Rapid ecological replacement of a native bumble bee by invasive species

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Despite rising global concerns over the potential impacts of non-native bumble bee (*Bombus* spp) introductions on native species, large-scale and long-term assessments of the consequences of such introductions are lacking. *Bombus ruderatus* and *Bombus terrestris* were sequentially introduced into Chile and later entered Argentina's Patagonian region. A large-scale survey in Patagonia reveals that, in 5 years post-arrival, the highly invasive *B terrestris* has become the most abundant and widespread *Bombus* species, and its southward spread is concurrent with the geographic retraction of the only native species, *Bombus dahlbomii*. Furthermore, a 20-year survey of pollinators of the endemic herb *Alstroemeria aurea* in northern Patagonia indicates that *B ruderatus* and *B terrestris* have replaced *B dahlbomii*, formerly the most abundant pollinator. Although the decline's underlying mechanisms remain unknown, the potential roles of exploitative competition and pathogen co-introduction cannot be ruled out. Given that invasive bumble bees can rapidly extirpate native congeners, further introductions should be discouraged.

Non-native bumble bees have been introduced and have become established in diverse geographic regions on many continents (Velthuis and van Doorn 2006). The worldwide trade in bumble bee colonies for crop pollination, in particular of *Bombus terrestris*, has elicited special concern about the potential for invasion by non-native bumble bees and their impacts on native pollinator species (Ings *et al.* 2006; Arbetman *et al.* 2013). Because native *Bombus* are ecologically more similar to introduced bumble bees than to non-*Bombus* native pollinators, native *Bombus* are expected to be more vulnerable, through either potential horizontal disease transmission (Schmid-Hempel and Schmid-Hempel 1993) or competitive displacement (Ings *et al.* 2006).

The bumble bee-sparse southern tip of South America (Patagonia region) is expected to be particularly susceptible to *Bombus* invasion. Its single native bumble bee species, *Bombus dahlbomii* (Figure 1a), inhabits the temperate forests of southern Argentina and Chile. This large, charismatic species has played a key role in plant–pollinator webs in many forest types in the region (Aizen *et al.* 2002; Morales and Aizen 2002; Vázquez and Simberloff 2003). Moreover, *B dahlbomii* interacts closely with the native endemic herb "amancay" (*Alstroemeria aurea*, Alstroemeriaceae; Figure 1b; Aizen 2001; Vázquez and Simberloff 2003), a wild lily species parental to cultivated hybrids commonly sold in flower shops around the world. This scenario presents a unique opportunity to explore the effects of non-native bumble bees in a bumble bee-impoverished region.

Southern Argentina's Patagonia region has been sequentially invaded by the European species *Bombus ruderatus* (Figure 1c) in 1993 (Roig-Alsina and Aizen 1996) and *B terrestris* (Figure 1d) in 2006 (Torretta *et al.* 2006), after both were intentionally introduced for crop-pollination purposes into Chile in 1982–1983 and 1997–1998, respectively (Figure 2; WebPanel 1). Earlier claims of an apparent decline of *B dahlbomii* (Ruz 2002; Montalva *et al.* 2011) have been anecdotal and its presumed link to the introduction of invasive bumble bees has been speculative (Arbetman *et al.* 2013).

In the absence of large-scale and long-term studies (Cameron *et al.* 2011), robust estimates of the invasions' temporal origin and spatial extent, as well as its impact on different components of the native fauna, are difficult to obtain. Moreover, plant–pollinator systems are inherently dynamic and therefore multi-year studies are necessary to demonstrate genuine trends of pollinator-population shifts in nature (Roubik 2001). Here we report the results of a recent large-scale (~1270-km north–south transect) survey of bumble bee distribution and abundance in Patagonia, in conjunction with a 20-year study of flower visitors to amancay in an old-growth forest in northern Patagonia's Challhuaco Valley (Figures 1b and 2). We provide strong evidence that non-native bumble bees that were introduced into Chile and spread east into Argentina – *B ruderatus* arriving first, followed by *B terrestris* – rapidly displaced the native bumble bee species from much of its Argentinean range.

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■ Methods

Regional assessment of bumble bee abundance

During the 2011 mid-to-late austral summer (late January to early March), we conducted a survey of bumble bee

Figure 1. *Study species and system. (a) The native bumble bee* Bombus dahlbomii. *(b) Understory of lenga* (Nothofagus pumilio) *deciduous forest during blooming of the native herb amancay* (Alstroemeria aurea) *in northern Patagonia's Challhuaco Valley, the site where the long-term study was performed (see location in Figure 2). (c and d) Invasive bumble bees (c)* B ruderatus *and (d)* B terrestris.

species abundance across the known range of *B dahlbomii* in Argentina, along the eastern slope of the Andes (Figure 2), according to the most extensive and recent survey of museum specimens for the species (Abrahamovich and Díaz 2001). We recorded all bumble bees observed during an approximate 4-hour period of walking along trails in 33 sampling sites, each one sampled once (WebPanel 2; WebTable 1).

We first evaluated whether the observed species differed in overall abundance. Colony initiation differed among species, with *B terrestris*, *B dahlbomii*, and *B ruderatus* emerging in sequential order (WebPanel 2) and, as a consequence, bumble bee abundance of different species

may vary during the season. To control for seasonal effects, we incorporated sampling date (WebTable 1) as an additional term in our model. We modeled bumble bee abundance as a function of species (categorical factor with three levels), latitude, and sampling date (continuous predictors) using generalized linear models (GLMs) with negative binomial error distribution (NBD) to account for overdispersion in count data. We selected the best model by comparing Akaike Information Criteria (AIC) values (WebTable 2).

We also explored whether there was a latitudinal trend in species abundance for each species separately. We modeled the abundance of each species as a function of

latitude and sampling date using GLMs with NBD, and selected the best model by comparing AIC values. Finally, we tested for statistical correlation between the abundance of *B dahlbomii* and *B ruderatus* and of *B dahlbomii* and *B terrestris*, using Spearman's rank correlation test. We corrected for multiple tests with Bonferroni's correction (WebPanel 2).

Local temporal trends in bumble bee composition and abundance on amancay flowers

We recorded bumble bees visiting amancay flowers during 17 flowering seasons, spanning 20 years, from 1994 to 2013 (excluding 1999, 2000, and 2012) in the Challhuaco Valley (Figure 2). In this area, *B dahlbomii* was reported to account for ~94.5% and 92% of all visits to flowers in 1994 and 1995, respectively (Aizen 2001); *B ruderatus* was reported to account for roughly <1% of all visits in the same years (Roig-Alsina and Aizen 1996; Aizen *et al.* 2002). Surveys were carried out between late January and early March during amancay blooming, which is highly synchronized in the population from year to year (Aizen 2001). The flowering of amancay, the dominant herbaceous species in this relatively species-poor plant community, does not overlap with the flowering of any other conspicuous insect-pollinated flower species. At this time of the season, workers of the three bumble bee species forage actively on amancay flowers. We counted the number of open flowers and recorded the number of individual bumble bees entering 3 m × 3 m plots during 10-minute intervals. For each species, we estimated the number of bumble bees per flower per hour, which allowed us to test for a trend in the yearly mean abundance of each bumble bee species in the time series.

Stochastic variation in bee abundance suggests that a minimum of 4 years is needed to capture temporal trends (Roubik 2001). Thus, the standardized sampling protocol followed over 17 flowering seasons, together with the relative simplicity and stability of this system, allowed us to reliably track changes in temporal trends of bumble bee abundance. We tested for a trend in the abundance of each bumble bee species over time with linear regression models. Time-series data may violate the independence assumption; thus, we tested for any autocorrelation using different approaches (WebPanel 2). Also, because of natural stochastic variation in bumble bee abundance (Roubik 2001), a population may remain undetectable during one or more years in the field, even when it is present in small numbers. To test the null hypothesis that failing to record *B dahlbomii* over a given
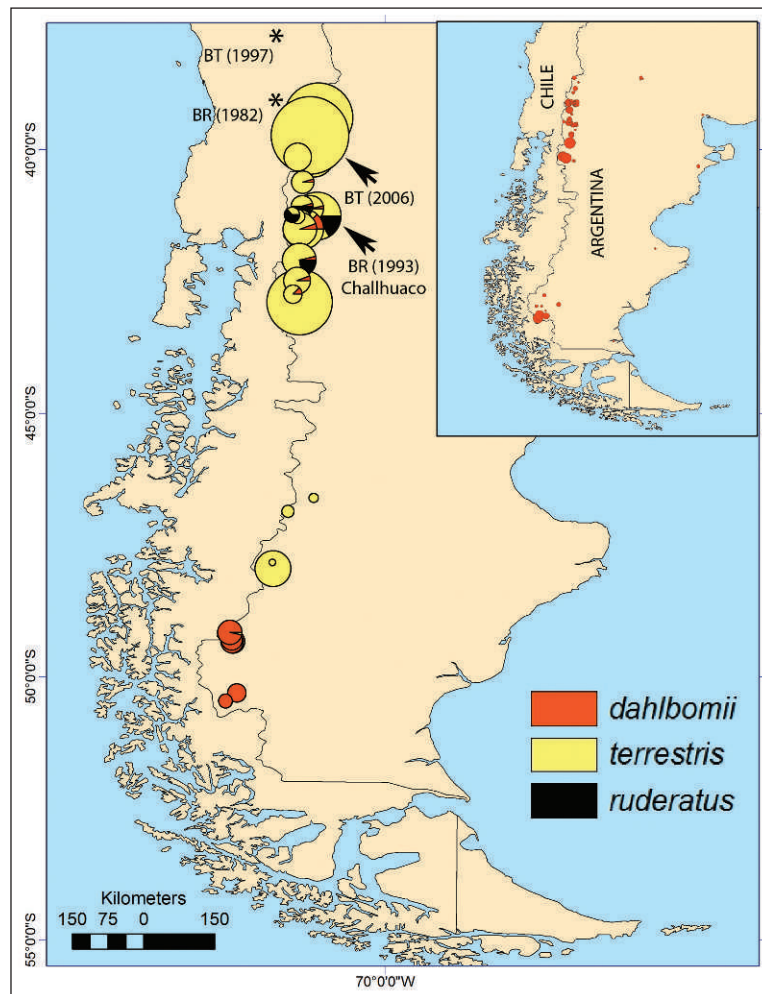

Figure 2. *Map of the region surveyed during the 2011 austral summer. "BR(1982)" and "BT(1997)" indicate the sites and years of first release of* Bombus ruderatus *and* B terrestris *in Chile, respectively (Ruz 2002; Montalva* et al. *2011); "BR(1993)" and "BT(2006)" indicate the sites and years of the first report of* B ruderatus *(Roig-Alsina and Aizen 1996) and* B terrestris *(Torretta* et al. *2006) in Argentina, respectively. Inset map shows the distribution of* B dahlbomii *and proxy abundance before the invasion of* B ruderatus *and* B terrestris, *according to Abrahamovich and Díaz (2001). Pie size is proportional to sampling size within each map, but pie sizes are not comparable between maps.*

time interval was the result of stochastic year-to-year variation, we calculated the probability of sampling zero *B dahlbomii* abundance during five consecutive sampling seasons after 12 consecutive seasons when we recorded at least one *B dahlbomii* (WebPanel 2).

■ Results

Regional-scale spatial patterns

The native *B dahlbomii* was recorded in 48% of 33 sampled sites (WebTable 1), but was locally dominant (ie > 90% of all individuals observed) at only 18% of sampled sites, the six southernmost (Figure 2). *Bombus ruderatus* was recorded in 24% of sampled sites and was domi-
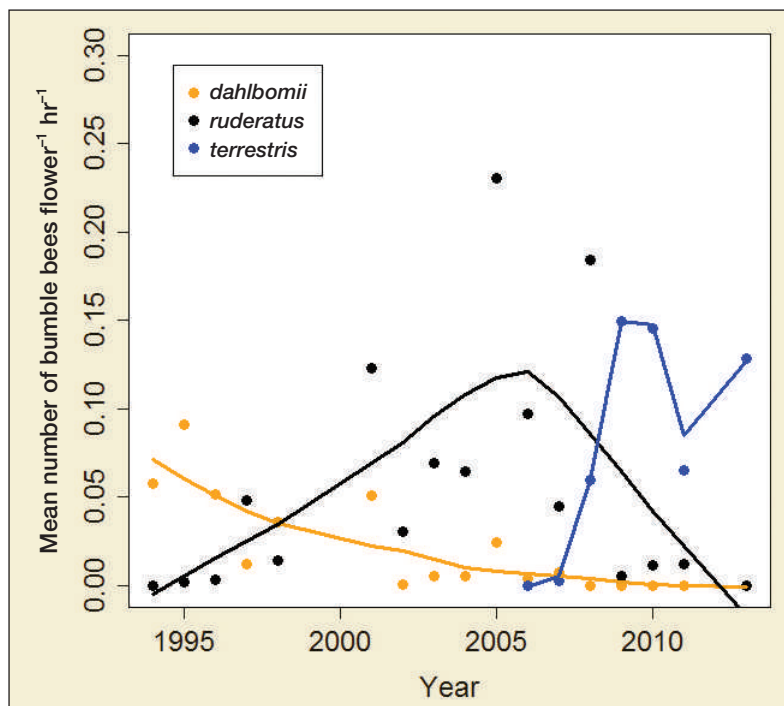
Figure 3. *Changes in abundance of the three bumble bee species visiting amancay flowers in the Challhuaco Valley. Each data point represents a yearly average. Fitted lines were obtained by nonparametric local regression smoother (LOESS) and observed data.*

nant at none of them, whereas *B terrestris* was by far the most widespread species throughout the sampling range; it was recorded in 88% of sampled sites and was locally dominant at 48% of them. In 29 years, *B ruderatus* has dispersed ~434 km beyond its 1982 release sites, while *B terrestris* has dispersed ~1320 km beyond its 1997 southernmost release site (Figure 2; WebPanel 1) in half the time (14 years), thus exhibiting a sixfold difference in invasion rate.

Bombus terrestris, which accounted for 86% of all individuals recorded, was an order of magnitude more abundant than *B ruderatus* and *B dahlbomii* combined (Figure 2; WebTable 1), being significantly more numerous than *B dahlbomii* ($Z = 4.42$; $P < 0.0001$); abundance of *B ruderatus* (the least numerous species), however, did not significantly differ from that of *B dahlbomii* ($Z = –1.603$; $P < 0.109$; WebTable 1).

The number of *B dahlbomii* exhibited a highly significant seasonal and southward increase (sampling date = $0.099 ± 0.036$, Bonferroni-corrected $P = 0.0015$; latitude = $0.511 ± 0.104$, Bonferroni-corrected $P < 0.001$; WebTable 2). Similarly to *B dahlbomii*, the abundance of *B ruderatus* increased significantly across the season and, unlike the native species, decreased southward (sampling date = $0.046 ± 0.010$, Bonferroni-corrected $P < 0.0001$; latitude = $–0.602 ± 0.156$, Bonferroni-corrected $P < 0.001$). We found no seasonal effect on *B terrestris* abundance (sampling date = $0.022 ± 0.018$, Bonferroni-corrected $P = 0.642$). Similarly to *B ruderatus*, the number of *B terrestris* significantly decreased southward (lati-

tude = $–0.305 ± 0.066$, Bonferroni-corrected $P < 0.001$).

The abundance of *B dahlbomii* was negatively and significantly correlated with the abundance of *B terrestris* ($r = –0.42$, Bonferroni-corrected $P = 0.030$) but was not significantly correlated with the abundance of *B ruderatus* ($r = –0.29$, Bonferroni-corrected $P = 0.197$) at the geographic scale. However, the only sites across the surveyed latitudinal range in which *B dahlbomii* populations were still abundant were those in which both invasive species were absent or very scarce (Figure 2; WebTable 1). Thus, the spatial survey provides observational evidence that links native bumble bee decline to invasive bumble bee success, especially to that of *B terrestris*.

Local-scale, long-term patterns

The negative association between the geographic trends in native and invasive bumble bee abundance is largely paralleled by the results of the local long-term survey of visitors to amancay flowers in the Challhuaco Valley. In total, we accumulated 1219 observation periods (each 10 minutes long) in 208 sampling days over 17 years. Figure 3 shows the yearly variation in the mean number of bumble bees of each species visiting amancay flowers per hour from 1994 to 2013. Incorporating autocorrelation failed to improve the initial linear regression model (WebTable 3; WebFigures 1–4); therefore, we report only the results of the linear regression model.

Between 1994 and 2013, *B dahlbomii* abundance declined significantly (year = $–0.004 ± 0.001$, $P < 0.001$, $n = 17$; Figure 3). Furthermore, since 2008, no *B dahlbomii* individuals have been recorded in the area in any of the five consecutive sampling seasons. The probability of this occurring as the result of stochastic year-to-year variation is equivalent to 0.017, suggesting the local population is no longer present. In contrast, from 1994 to 2005, *B ruderatus* abundance exhibited a significant positive trend (year = $0.013 ± 0.004$, $P = 0.014$, $n = 10$; Figure 3).

From 2006, when *B terrestris* was first detected in the region, to 2013, *B ruderatus* abundance appeared to decline, but this negative trend was not significant (year = $–0.017 ± 0.010$, $n = 7$, Bonferroni-corrected $P = 0.324$); in the same period, *B terrestris* abundance exhibited an increasing, although not significant, trend (year = $0.019 ± 0.009$, $n = 7$, Bonferroni-corrected $P = 0.159$). This is likely due to the high year-to-year fluctuation in abundance and the shorter length of the post-invasion as compared with the pre-invasion time series and concomitant lower statistical power of the test (Figure 3).

Bombus dahlbomii abundance over the two decades was negatively correlated with that of both invasive species

pooled (Spearman's $r = -0.68$, $P = 0.002$, $n = 17$). *Bombus dahlbomii* abundance was negatively, although marginally, correlated with *B ruderatus* abundance before *B terrestris* arrived ($r = -0.56$, $P = 0.096$, $n = 10$) but not after its arrival ($r = 0.44$, Bonferroni-corrected $P = 0.633$, $n = 7$). However, *B dahlbomii* abundance was negatively, although marginally, correlated with *B terrestris* abundance after its arrival ($r = -0.76$, Bonferroni-corrected $P = 0.097$, $n = 7$).

■ Conclusions

This study documents the extent and speed of invasion of two introduced bumble bee species in southern Argentina, showing that the most recent invader to arrive in the Patagonia region, *B terrestris*, has rapidly become the most abundant and widespread species, partly replacing not only the native *B dahlbomii* but also *B ruderatus*, the previously introduced species. The speed of *B dahlbomii*'s decline throughout much of its range is noteworthy (Figure 2); this process began, at least in the Challhuaco Valley, with the introduction of *B ruderatus* and was later enhanced by the arrival of *B terrestris*. Two years after the detection of *B terrestris* in northern Patagonia, *B dahlbomii* had completely disappeared from the Challhuaco Valley (Figure 3).

Our regional survey extends the southward front of invasion of *B ruderatus* and *B terrestris* by about 120 km and 650 km, respectively, beyond the distributions presented in Montalva *et al.* (2011), and reveals that – in Argentina – the rate and extent of spread of *B terrestris* is higher than that of *B ruderatus*. This pattern is similar to that found in New Zealand, where both species were introduced in the 19th century (Macfarlane and Gurr 1995). We hypothesize that larger colonies (WebPanel 2) and a more generalist use of floral resources (Montalva *et al.* 2011) by the short-tongued *B terrestris* may have partly contributed to its greater success.

The dramatic collapse of *B dahlbomii* populations over the past two decades in northern Patagonia is temporally and spatially concurrent with the spread of the two invasive bumble bee species. In fact, the historical distribution (Abrahamovich and Díaz 2001) depicted in the inset map of Figure 2 and the long-term survey summarized in Figure 3, in addition to other northern Patagonia studies (Aizen 2001; Morales and Aizen 2002; Vázquez and Simberloff 2003), reveal that at the onset of this millennium, *B dahlbomii* was common in regions where it is absent today, including those areas where *B ruderatus* and *B terrestris* were first recorded (Figure 2; WebPanel 1; WebTable 1). Our results provide evidence of a substantial contraction in *B dahlbomii*'s historical range.

Although it has yet to be determined whether the invasive species are driving *B dahlbomii*'s collapse, our results, based on correlations, strongly suggest that this is the case. The decline of pollinators in general, and of native bumble bees in particular, is a global trend; along with invasive species, other related and concurrent factors – such as competition, changes in land use and habitat fragmentation, agrochemicals, climate change, and pathogens – have received considerable attention as potential causal factors of these declines (reviewed in Williams and Osborne 2009; Potts *et al.* 2010; Cameron *et al.* 2011).

Our study was conducted principally in national parks and relatively unspoiled areas lacking intensive agriculture or large urban settlements. Thus, pesticides and habitat fragmentation do not seem likely causes in this case. Furthermore, because invasive bumble bees in this region may benefit from habitat disturbance (Morales and Aizen 2002; Aizen and Feinsinger 2003), we expect that if habitat disturbance plays a role in *B dahlbomii* decline, this is mediated by increasing abundance of invasive bumble bees. Alternatively, poleward migrations of cold-adapted species, as the current distribution of *B dahlbomii* might suggest, are also expected under a global warming scenario. However, the rapid collapse reported here suggests climate change is an unlikely explanation for the decline of the native *B dahlbomii*, although changing climatic factors merit further attention.

Disentangling the underlying mechanisms linking the decline of *B dahlbomii* with the spread of invasive bumble bees exceeds the scope of this paper. Yet a comparison of spatiotemporal patterns suggests that the importance of different invasive species and associated mechanisms might vary at different spatial scales. At the regional scale, Figure 2 suggests that *B ruderatus*' spread has been relatively limited to the northern portion of *B dahlbomii*'s range, whereas *B terrestris* has invaded almost its entire range. Thus, in areas where *B ruderatus* has not invaded, the demise of *B dahlbomii* appears to be directly linked to *B terrestris*' thriving. Because this very invasive species has reached extremely high local abundances (Figure 2), has larger colonies, is characterized by a longer colony cycle than *B dahlbomii* (WebPanel 2), and seems to be a generalist forager (Montalva *et al.* 2011), exploitation competition cannot be dismissed.

However, circumstantial evidence implies a potential role of a novel pathogen co-introduced by *B terrestris*. A recent investigation of bumble bees collected in northern Patagonia, including the Challhuaco Valley, revealed that *B terrestris* specimens harbor the deleterious pathogen *Apicystis bombi*, which seems to have spread to *B dahlbomii* and *B ruderatus* populations (Arbetman *et al.* [2013] and references therein). The replacement of *B dahlbomii* by *B terrestris* in a large portion of its range may therefore be interpreted as a result of the interplay between resource competition and possible pathogen spillover; additional research is necessary to ascertain all the possible causal factors of decline.

At the local scale, *B ruderatus* seems to have initially triggered the population decline of *B dahlbomii*, which may have been reinforced after the *B terrestris* invasion. The extraordinary success of *B ruderatus* in the

Challhuaco Valley (Figure 3) as compared with other regions may be explained by the fact that the dominant floral resource is a species with deep corollas (Figure 1b), easily accessible to the unusually long tongues of *B ruderatus* (WebPanel 2). A comparison of foraging efficiency between *B dahlbomii* and *B ruderatus* before the arrival of *B terrestris*, however, rejects competition for resources with *B ruderatus* as an explanation of the initial *B dahlbomii* decline (Aizen *et al.* 2011). Other factors, including competition for nesting sites and other undetected introduced diseases, require investigation.

From a broader perspective, our results highlight the risks associated with current introductions of non-native *Bombus* around the world. Declines of native pollinators can occur at an unexpectedly fast rate, thereby complicating or prohibiting the restoration of their populations. We advise against further introductions of bumble bees outside their native ranges.

■ Acknowledgements

■ References

Abrahamovich AH and Díaz NB. 2001. Distribución geográfica de las especies del género *Bombus* Latreille (Hymenoptera, Apidae) en Argentina. *Rev Bras Entomol* **45**: 23–36.

Aizen MA. 2001. Flower sex ratio, pollinator abundance, and the seasonal pollination dynamics of a protandrous plant. *Ecology* **82**: 127–44.

Aizen MA and Feinsinger P. 2003. Bees not to be? Responses of insect pollinator faunas and flower pollination to habitat fragmentation. In: Bradshaw GA and Marquet PA (Eds). How landscapes change: Ecological Studies 162. Berlin, Germany: Springer Berlin Heidelberg.

Aizen M, Lozada M, and Morales C. 2011. Comparative nectar-foraging behaviors and efficiencies of an alien and a native bumble bee. *Biol Invasions* **13**: 2901–09.

Aizen MA, Vázquez DP, and Smith-Ramírez C. 2002. Historia natural y conservación de los mutualismos planta–animal del bosque templado de Sudamérica austral. *Revista Chil Hist Nat* **75**: 79–97.

Arbetman M, Meeus I, Morales CL, *et al.* 2013. Alien parasite hitchhikes to Patagonia on invasive bumblebee. *Biol Invasions* **15**: 489–94.

Cameron SA, Lozier JD, Strange JP, *et al.* 2011. Patterns of widespread decline in North American bumble bees. *P Natl Acad Sci USA* **108**: 662–67.

Ings TC, Ward NL, and Chittka L. 2006. Can commercially imported bumble bees outcompete their native conspecifics? *J Appl Ecol* **43**: 940–48.

Macfarlane RP and Gurr L. 1995. Distribution of bumble bees in New Zealand. *New Zeal Entomol* **18**: 29–36.

Montalva J, Dudley L, Kalin Arroyo M, *et al.* 2011. Geographic distribution and associated flora of native and introduced bumble bees (*Bombus* spp) in Chile. *J Apicult Res* **50**: 11–21.

Morales CL and Aizen MA. 2002. Does invasion of exotic plants promote invasion of exotic flower visitors? A case study from the temperate forests of the southern Andes. *Biol Invasions* **4**: 87–100.

Potts SG, Biesmeijer JC, Kremen C, *et al.* 2010. Global pollinator declines: trends, impacts and drivers. *Trends Ecol Evol* **25**: 345–53.

Roig-Alsina A and Aizen MA. 1996. *Bombus ruderatus* Fabricius, un nuevo *Bombus* para la Argentina (Hymenoptera: Apidea). *Physis* **5**: 49–50.

Roubik DW. 2001. Ups and downs in pollinator populations: when is there a decline? *Conserv Ecol* **5**: 2.

Ruz L. 2002. Bee pollinators introduced to Chile: a review. In: Kevan PG and Imperatriz-Fronseca VL (Eds). Pollinating bees – the conservation link between agriculture and nature. Brasília, Brazil: Ministry of Environment.

Schmid-Hempel P and Schmid-Hempel R. 1993. Transmission of a pathogen in *Bombus terrestris*, with a note on division of labour in social insects. *Behav Ecol Sociobiol* **33**: 319–27.

Torretta JP, Medan D, and Abrahamovich AH. 2006. First record of the invasive bumblebee *Bombus terrestris* (L) (Hymenoptera, Apidae) in Argentina. *T Am Entomol Soc* **132**: 285–89.

Vázquez DP and Simberloff D. 2003. Changes in interaction biodiversity induced by an introduced ungulate. *Ecol Lett* **6**: 1077–83.

Velthuis HHW and van Doorn A. 2006. A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie* **37**: 421–51.

Williams PH and Osborne JL. 2009. Bumblebee vulnerability and conservation world-wide. *Apidologie* **40**: 367–87.