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Extremely frequent bee visits increase pollen deposition but reduce drupelet set in raspberry

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Summary

1. Production of many flowering crops often benefits from elevated pollinator diversity and abundance. Nevertheless, the opposite relationship may arise if bees impair fruit or seed production and/or quality by damaging flowers during visitation, despite transferring pollen.

2. We assessed pollination and drupelet set (i.e. the number of drupelets per fruit) in 16 raspberry *Rubus idaeus* fields along a gradient of bee abundance in north-west Patagonia, Argentina. Using pollen supplementation, we also tested whether drupelet set was pollen limited in a subset of six fields.

3. Managed *Apis mellifera* and the invasive bumblebee *Bombus terrestris* accounted for 50% and 45% of all bee visits, respectively, to raspberry flowers. Pollen loads on stigmas increased with visit frequency of all bees combined and particularly with visitation by *A. mellifera,* but not by *B. terrestris*. Drupelet set was not pollen limited along the gradient of bee abundance.

4. Instead, drupelet set decreased with the proportion of damaged styles, which varied more strongly with the frequency of visits by *B. terrestris* than by *A. mellifera*. In fields with the highest bee frequency of visits (~300 visits flower^{-1} day^{-1}), ~80% of styles were damaged in flowers and these developed into fruits with ~30% fewer drupelets compared to flowers in fields with the lowest bee visitation rates (~4 visits flower^{-1} day^{-1}).

5. *Synthesis and applications*. Extreme bee visitation, particularly by *Bombus terrestris*, damaged the styles of raspberry flowers, precluding ovule fertilization by deposited pollen and limiting crop production by reducing drupelet set. Only a few bee visits are required to maximize fruit production in raspberry plants, therefore, pollinator management in north-west Patagonia should focus principally on reducing the abundance of the invasive bumblebee *B. terrestris* and secondarily controlling the number of honeybee hives in nearby cultivated fields. Although mainstream pollinator management relies on the assumption that more visits enhance fruit set, high bee visitation rates can be detrimental for fruit development and, consequently, for crop yield.

Key-words: *Apis mellifera*, bee frequency of visits, *Bombus terrestris*, crop pollination, drupelet set, flower damage, raspberry

Introduction

Global production of pollinator-dependent crops has increased during the last 50 years (Aizen *et al.* 2008; Aizen & Harder 2009; Garibaldi *et al.* 2011), while the abundance and diversity of wild pollinators have decreased in many regions, mainly due to agricultural expansion and intensification (Potts *et al.* 2010). In combination, these contrasting trends have the potential to be detrimental for long-term global food production, because increasing pollination limitation could decrease yield of pollinator-dependent crops (Kremen & Ricketts 2000; Holden 2006; Garibaldi *et al.* 2011; Bommarco *et al.* 2012). The conflict between pollination requirements and agricultural intensification could be mitigated through active pollinator management, which in many instances involves introduction of domesticated bees to supplement the pollination services provided for free by wild bees inhabiting adjacent remnants of natural and semi-natural habitats. Honeybees *Apis mellifera* have been historically managed for both honey production and crop pollination,

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but in the last three decades some species of bumblebees (*Bombus* spp.) are increasingly being reared and sold specifically for pollination services (Velthuis & van Doorn 2006).

Although the introduction of commercial pollinators beyond their native ranges for crop pollination may have increased the production of some crops (see Velthuis & van Doorn 2006 and references therein; Southwick & Southwick 1992), these introductions have resulted in some recent major invasion events (Dafni *et al.* 2010). In addition to negative consequences for native bees (Goulson 2003; Morales *et al.* 2013), the quality of the pollination service that these invasive pollinators provide has been questioned (Vergara 2008). Indeed, these invasions may have resulted in an unusually high abundance of flower visitors particularly adapted to large-scale disturbed environments like most agroecosystems. At first glance, an increase in pollinator availability is expected to increase pollination and, consequently, the yield of pollen-limited crops (Velthuis & van Doorn 2006 and references therein). However, excessively frequent visits can be detrimental to fruit or seed set if pollinators damage flowers during visitation or if a surplus of pollen deposition leads to stagnation of growing pollen tubes (Young 1988; Young & Young 1992; Morris, Vázquez & Chacoff 2010). Given these opposing effects, the simplistic assumption that increasing bee density, through the introduction of managed bees, will promote crop productivity should be assessed to fully evaluate the short- and long-term consequences of this management practice (Steffan-Dewenter 2003).

Raspberry '*Rubus idaeus*' crops are known to benefit from pollinator visitation (Crane & Walker 1984; Cane 2005; Morales 2009). Each of the 70–90 pistils on the multicarpelled raspberry flower can develop into a drupelet if properly pollinated. The whole raspberry flower develops into an aggregate fruit known as a polidrupe. To ensure production of commercial-quality fruits, most if not all of a flower's many ovules have to be fertilized (Cane 2005). Despite being fully self-fertile, the production of well-formed fruits depends completely on animal pollination (Cane 2005; Morales 2009). Therefore, honeybee or bumblebee hives are commonly deployed in raspberry fields to ensure an adequate pollination (Velthuis & van Doorn 2006).

Intermountain valleys on the eastern slope of the Patagonian Andes, Argentina, are suitable habitat for cultivation of different berries, including raspberry, one of the most important regional crops (Secretaría de Agricultura, Ganadería, Pesca y Alimentos 2006; Instituto Interamericano de Cooperación para la Agricultura 2012). In this region, raspberry pollination currently relies mostly on two non-native pollinator species, the managed honeybee and the invasive bumblebee *Bombus terrestris* (Morales 2009). *Apis mellifera* was introduced in Patagonia by European settlers more than a century ago and has been reported as one of the main raspberry flower visitors in the region (Morales 2009). Although this pollinator has naturalized, its local abundance is positively associated with habitat disturbance (Morales & Aizen 2002) and the presence of managed hives (see Results). On the other hand, *B. terrestris* arrived in Patagonia in 2006 (Torretta, Medan & Abrahamovich 2006), after being introduced in neighbouring Chile for crop pollination. Currently, in north-west Patagonia, *B. terrestris* is at least one order of magnitude more abundant than the only native bumblebee *B. dahlbomii* and another previously introduced bumblebee, *B. ruderatus*, and it is a frequent flower visitor of many native and exotic plant species (Morales *et al.* 2013). However, abundance of *B. terrestris*, and many other wild invertebrates, in north-west Patagonia was reduced after the 2011 eruption of the Puyehue volcano (Buteler *et al.* 2011; Morales *et al.* 2014), which generated a strong spatial gradient of decreasing abundance of bumblebees and native bees in association with increasing ash deposition (Morales *et al.* 2014).

Due to the introduction of domesticated bees to supplement the pollination service provided by native pollinators has become increasingly frequent (Velthuis & van Doorn 2006), bee invasion events have also become more frequently reported (Goulson 2003; Stout & Morales 2009). Therefore, the evaluation of the effectiveness of this management practice and of its unintended consequences for crop pollination and yield should be an immediate research priority. Particularly, bee introduction and ensuing invasion can create situations of bee superabundance that can neglect presumed pollination benefits because of increasing interaction costs (e.g. flower damage), associated with intense visitation (Young 1988; Young & Young 1992; Morris, Vázquez & Chacoff 2010). Here, we studied the consequences of spatial variation in visit frequency of managed *A. mellifera* and the invasive *B. terrestris* on pollen deposition, flower damage and fruit production in raspberry fields. Specifically, we asked the following questions: (i) Does bee frequency of visits affect the number of pollen grains deposited on raspberry stigmas, and does this effect differ between these two main flower visitors? (ii) Do bees damage flower styles during visitation, and does this damage differ between the two main flower visitors? (iii) Does the number of pollen grains per stigma affect the number of drupelets per fruit (i.e. drupelet set)? (iv) Does the number of damaged styles per flower affect drupelet set? More generally, we investigated whether interaction costs surpass benefits at high visit frequencies, thus impairing crop yield.

Materials and method

STUDY CROP

Raspberry *Rubus idaeus*, Rosaceae is a temperate-zone shrub cultivated for its fruit (Crane & Walker 1984). Despite being fully self-fertile, the structure of a raspberry flower (numerous pistils around a central core) precludes complete autonomous self-pollination. Specifically, pollen from the peripheral ring of anthers cannot contact the centermost pistils from the same flower in the absence of a pollen vector (Cane 2005). Thus, even though most raspberry flowers can set fruit independently of bee visitation, non-visited flowers set more malformed fruits (i.e. with fewer drupelets) than open-pollinated flowers (Morales 2009). As stated above, honeybee or bumblebee hives are commonly deployed in raspberry fields to increase production of commercial-quality fruits, mainly by increasing the number of drupelets per fruit (Chagnon, Gingras & Oliveira 1991; Cane 2005; Velthuis & van Doorn 2006; Lye *et al.* 2011).

STUDY AREA AND SITES

Field work was conducted during the 2012 austral summer (January–March) in 16 raspberry fields located in north-west Patagonia, Argentina, near (from hundreds of metres to a few kilometres away) National Parks (i.e. Nahuel Huapi, Lanin and Puelo) and other conservation areas, and thus surrounded by, or nearby large extensions of natural habitat, mainly forests of the Subantarctic domain (Cabrera 1976). These fields were scattered over ~1000 km^2 encompassing an elevation range of 300–1400 m and were planted with the 'Autumn bliss' variety, which produces two blooming peaks per season. Annual mean precipitation among fields ranges from 650 to 1500 mm and annual mean temperature from 7 to 10·4 °C (Hijmans *et al.* 2005). The cultivated area of the sampled fields varied approximately between 0·05 to 0·5 hectares. All fields were managed organically, with irrigation and periodic fertilization applications. Distances between sampled fields were always >1 km, averaging 94·5 km, exceeding the expected mean foraging distance of most foraging social bees (Walther-Hellwig & Frankl 2000; Steffan-Dewenter & Kuhn 2003; Osborne *et al.* 2008) (Fig. 1). Thus, each field can be considered as an independent replicate regarding its bee fauna.

As a consequence of the eruption of the Puyehue volcano in June 2011, a large part of the study area was covered by volcanic ash (Gaitan *et al.* 2011), which has insecticidal properties (Buteler *et al.* 2011; Fernandez-Arhex *et al.* 2013; Martínez *et al.* 2013). The thickness of the ash layer varied between 0 and 13 cm among study sites (Fig. 1). Thus, this event generated a strong gradient of decreasing abundance of bumblebees and native bees with increasing ash deposition (Morales *et al.* 2014, see Appendix S1 in Supporting Information).

FIELD SAMPLING

In each field, we conducted 20 five-minute pollinator censuses, during which we recorded and identified all flower visitors and the number of flowers visited by each individual to a pair of neighbouring raspberry stems (<20 cm apart). There was natural variation in the number of open flowers per stem, and the number of observed flowers during a census ranged from 4 to 10 (mean ± SD = 6·4 ± 1·8). Within a field, each census involved a different randomly selected pair of stems. We included pollinator observations in edge raspberry rows and did not evaluate edge effects because of the limited area of the study fields (see Study Area and Sites above). All censuses were performed from 10:00 to 18:00 during sunny or slightly cloudy days with, at most, light wind. Flower visitors in each field were surveyed during one day of the blooming season, between January 11 and February 23 due to logistic and time limitations, and the geographic scale of our study. However, the temporal order we sampled the fields did not follow any particular spatial gradient. During pollinator censuses, we recorded only flower visitors that contacted anthers and stigmas. Flower visitors were identified in the field with the aid of a reference collection.

To assess the consequences of flower visits, we quantified stigmatic pollen loads (i.e. pollen deposition) and the proportion of damaged styles. We collected three senescent flowers from each of 10 randomly selected stems in each field and stored them in individual centrifuge tubes containing 70% alcohol. In the laboratory, we randomly selected five pistils from each flower, placed between two slides with Alexander's stain (Alexander 1980) and observed them under a microscope at 400× magnification. Each

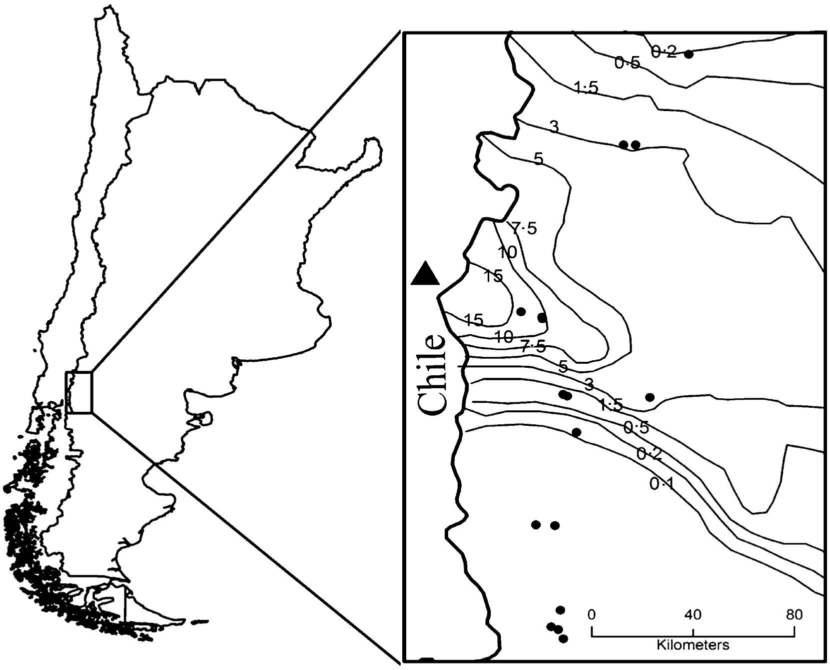

Fig. 1. Study region in north-west Patagonia, Argentina, including the location of the 16 raspberry fields (black points). Isolines illustrate variation in the thickness (cm) of the ash layer deposited after the 2011 eruption of the Puyehue volcano (black triangle) in neighbouring Chile.

pistil was classified as 'undamaged' if style was intact and the stigma was present, or 'damaged' if the style was broken and/or the stigma missing. We counted the pollen grains deposited on the stigmas of undamaged pistils. In total, we assayed approximately 2400 pistils from 477 flowers.

We evaluated pollination limitation (i.e. limitation due to flowers receiving too few pollen grains or pollen grains of insufficient quality to maximize either seed or fruit production) in a subset of six raspberry fields (see Table S1, Supporting information), spaced along the gradient of ash deposition and ensuing bee abundance. Although this technique confounds components of pollen quantity and quality associated with pollen limitation (Aizen & Harder 2007), raspberry quality limitation is not expected to be highly relevant because of complete self-compatibility. Therefore, here pollen supplementation is a proper protocol to estimate quantity pollen limitation (Aizen & Harder 2007). Experiments were carried out by comparing the drupelet set (i.e. number of drupelets per fruit) of 40 open-pollinated flowers vs. 40 open-pollinated flowers supplemented with cross-pollen (Ashman *et al.* 2004) from 10 randomly selected stems per field. We supplemented flowers in the same stems where we, at the same time, tagged open-pollinated flowers to control for individual stem variation. Pollen was supplemented during the same day that we visited the field to perform the pollinator census. The pollen used for supplementation was collected from flowers on at least five other stems in the same field. Each experimental flower was marked with a paper tag for later harvesting.

Approximately four weeks after surveying flower visitors, we collected all ripe fruits from 10 randomly selected raspberry stems in 12 of the 16 study fields, totalling 451 fruits (i.e. 30–60 fruits per field), including fruits from the experimental flowers in the pollination-limitation study. Fruits were harvested in the same stage of ripening (i.e. when the polidrupe detaches easily from the receptacle and each drupe's colour is bright red), transported in an electric cooler to the laboratory, and kept in a freezer. We used a magnifying glass (20×) to count the number of drupelets from each fruit and an electronic scale to weigh the fruits. Number of drupelets per fruit (i.e. drupelet set) correlates strongly with fruit weight ($r = 0{\cdot}77$, $n = 387$, $t = 24{\cdot}3$, $P < 0{\cdot}001$), which determines the commercial quality of the fruit (Milivojevic *et al.* 2011).

DATA ANALYSIS

We used generalized linear mixed-effects models (GLMM) to assess the influences of visit frequency on the number of pollen grains deposited per stigma, the proportion of damaged styles and drupelets per fruit, given the discrete nature of these variables and the hierarchical sampling design (i.e. repeated measures of flowers and fruits per field but only one mean value of visit frequency) (Pinheiro & Bates 2000; Gelman & Hill 2007). All models included 'field' as a random effect and those analysing drupelets per fruit, as the response variable, also included the random effect of 'stem' within 'field', following the nested structure of the sampling design. Based on graphical analysis (i.e. residuals vs. predicted values), all models satisfied the underlying statistical assumptions, including linearity and the expected relation of the variance to the mean given the nature of the dependent variable error distribution. We also found no evidence of spatial autocorrelation in visit frequency among fields after accounting for the effect of ash deposition (see Appendix S2, Supporting information). All models were implemented with the statistical software R version 2.15.1 (R, Development Core Team 2012) using the glmmadmb function (library: glmmADMB; Bolker *et al.* 2012).

We separately assessed the effects of visit frequency of all Apoidea, or *A. mellifera* and *B. terrestris* in relation to the number of pollen grains per stigma, the proportion of damaged pistils and drupelet per fruit. In the latter analyses, we used the partial coefficients for the visit frequencies by *A. mellifera* and *B. terrestris* to discriminate between the differential effects of the two main flower-visiting species. We did not assess the specific effect of native bees, because *A. mellifera* and *B. terrestris* accounted for approximately 95% of all bee visits to raspberry flowers (see Results). Analyses of the numbers of pollen grains per stigma and drupelets per fruit considered negative-binomial distributions and a *ln*-link function (see Table S2, Supporting information). The analysis of the proportion of damaged pistils considered a beta-binomial distribution with a *logit*-link function. Both distributions simplify to the Poisson and binomial distribution, respectively, in the absence of overdispersion (Bolker 2008). Analysis of drupelet set also included the effects of stigmatic pollen deposition and the proportion of damaged styles as predictors.

We also evaluated whether the number of drupelets per fruit was differentially limited by pollination along the gradient of bee abundance with an analysis that considered the effect of pollination treatment (i.e. pollen-supplemented *vs* open-pollinated flowers), frequency of visits and their interaction. Drupelet number was modelled assuming also a negative-binomial distribution (see Table S2, Supporting information).

Path analysis was used to assess the overall beneficial vs. detrimental effects of bee visitation on drupelet set. The initial path model proposed that visit frequency of *A. mellifera* and *B. terrestris* affected drupelet set positively via increased stigmatic pollen deposition and negatively via increased pistil damage (Fig. 2a). Model fit to the data was assessed using the model proposed by Shipley (2009), which unlike classical SEM (structural equation modelling) allows consideration of the hierarchical structure of the data and variables with different sampling distributions. Following this method, we first identified all k possible 'independence claims', which are pairs of variables not connected by an arrow and are therefore expected to be statistically independent after accounting for the effects of any direct causal variables in the proposed path diagram (Shipley 2009). We then tested each claim separately by fitting a GLMM, assuming for each response variable the sampling distribution and link function outlined above (see Table S2, Supporting information). For each claim, we obtained the probability (p_i) that variable X has not direct effect on Y after conditioning by all variables with direct effects. Finally, we combined the probabilities (p_i) of all claims according to

$$C = -2\sum_{i=1}^{k} \ln(p_i).$$

We tested whether the observed C differed significantly from that expected by chance by comparing it to a χ^2 distribution with $2k$ degrees of freedom (Shipley 2009).

Results

We observed 3216 bee visits to *R. idaeus* flowers. *Apis mellifera* and *Bombus terrestris* accounted for 50 and 45%

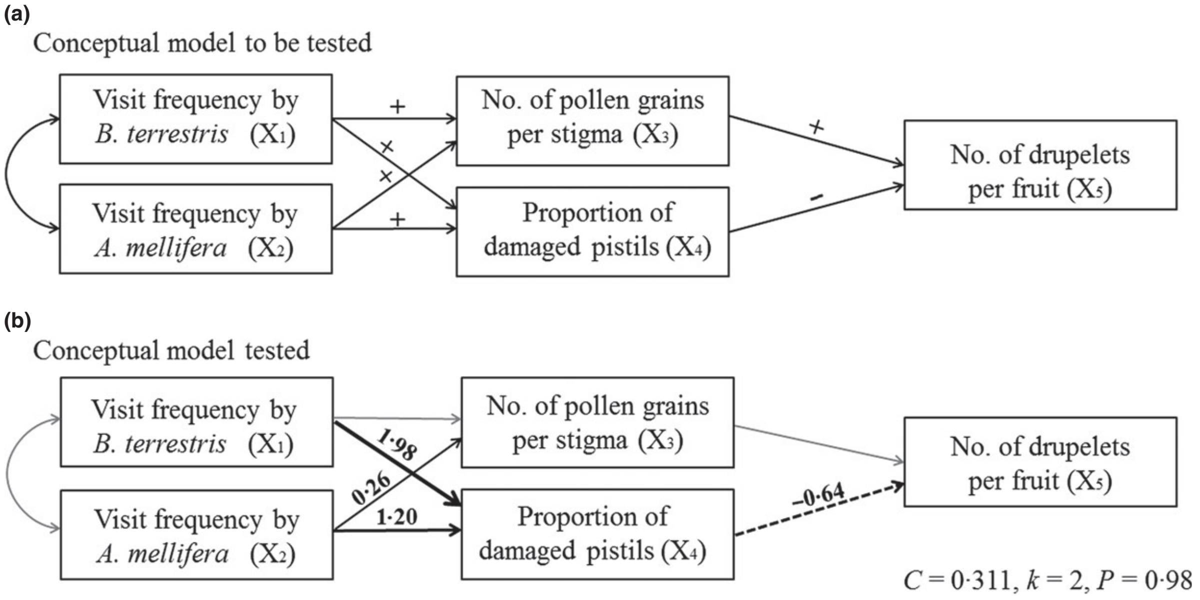

Fig. 2. Conceptual model examined by path analysis (a and b, pre- and post-tested, respectively), showing the hypothesized sequence of effects of visitation by the two most important flower visitors to raspberry flowers, *Bombus terrestris* (X_1) and *Apis mellifera* (X_2), on stigmatic pollen deposition (i.e. number of pollen grains per stigma) and flower damage (i.e. proportion of damaged pistils) (variables X_3 and X_4, respectively), and their effects on the number of drupelets per fruit (X_5). (a) The conceptual model to be tested. One-headed arrows represent directional effects, and two-headed curved arrows represent correlations between pairs of variables. The symbol (+, –) associated with an arrow indicates the expected effect (positive or negative, respectively) on the response variable. (b) Final model, where the continuous and dashed arrows indicate positive or negative effects, respectively, whereas grey arrows indicate non-significant effects. Thickness of the arrows indicates the magnitude of the effect (significant partial regression coefficients shown above the arrows).

of all bee visits, respectively, and native bees (mainly *Cadeguala albopilosa*, Colletidae, and *Ruizantheda proxima*, Halictidae) accounted for the remaining 5%. Frequency of visits varied extensively among fields, ranging from 0·01 to 3·13 visits flower^{-1} 5 min^{-1} for all bees combined, from 0 to 2·24 visits flower^{-1} 5 min^{-1} for *A. mellifera*, from 0·01 to 2·33 visits flower^{-1} 5 min^{-1} for *B. terrestris* and from 0 to 0·45 visits flower^{-1} 5 min^{-1} for native bees. Visitation by *A. mellifera* depended on the type of management (i.e. hives present or not), whereas visitation by *B. terrestris* and native bees varied negatively with the amount of ash deposited (see Appendix S1, Fig. S1, Supporting information).

As expected, the mean number of pollen grains per stigma increased with increasing visit frequency of all bees combined (estimate ± SE = 0·17 ± 0·06, $P = 0{\cdot}01$) (Fig. 3, Table S2, Supporting information). On average, flowers in fields with the highest frequency of visits received 60% more pollen grains than those with the lowest frequency of visits, increasing from ~30 to 50 pollen grains/stigma. Specifically, stigmatic pollen deposition increased significantly with increasing frequency of *A. mellifera* visits (partial regression coefficient ± SE = 0·26 ± 0·09, $P = 0{\cdot}004$), but not with those of *B. terrestris* (partial regression coefficient ± SE = 0·12 ± 0·1, $P = 0{\cdot}22$) (Fig. 3, Table S2, Supporting information). Thus, pollen deposition is more related to visitation by *A. mellifera* than *B. terrestris*.

The proportion of damaged pistils increased with increasing total frequency of visits (estimate ± SE = 1·48 ± 0·22, $P < 0{\cdot}001$) (Fig. 3, Table S2, Supporting information). On average, ~80% of styles were damaged in flowers from fields with the highest visitation rates, whereas almost all styles were intact in fields with the lowest visitation. In particular, the proportion of damaged pistils varied positively with visit frequency of *A. mellifera* (partial regression coefficient ± SE = 1·20 ± 0·31, $P < 0{\cdot}001$), but more strongly with the visit frequency of *B. terrestris* (partial regression coefficient ± SE = 1·98 ± 0·34, $P < 0{\cdot}001$) (Fig. 3, Table S2, Supporting information). Thus, although both flower visitors damaged flowers, *B. terrestris* was more detrimental than *A. mellifera*.

The number of drupelets per fruit did not vary significantly with stigmatic pollen deposition (estimate ± SE = 0·007 ± 0·005, $P = 0{\cdot}13$) (Fig. 4a, Table S2, Supporting information), but was negatively affected by the incidence of pistil damage (–0·64 ± 0·18, $P < 0{\cdot}001$) (Table S2, Supporting information). Fields with the highest pistil damage produced, on average, 40% fewer drupelets per fruit than fields with the lowest damage, decreasing from about 70 to 40 drupelets per fruit (Fig. 4b). Drupelet number also declined with increasing total frequency of visits (–0·12 ± 0·04, $P = 0{\cdot}008$) (Table S2, Supporting information). As expected from the preceding results, fields with the highest visitation produced, on average, 30% fewer drupelets per fruit than fields with the lowest visitation, decreasing from about 70 to 50 drupelets per fruit (Fig. 4c). Specifically, drupelet set var-

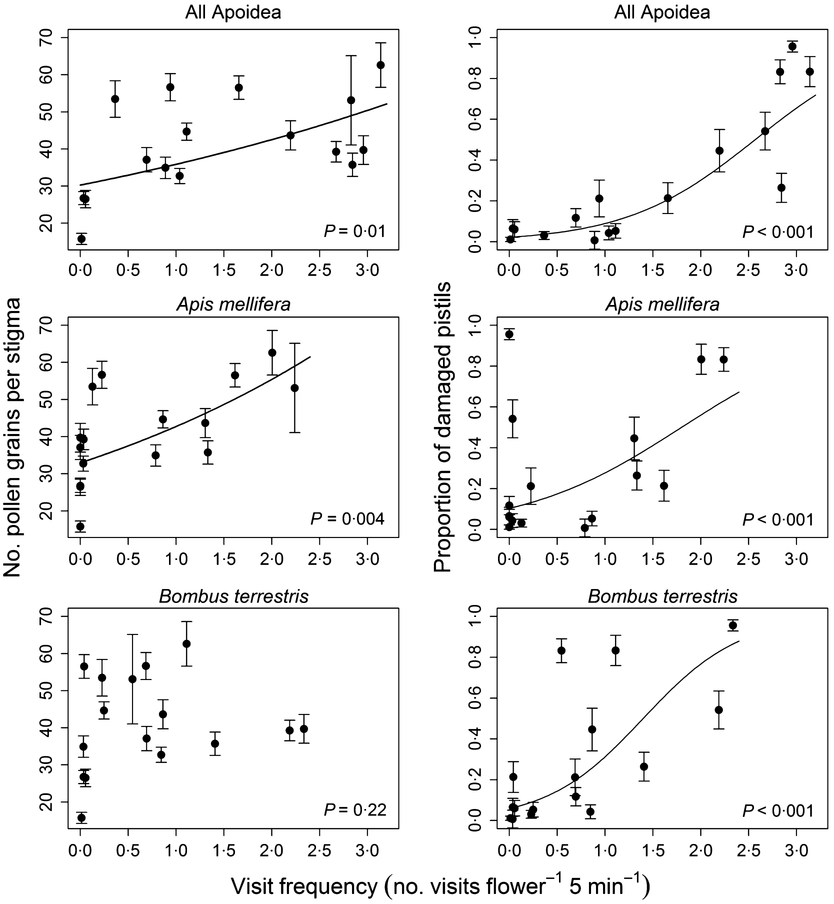

Fig. 3. Relations of mean (±SE) pollen deposition (left panels) and flower damage (right panels) to visit frequency of all Apoidea (upper panels), *Apis mellifera* (middle panels) and *Bombus terrestris* (lower panels). Solid lines indicate the predicted relations from generalized mixed linear models that considered a negative-binomial distribution (pollen deposition) and beta-binomial distribution (flower damage).

ied negatively with visit frequency of both *A. mellifera* (partial regression coefficient ± SE = −0·12 ± 0·06, $P = 0·05$) (Table S2, Supporting information) and *B. terrestris* (partial regression coefficient ± SE = −0·15 ± 0·07, $P = 0·04$) (Table S2, Supporting information). Furthermore, the negative effect of visit frequency did not differ between pollen-supplemented and open-pollinated flowers across the gradient of bee abundance (0·006 ± 0·03, $Z = 0·20$, $P = 0·84$) (Fig. 5). Correspondingly, pollen supplementation did not increase drupelet set overall (0·021 ± 0·05, $Z = 0·4$, $P = 0·69$).

The conceptual model fitted by path analysis explained the data adequately ($C = 0·311$, $k = 2$, $P = 0·98$) (Fig. 2b). Mean visit frequency of *A. mellifera* and *B. terrestris* varied largely independent of each other ($r = 0·15$, $n = 16$, $P = 0·57$). Visit by *A. mellifera* contributed significantly to raspberry pollination by increasing pollen deposition, whereas visits by *B. terrestris* did not affect pollen

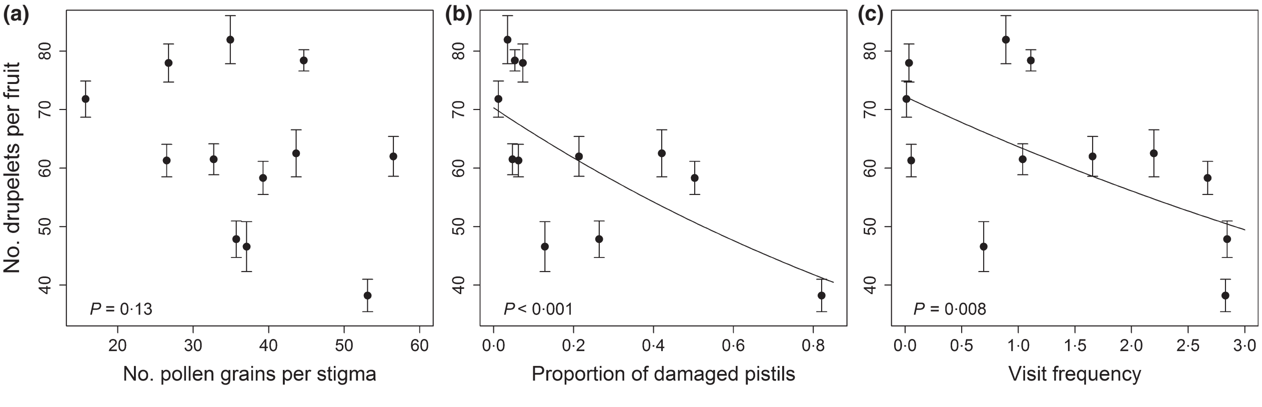

Fig. 4. Relations of the mean (±SE) number of drupelets per fruit to (a) stigmatic pollen deposition, (b) the proportion of damaged pistils per flower and (c) frequency of visits (no. visits flower^{-1} 5 min^{-1}) of all bees. Solid lines indicate the predicted relationships estimated from the negative-binomial models.

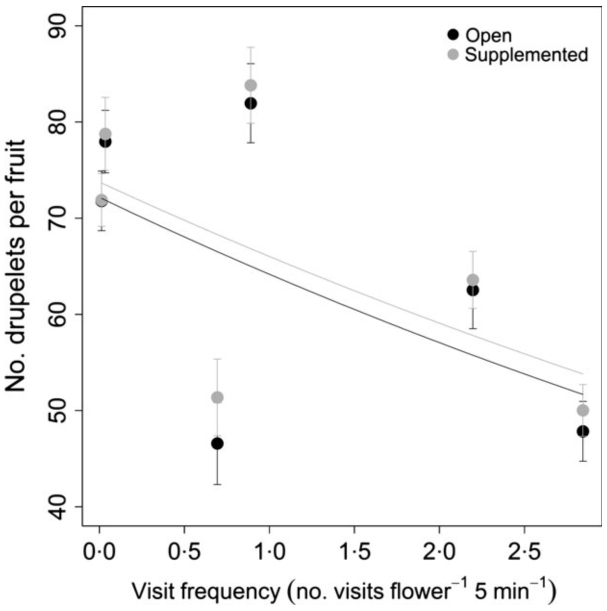

Fig. 5. Effect of pollen supplementation (pollen-supplemented vs. open-pollinated flowers) on the mean (±SE) number of drupelets per fruit along a gradient of bee frequency of visits. Solid lines indicate the relations predicted for pollen supplementation (grey) and open pollination (black) based on the negative-binomial model.

deposition significantly. The main influence on variation in drupelet set was pistil damage, rather than pollen deposition. Visits by both *A. mellifera* and *B. terrestris* increased the proportion of damaged pistils in raspberry flowers, in turn reducing drupelet set. However, this process depended more strongly on the frequency of visits of *B. terrestris* than *A. mellifera*. Thus, excessive visitation by both flower visitors can generate more detrimental than beneficial effects.

Discussion

Honeybees and bumblebees were the most important raspberry flower visitors in the study fields (see also Crane & Walker 1984; Willmer, Bataw & Hughes 2008; Morales 2009; Lye *et al.* 2011), and their visits, particularly those of *A. mellifera*, consistently increased pollen deposition on raspberry stigmas. However, extremely frequent visits by the managed *A. mellifera* and particularly by the invader *B. terrestris* to raspberry flowers reduced drupelet set and, consequently, fruit production by damaging flower styles. Thus, raspberry drupelet set should be expected to exhibit a humped relation with the frequency of bee visits across the whole range of potential visit frequency.

Flowers in the fields with the lowest total frequency of visits, which experienced ~10 visits during their ~2·5 day life span (i.e. assuming a daily period of visitation of 10 h) received, on average, 30 pollen grains per stigma, which should be more than enough to fertilize the single ovule per carpel. Indeed, this number of visits agrees with that estimated by Chagnon, Gingras & Oliveira (1991) as the minimum frequency of visits to ensure adequate pollination and maximum fruit quality and production in raspberry. Also, drupelet set was not pollination limited, even in fields with the lowest bee visitation, indicating that all fields had sufficient pollinator abundance to ensure sufficient pollen transfer. In contrast, frequent bee visits can damage raspberry flowers, reducing the number of drupelets per fruit. Raspberry flowers in fields with the highest visitation received ~170 and ~140 daily visits by *B. terrestris* and *A. mellifera*, respectively, and the incidence of pistil damage increased strongly with frequency of visits, particularly of *B. terrestris*. Indeed, we can blame pistil damage, specifically style breakage, as the main determinant of drupelet set failure after discarding other factors associated with ash deposition (see Appendix S1, Supporting information). This damage should be inconsequential if it occurred after ovule fertilization; however, the negative relation of drupelet set to the proportion of damaged styles indicates that much damage occurred before fertilization (i.e. during the first hours after flower anthesis). Thus, early style breakage precludes the arrival of pollen tubes into the ovary.

Morris *et al.* (2010) reported that excessive flower visitation can impose a mutualism cost, strongly decreasing reproductive success below the maximum possible in a Capparaceae after a few hymenoptera visits. Similarly, Young (1988) found a humped relation in the reproductive success of an Araceae with beetle abundance. However, no specific mechanisms have been uncovered for these effects. In raspberry, a positive effect of visit frequency on flower damage, specifically style damage, could determine such an optimal frequency. Although several studies have reported flower damage, they have mostly considered the effects of non-legitimate flower visitors, specifically nectar robbers (Cunningham 1995; Traveset, Willson & Sabag 1998; Kenta *et al.* 2007), which do not transfer pollen effectively. In contrast, the two main flower visitors in our study, *A. mellifera* and *B. terrestris*, are legitimate raspberry pollinators, with visitation by *A. mellifera* being more related to increasing pollen deposition than visitation by *B. terrestris*. In other raspberry studies, it has been shown that sporadic flower visitation by either bee species enhances fruit quality by increasing the number of drupelets per fruit (Crane & Walker 1984; Willmer, Bataw & Hughes 2008; Lye *et al.* 2011). However, the positive association of pistil damage with the frequency of visits by these pollinators, especially *B. terrestris*, suggests the existence of an intermediate optimal visit frequency that depends on the number of visits as well as pollinator identity. Unfortunately, we could not identify this optimum, because even the lowest observed frequency of visits provided adequate pollination, so that the optimum seems to be ≤10 visits during a flower's life span (see also Chagnon, Gingras & Oliveira 1991).

In the absence of natural enemies and other regulatory factors, invasive species can become extremely abundant, achieving densities never observed in their native ranges

(Pimentel, Zuniga & Morrison 2004). This seems to be the case of *B. terrestris* in north-west Patagonia, where it is now extremely abundant (Morales *et al.* 2013). Furthermore, the synchrony in the timing of highest bumblebee density and the blooming peak of this raspberry variety (January–February), which occurs after the flowering of most wild and other cultivated plants (Aizen & Vázquez 2006; Morales & Aizen 2006), generates flower overexploitation by excessive visitation, exceeding several hundred visits during a flower's life span by this bumblebee species alone. Indeed, in raspberry fields with high abundance, bumblebees break the sepals of raspberry buds to reach nectar, even damaging pistils before anthesis. Regrettably, comparable estimates of visitation rates to raspberry in their native range were not available. However, Schulze *et al.* (2012) estimated frequency of visits in wild and cultivated strawberry, another Rosaceae with multipistilar flowers, reporting mean total visit frequency of 0·07 visits flower^{-1} 5 min^{-1}, a value comparable to those found in the raspberry fields with the lowest visitation. For this reason, present densities of *B. terrestris* in most raspberry fields of north-west Patagonia, a product of a biological invasion, are clearly excessive. Thus, sound pollination practices should focus primarily on controlling populations of this alien bumblebee. However, to our knowledge, there are still not developed practices to manage invasive bumblebees. The development of such practices will be hindered as long as the agronomic view of *B. terrestris* as an always 'beneficial' bee prevails.

On the other hand, *A. mellifera* contributed more effectively to raspberry pollination while being less damaging than *B. terrestris*. Differences in food gathering could not explain differential pollination efficiency because both honeybees and bumblebees forage mainly for nectar in raspberry. Perhaps, differences in pollination efficiency could be attributed to more pollen being removed from than deposited on the stigmas by *B. terrestris* after a few visits (Fig. 3; see also Young & Young 1992). This apparent trade-off between pollination service and pistil damage requires more attention for adequate implementation of pollination practices. To our knowledge, however, stocking densities of honeybee hives in raspberry fields are not based on the quantitative relation of fruit production to bee density, but rather focused on honey production. Furthermore, as visitation by *A. mellifera* and *B. terrestris* was not correlated, deployment of honeybees is unlikely to reduce the local abundance of the invasive bumblebee. Indeed, we expect that in the absence of *B. terrestris*, the few visits provided by the background pollinator assemblage of native bees and flies, together with properly managed honeybees, could provide adequate pollination to maximize the production of commercial-quality fruits in raspberry fields.

Although less detrimental than *B. terrestris*, visits of *Apis mellifera* caused some style damage. In a recent analysis of pollination service in 41 crop systems worldwide, Garibaldi *et al.* (2013) reported that honeybee visitation increased pollen deposition more frequently and more strongly than it increased fruit set, which was interpreted as the result of the deposition of high amounts of poor-quality (i.e. self) pollen. Our results provide an alternative explanation for this apparent inconsistency between pollen deposition and fruit production that might also apply to other crops: too many visits can impair fruit set by damaging flowers, in spite of increasing pollen deposition. Evidence presented in our study opens the necessity to evaluate the response of different crops to pollinator density and encourages the use of native insects for crop pollination through local management practices (Carvalheiro *et al.* 2011, 2012; Garibaldi *et al.* 2013) as opposed to the introduction of alien bees. This will prevent further invasion events and, consequently, flower over-visitation.

Animal pollination is necessary for increasing and sustaining yield in diverse crops (Klein *et al.* 2007; Aizen *et al.* 2008), and the pollination demands of global food production increasingly exceed apparently declining pollinator availability (Aizen & Harder 2009; Garibaldi *et al.* 2013). Although the introduction of pollinators has decreased the pollination deficit and increased the production of many crops (Velthuis & van Doorn 2006 and references therein; Southwick & Southwick 1992), our raspberry study provides novel perspectives in relation to current concerns about the global decline in the abundance of pollinators and an ensuing pollination crisis. It clearly illustrates that an excess, rather than a deficit, of flower visitors associated with the invasion of a bumblebee species and incorrect management of honeybee hives can reduce crop yield. As several pollinator species, besides *A. mellifera*, are now reared on industrial scales and introduced on almost all continents for crop pollination, these results raise the possibility of negative effects on agricultural production besides the negative impacts on native bee faunas (Goulson 2003; Morales *et al.* 2013). Until the incidence and severity of such detrimental effects on crop yield is assessed in other crop species, extreme caution should be exercised in uncritically adopting this management practice.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1. Effects of ash deposition on bee frequency of visits.

Table S1. Characteristics of the study raspberry fields.

Table S2. Regression models.

Appendix S1. Ash effects on bees and possible confounding effects on response variables.

Appendix S2. Spatial autocorrelation analysis.