important to note that *C. vulgare*, which was mainly visited by both categories of invasive bees, had the highest proportion of heterospecific pollen deposition. Native pollinators visitation rate related positively to heterospecific pollen deposition in *O. valdiviensis*, probably because different species with different behavior and morphology were grouped in this category.

An increase in exotic bee visitation rates may be beneficial for the reproductive success of *O. valdiviensis*. All the pollinator categories contributed to conspecific pollen deposition and there was a significant effect of conspecific and heterospecific pollen deposition on reproductive success. Despite the high conspecific pollen deposition, pollen quality limitation can be important cause *O. valdiviensis* presents tristly as a cross-breeding system: three different morphotypes differ in the length of the style and the length of the two whorls of the stamen (Fyfe 1950). Effective pollination occurs when pollen comes from individuals with different morphotypes. Honey bees tend to forage several flowers of the same inflorescence or individual before moving to another, which could reduce the quality of the pollen (i.e., increasing geitonogamy) (Paton 1997). Another interesting aspect is that heterospecific pollen grains, although mean grains were high, were related to reproductive success. Heterospecific pollen can reduce reproductive output

by different mechanisms such as allelopathic inhibition of conspecific pollen (Morales & Traveset 2008). Those detrimental effects can even be independent of conspecific pollen load size (Arceo-Gómez & Ashman 2011).

We observed no effect of conspecific pollen deposition on the reproductive success of *M. spinosa*. Their primary pollinator is *B. dahlbomii*, whose population is declining, and it has been suggested that invasive bumblebees may not be able to replace it (Madjidian *et al* 2008). In our study mean reproductive success was 64%, even though exotic bumblebees were the main pollinators. The number of deposited conspecific pollen grains on stigma was very high, suggesting that other factors are limiting its reproductive success such as pollen quality or nutrient limitation (De Jong and Klinkhamer 1989; Niesenbaum 1993; Morales & Traveset 2008).

*Cirsium vulgare*, an exotic species mainly visited by both exotic bees, had an extremely high reproductive success showing that this plant species does not suffer from a limitation in pollen quantity or quality. Positive interaction between invasive bees and exotic plant species has already been demonstrated (Goulson 2003, Morales *et al* 2009, Traveset & Richardson 2014). Even though we did not found a positive relation between exotic bees and conspecific pollen deposition, this data may suggest that both honey bees and bumblebees are contributing positively to the reproductive success of *C. vulgare*.

Based on our data, we can conclude that invasive bees do not compromise the reproductive success of the studied plant species. It is important to bear in mind that our work is based on variation in the visitation rates at the individual level during one growing season and native pollinators were analyzed as “one group”. However, it is an important contribution to understanding the processes associated with the potential impact of invasive bees in the area and serves as a guide for future research.

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## Appendix

Fig 4 Theoretical path diagram incorporating all the hypothetical causal relationships between the variables of interest for the three studied plant species. Variables: Simpson's diversity (1-D), honey bees v. r. (honey bees visitation rate), native pollinators v. r. (mean native pollinators visitation rate), bumblebees v. r. (mean *Bombus terrestris* and *B. ruderatus* visitation rate), conspecific pollen grains, heterospecific pollen grains and reproductive success (number of viable seeds/ total number of ovules for *O. valdiviensis* or healthy/ total number of achenes for *M. spinosa* and *C. vulgare*). Full lines represent positive effects and dotted lines negative effects

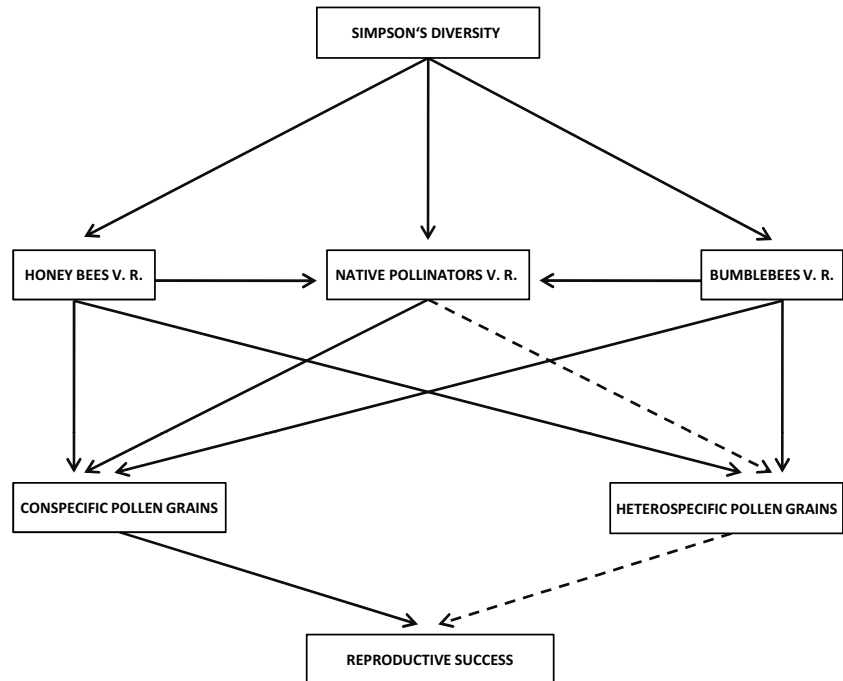


Table 2 Pearson correlation coefficients for all transformed variables of interest for each of the three plant species (a) *Oxalis valdiviensis*, (b) *Mutisia spinosa* and (c) *Cirsium vulgare*. Asterisks indicate significant differences (\*\*:  $P$  value  $<0.05$ ) or marginal significant differences (\*:  $P$  value  $0.05-0.1$ )

	Simpson's diversity	Honey bees visitation rate	Native pollinators visitation rate	Bumblebees visitation rate	Conspecific pollen grains	Heterospecific pollen grains
<b>(a) <i>Oxalis valdiviensis</i></b>						
Honey bees visitation rate	0.01					
Native pollinators visitation rate	0.57*	-0.11				
Bumblebees visitation rate	-0.05	-0.15	0.30			
Conspecific pollen grains	0.14	0.66**	0.38	0.32		
Heterospecific pollen grains	0.70**	0.27	0.55*	0.26	0.49	
Reproductive success	-0.30	-0.07	0.07	0.30	0.27	-0.25
<b>(b) <i>Mutisia spinosa</i></b>						
Honey bees visitation rate	0.58**					
Native pollinators visitation rate	-0.05	-0.25				
Bumblebees visitation rate	-0.50*	-0.40	0.55**			
Conspecific pollen grains	0.36	0.02	-0.16	-0.27		
Heterospecific pollen grains	0.13	-0.15	-0.06	-0.08	0.46*	
Reproductive success	0.01	0.13	0.22	0.04	-0.29	-0.39
<b>(c) <i>Cirsium vulgare</i></b>						
Honey bees visitation rate	0.65**					
Native pollinators visitation rate	0.33	0.31				
Bumblebees visitation rate	-0.09	0.55**	-0.04			
Conspecific pollen grains	0.06	0.27	-0.01	0.18		
Heterospecific pollen grains	-0.68**	-0.44*	-0.21	0.10	-0.15	
Reproductive success	-0.45*	-0.30	-0.05	-0.06	-0.01	0.22

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