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Decadal trends in the pollinator assemblage of *Eucryphia cordifolia* in Chilean rainforests

Cecilia Smith-Ramírez · Rodrigo Ramos-Jiliberto · Fernanda S. Valdovinos · Paula Martínez · Jessica A. Castillo · Juan J. Armesto

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Abstract Long-term studies of plant–pollinator interactions are almost nonexistent in the scientific literature. The objective of the present study was to determine changes and trends in the pollinator assemblage of ulmo (*Eucryphia cordifolia*; Cunoniaceae), a canopy-emergent tree found in Chilean temperate rainforests. We assessed the temporal variability of the pollinator assemblage and identified possible modulators of the observed temporal shifts. We sampled insect visitors to the flowers of 16 individual trees of *E. cordifolia* during 10 consecutive flowering seasons (2000–2009), recording a total of 137 pollinator species with a mean number of species per year of 44. Only three pollinator species (2.2 %) were recorded every year. Two bee species accounted for 50 % of all insect visits to flowers. One bee species, *Bombus dahlbomii* (native), was dominant in one season, whereas *Apis mellifera* (exotic) dominated during the next season. These interannual shifts in population abundances presented first-order dynamics that were characterized by oscillations with a period of 2 years. Changes in the abundances of the dominant pollinators, as well as differences in temperature and precipitation during insect emergence and flowering, led to a nested temporal structure of pollinator composition. Furthermore, the abundances of less common pollinators were sensitive to the abundance of the dominant bee species and to monthly maximum temperatures and the average precipitation during spring and summer. Based on our results and those from other studies, we predict a decline in the numbers of *Bombus dahlbomii* and nondominant native pollinators in response to new exotic arrivals.

Keywords Bipartite networks · Exotic species · Hymenopterans · Nested community · Parasites · Ulmo

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C. Smith-Ramírez (✉) · J. A. Castillo · J. J. Armesto
Instituto de Ecología y Biodiversidad (IEB), Facultad de Ciencias, Universidad de Chile, Santiago, Chile
e-mail: csmith@willnet.cl

C. Smith-Ramírez · P. Martínez · J. J. Armesto
Fundación Senda Darwin, Chiloé Island, Chile

C. Smith-Ramírez
Facultad de Ciencias Forestales y Recursos Naturales, Instituto de Conservación, Biodiversidad y Territorio, Universidad Austral de Chile, Valdivia, Chile

R. Ramos-Jiliberto · F. S. Valdovinos
Centro Nacional del Medio Ambiente, Fundación de la Universidad de Chile, Santiago, Chile

R. Ramos-Jiliberto · F. S. Valdovinos
Facultad de Ciencias, Universidad de Chile, Santiago, Chile

R. Ramos-Jiliberto
Instituto de Filosofía y Ciencias de la Complejidad (IFCC), Santiago, Chile

J. A. Castillo
Escuela de Graduados, Facultad de Ciencias Forestales y Recursos Naturales, Universidad Austral de Chile, Valdivia, Chile

J. J. Armesto
Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile

Introduction

Traditionally, plant–pollinator studies rely on observations made during a single reproductive season, with salient exceptions. For instance, Herrera (1988) found that 35.7 % of the pollinators of *Lavandula latifolia* Medik. co-occurred during 6 years of sampling. Although information on long-term changes in plant–pollinator systems remains limited, five community studies have described the temporal patterns of pollination networks during periods of 2–4 years. These studies reveal striking similarities in network structure among years despite important changes in species composition (Basilio et al. 2006; Alarcón et al. 2008; Olesen et al. 2008; Petanidou et al. 2008; Burkle and Irwin 2009; Dupont et al. 2009). In accord with Herrera (1988), in these plant–pollinator networks, the number of plant pollinators and their identities differed significantly among years, whereas the numbers of interacting species and network links remained constant. Petanidou et al. (2008) reported that only 20.5 % of the pollinators of 38 plant species were recorded during all 4 years of their study. Dupont et al. (2009) found that 30 % of the pollinators recorded in year 1 were recorded again in the subsequent year at six different study sites. On the other hand, Olesen et al. (2008) found that 80 % of the pollinator species observed in a Mediterranean plant community during the first reproductive season were present in the following year. The greatest interannual variability in the frequency of visits was seen for the less frequent insect pollinators. In contrast, generalist pollinators usually presented lower interannual variability (Herrera 1988; Petanidou et al. 2008).

Changes in insect abundance and composition among years in the same plant community can be attributed to differences in weather conditions, flower resource (nectar or pollen) abundance, and less frequently to the influences of exotic species and disease transmission. An extensive study of population fluctuations of euglossine bees over 22 years in Panamanian forests found that drier periods (associated with the El Niño Southern Oscillation) increased bee abundance (Roubik 2001). Furthermore, González et al. (2009) and Devoto et al. (2005, 2009) found that differences in rainfall across pronounced topographic gradients greatly influenced species prevalence and the turnover of pollinators in plant communities from the West Indies and Patagonia, respectively.

Studies of interspecific competition as a cause of insect pollinator fluctuations are relatively scarce, but it has been shown that the introduction of some bee species can have negative impacts on the abundances of native species (Dupont et al. 2004; Morales 2007). Several studies have reported that competition for floral resources and nesting places between native pollinators and European honeybees frequently results in reductions in the seed sets of indigenous plant species (Gross and Mackay 1998; Goulson 2003; Celebrezze and Paton 2004; Dupont et al. 2004). Another factor that intensifies interspecific competition between pollinators is introduced diseases, which are known to reduce populations or produce fluctuations in them (Sisojevic 1979; Meeus et al. 2011; Williams and Osborne 2009). Another potential explanation for changes in insect pollinator assemblages among years is insufficient sampling effort in any given year or among years (Petanidou et al. 2008; Burkle and Alarcón 2011).

Although some studies of plant–pollinator systems encompassing several reproductive seasons are now available (Petanidou and Ellis 1993, 1996; Petanidou et al. 1995, 2008; Medan et al. 2006), the time frames used in them were too short to enable the long-term effects of climatic and biotic drivers on pollinator assemblage structure and composition to be explored. Such information is urgently needed to assess the impacts of impending climatic trends and exotic species invasion on plant–pollinator systems. Burkle and Alarcón (2011) called for a significant increase in the time frames of these studies from the current norm of 2–4 years to allow the identification of long-term temporal patterns in network structure and thus address their ecological causes and consequences.

The objective of the present study was to determine decadal changes and trends in the pollinator assemblage of one generalist, long-lived tree species that characterizes the canopy-emergent layer of lowland temperate rainforests in southern Chile. The study was conducted over ten consecutive reproductive seasons in the summer of the Southern Hemisphere. Due to the huge sampling effort required to study a rich insect pollinator community over several years, we decided to focus on one tree species, *Eucryphia cordifolia* Cav. In terms of the number of flower visitors it attracts, this species is among the most attractive in South American temperate forests, due to its large size and lavish floral display. Therefore, its presence is considered crucial to maintaining a rich community of pollinators for rainforest trees (Smith-Ramírez et al. 2005). We attempted to answer several questions about the pollinator assemblage of this canopy-emergent tree. (1) What is the magnitude of interannual differences in species richness and composition of its pollinator assemblage? (2) What is the contribution of interannual climate variability to the observed changes in the composition and abundances of its pollinator species? (3) How did the degree of nestedness of its pollinator assemblage vary over the 10 years of the study? (4) What is the effect of interannual changes in the abundances of dominant pollinators on the abundances and composition of the less frequent pollinator species?

This study examines decadal trends in the pollinator assemblage of the most generalist rainforest tree species in temperate Chile, the fruit crop of which depends on a rich community of insect pollinators (Smith-Ramírez et al. 2005; Ramos-Jiliberto et al. 2009). Based on this diverse array of pollinators, we expected to see large interannual fluctuations in the pollinator network associated with the flowering of *E. cordifolia*. These large fluctuations would be produced when the pollinator assemblages comprise numerous species that make relatively few visits to the flowers of one tree species. We also predicted that the dominant (most frequent) pollinator species should exhibit lower interannual variabilities than those of many less common species, and that differences in the abundances of the common species could have an effect on the abundances and composition of the entire assemblage, as reported by Petanidou et al. (2008) and Herrera (1988). We also expected that the diversity and abundances of pollinators would be strongly influenced by variations in summer rainfall and temperature during successive flowering seasons, particularly because summer is the most favorable period for insect activity in temperate rainforests. To our knowledge, question (3) has not been addressed in similar studies, and it is considered here as an exploratory question. The nestedness of plant–pollinator networks has traditionally been regarded as an indicator of whether some pollinator species in the assemblage are subsets of a larger group of insect species. Studies have documented a general pattern in plant–pollinator networks: the specialist species are usually a subset of the most generalist species (Bascompte et al. 2003), and this pattern has been consistent among years (Petanidou et al. 2008). However, here we use a different approach to analyze nestedness over time—we assess whether the pollinator assemblages of years with low biodiversities are subsets of the corresponding assemblages in years with higher biodiversities. A nested structure of pollinator assemblages across years may suggest possible interactions among pollinator species and, when data are analyzed in relation to interannual climatic differences, nestedness also can reveal tendencies of particular pollinator species to co-occur in certain years or to respond to the same specific environmental cues. All of these are important questions to answer when attempting to forecast the potential effects of future climate trends and exotic species invasions on interspecific interactions and plant–pollinator systems.

Materials and methods

Study sites, focal species, and climate

We selected individuals of *E. cordifolia* growing in a mosaic of old-growth and second-growth forest patches in an area of approximately 105 km^2 (15 × 7 km). We believe that the activities of several families of bees (Pedersen et al. 1972; Osborne et al. 2001; Hagler et al. 2011) and other pollinators in this area of landscape can be recorded. For logistic reasons (i.e., accessibility to flowering branches), the sample included 10–15 m tall trees along edges and some isolated trees in grazing pastures within a rural landscape in northern Chiloé Island, southern Chile (42°S, 73°35′W). The species *E. cordifolia* (Cunoniaceae) is an evergreen broad-leaved tree from lowland forests that reaches up to 35 m in height and occupies an emergent position in the forest canopy (Díaz et al. 2010). This species has relatively large, disk-shaped, symmetric white flowers (5–6 cm in diameter) with many stamens, and offers abundant nectar and pollen rewards to pollinators, which are nearly all insects (Smith-Ramírez et al. 2005). Each tree produces many flowers, which makes this blooming period highly conspicuous to an observer and to the insect community. Peak flowering usually occurs in late January to early February (Smith-Ramírez and Armesto 1994). *E. cordifolia* has a self-incompatible reproductive system and is strongly dependent on insect pollinator visits for setting fruits, which are small pods containing many winged, wind-dispersed seeds (Riveros 1991).

Mean monthly precipitation based on records from Puerto Montt (41°28′, 72°56′), the nearest meteorological station, varied during the decade of study between 30 mm during the austral summer months (January–March) to 500 mm during the austral winter months (June–August). Annual precipitation showed marked differences among years. For example, the year 2002 stands out as one of the rainiest in 50 years of records for Puerto Montt, with 2,034 mm. During the study period (2001–2010), about 65 % of the annual precipitation fell between April (early fall) and December (southern spring) each year. Mean air temperatures (based on records from Puerto Montt) were fairly homogeneous between years from 2001 to 2010, with 0.2–1 °C differences in mean maximum monthly temperatures between consecutive years and 0.02–0.7 °C differences in mean minimum monthly temperatures (Table 1).

Field methods

To assess the identities, species richness, and abundances of animal pollinators of *E. cordifolia*, field observations were concentrated into a period of approximately one week per year when this tree species was at maximum bloom in the study area (Smith-Ramírez and Armesto 1994). Each individual tree flowers regularly for about 3 weeks each year, but the peak flowering time of the *E. cordifolia* population may vary by up to 1 month among years. The identities and visit frequencies of floral visitors to individual trees were recorded during a variable number of fixed 20-min observation periods. These periods were uniformly distributed

Table 1 Pollinator species richness and climatic data from Puerto Montt. PP is the total annual precipitation (in mm), $T_{\max}$ and $T_{\min}$ are the monthly average maximum and minimum temperatures (°C), and R is the pollinator richness

Year	PP	$T_{\max}$	$T_{\min}$	R
2000	1615.2	6.0	14.6	–
2001	1,652.7	6.1	14.8	44
2002	2,034.3	6.6	14.6	55
2003	1,411.4	6.5	15.0	56
2004	1,557.5	6.7	15.6	48
2005	1,775.4	6.3	14.8	74
2006	1,930.4	6.3	15.0	51
2007	1,245.6	5.6	14.3	42
2008	1,591.3	6.5	16.0	40
2009	1,593.1	6.2	14.8	73
2010	–	–	–	23

across the entire day, but greater sampling efforts were made from 10 am to 6 pm, when the insects were more active. Observations were made from the ground and sometimes from a short platform (2 m tall) with the naked eye. To facilitate sampling, we preferred trees located in forest edges, so records of visitors were most often limited to profusely flowering branches located 4–5 m above the ground.

During each 20-min sample period, a group of two or three neighboring trees within a radius of 5 m in the field were closely inspected by 1–3 trained observers. We randomly assigned one or two trees to each trained observer. A previously defined number of easily visible open flowers (20) from one branch in each sampled tree were the targets when assessing the frequency of pollinator visits. We defined a putative pollinator as an animal that contacted the anthers or stigma of the flowers. Due to the morphology of the flowers (an open corolla with many short stamens), it was easy to record contacts with the anthers and stigma when insects were looking for nectar and pollen.

The number of 20-min periods sampled during one day depended on the number of hours with suitable weather conditions; rainy and/or windy periods of the day were avoided. Overall, we distributed the total number of sampling periods among flowering individuals as tohave similar sample sizes per tree. In the first year of study, we collected voucher specimens of each insect flower visitor for identification, but in subsequent years we concentrated on specimens of confirmed or previously undetected pollinators, or species with doubtful assignments. Specimens were identified with the assistance of specialists, and vouchers are kept in the entomological collection of Senda Darwin Biological Station. All of the specimens were identified to family level, but it was not always possible to obtain an assignment to genus or species level, although putative species were always kept separate using a morphospecies criterion, following consultation with specialists.

To assess the interannual variation in the pollinator assemblage of *E. cordifolia*, the identity of each pollinator and the relative frequency of pollinator visits were recorded for a total of 16 individual trees each year. As far as possible, the same trees were sampled each year, but some of the trees did not flower in subsequent years with the same intensity, and flowering overlaps among individual trees were not always constant. Each year, except in 2010, we completed 2,880 min (48 h) of observations during the flowering season and a minimum of 200 min of observations per tree. In 2010, a strong earthquake in Chile interrupted our field sampling routine, so we completed only 1,800 min of observation (30 h). The accumulated species richness curve reached an asymptote each year at around 1,000–1,400 min of observations for the total sample of trees, and at 80–100 min of records for each individual tree. After these observation times, no new insect species were recorded, only the less frequent pollinators.

Analysis

Frequency data for each insect pollinator species observed in the flowers of *E. cordifolia* were used as a proxy for its population abundance in each year. We treated these data in two ways. Absolute abundance was the number of observations recorded for a particular species standardized by observation time. Relative abundance is the absolute abundance of the species divided by the sum of the absolute abundances of all species during a given year of observation. We tested the correlations of the relative and absolute abundances of the two most common species in the pollinator assemblage (two hymenopterans) as well as their associations with the species richness and abundance of the whole set of pollinators over the 10 years of study. We also tested the relationship between the two dominant species and the relative abundances of insect taxonomic groups (coleopterans, dipterans, and hymenopterans) excluding the two dominant species. We also assessed the correlations between the structure of the pollinator community (all of the above dependent variables) and selected climatic variables, including the total annual precipitation (PP), the mean maximum ($T_{\max}$) and minimum ($T_{\min}$) air temperatures for each year, and those temperatures for the summer season only (Table 1). Lepidopterans were excluded from the analysis due to their scarcity in the pollinator assemblage of *E. cordifolia*. We also tested the correlations between the relative and absolute abundances of the two most common species in the assemblage and the same climatic variables. Spearman's rank correlations were performed with and without the inclusion of a 1-year delay in the effects of the climatic variables.

Temporal structure of the pollinator assemblage

To graphically visualize and analyze the interannual variation and decadal trends in the pollinator community structure for *E. cordifolia*, we constructed a bipartite network with one set of nodes representing the pollinator species and a second set of nodes representing the years in which observations were performed. A link between any pair of nodes of the two distinct sets represents the occurrence of a pollinator species in a given year. Figure 2 illustrates the temporal network structure of the community of pollinators of *E. cordifolia* from 2001 to 2010. Quantitative information on the frequency of visits is provided by the widths of links and nodes (Fig. 2). Hereafter, we assumed that the population abundance of each pollinator species was proportional to its observed visitation rate to all flowers in one reproductive season.

Nestedness analysis

We constructed binary adjacency matrices in which pollinator species were organized as rows and sampling years were arranged as columns. Therefore, an individual element (i, j) in the matrix represented the presence (1) or absence (0) of species i in year j. Matrices of this kind were constructed for the entire pollinator species assemblage (including and excluding the two most common pollinators), and for each of three distinct and most species-rich taxonomic groups in the sample (coleopterans, dipterans and hymenopterans).

Changes in nestedness in the pollinator assemblage structure among years were tested using the NODF index (Almeida-Neto et al. 2008). This metric allows the nestedness between rows and/or columns of the adjacency matrix described above to be measured. In our case, we were interested in evaluating nestedness over the 10 years of the study, represented by the columns of the matrix, so we used this component of the metric in the present analysis. The significance level of the nestedness index (the probability that the observed nestedness is greater than that obtained by chance from a randomly assembled matrix) was calculated by contrasting the observed NODF index values with a set of 1,000 null matrices in which columns were randomly sorted. The z-scores of the NODF indices were calculated as $z = (x - \bar{X})\sigma^{-1}$, where x is the observed NODF value and $\bar{x}$ and σ are the mean and the standard deviation of the NODF values from the null matrices, respectively. A value of z that is larger than 1.64 indicates that the observed nestedness in the sample is significantly larger (with $\alpha = 0.05$) than that of the null matrices.

We evaluated nestedness in matrices of pollinator species composition in which the years (columns) were sorted into ascending and descending values of the reported climatic variables calculated for three different time periods (Table 1), as follows. (1) The 10 months (April to January) preceding the flowering season of *E. cordifolia*. We used this interval to assess whether the yearly environmental variables (precipitation and temperature) affected insect emergence in a given year. (2) The previous season of insect pollinator emergence (austral spring: October to January). (3) The current flowering period (February). The matrices analyzed comprised the full pollinator species assemblage as well as each major taxonomic group (coleopterans, dipterans and hymenopterans).

Multivariate analysis

We conducted semi-parametric multivariate analysis of variance (MANOVA with the R statistics program) to determine whether the total or relative abundances of the different pollinators and taxonomic groups were determined by the abundances of the two most common species and/or by the selected environmental variables. We performed these analyses for the total assemblage of pollinators in the sample and for the main insect orders (see above). The environmental variables used here were the same variables described in the previous section for nestedness analysis. Upon the recent arrival of a exotic species in the pollinator assemblage, *Bombus terrestris*, the bimodal pattern observed previously for the two dominant species (*Apis mellifera* and *Bombus dahlbomii*) changed. Therefore, we conducted an analysis of the 10-year matrix, and of the 7 years of data obtained before the new invasive species of *Bombus* was recorded. We used non-metric multidimensional scaling (NMDS) analysis to examine the overall pattern of dispersion in the species abundances of pollinators. Data that are clustered together in the resulting plot reflect species that are similarly represented over the study period (McCune and Grace 2002). We interpreted the ordination by considering the significant biotic and environmental variables identified in the MANOVA. Based on the NMDS plot, we generated smooth surfaces for the significant biotic and environmental variables by fitting thin plate splines using general additive models and interpolating the fitted values on the unconstrained ordination diagram. NMDS was implemented by the R package ‘vegan’ (Oksanen 2013).

Results

We recorded a total of 137 pollinator species on the flowers of *E. cordifolia* during one decade of study: 67 species of dipterans, 32 coleopterans, 26 hymenopterans, 7 lepidopterans, and 5 infrequent visitors from other insect groups. The mean number of pollinator species recorded per year was 43.9, with a standard deviation of 9.1, indicating little

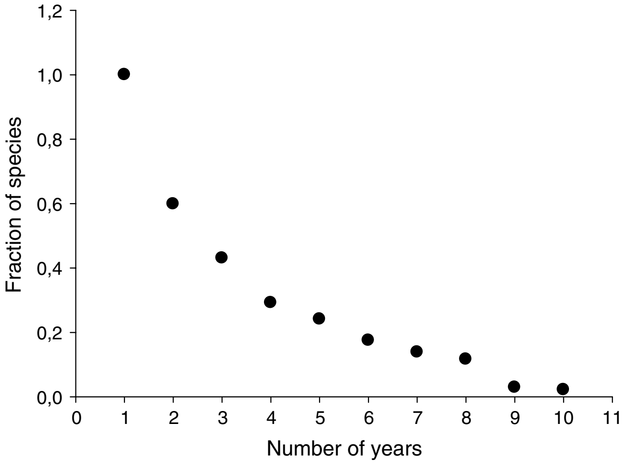

Fig. 1 Fraction of the insect species that visited flowers of *Eucryphia cordifolia* during its flowering season (February) and were observed in *n* or more years in Chiloé forest

variation in the total richness of the species assemblage among years. We obtained the highest pollinator species richness in the annual samples (58) in 2005 and 2009, while the lowest number of flower visitor species (30) was recorded during 2008. Figure 1 shows the fraction of new species that were added to the sample in any given year. Half of all the species were found during the first 2 years of sampling. Only 3 species were present over the entire period of 10 years of study. Two of them were the dominant hymenopterans while the other was a less frequent species, although it was not rare.

By far the two numerically most common and constant pollinators that visited the flowers of *E. cordifolia* were the introduced honeybee *Apis* and the native, larger bumblebee *B. dahlbomii* (species 2 and 1, respectively, in Fig. 2c). The temporal fluctuations in population abundance of these two species (estimated from flower visitation frequencies) show first-order dynamics characterized by oscillations with a period of 2 years. Such a pattern was most evident when their respective changes in absolute and relative frequencies during the first 7 years of the study were analyzed, whereas the pattern disappeared during the last 3 years of this study (Fig. 3). The following pattern emerged: first, the relative abundance of *B. dahlbomii* in the preceding year was inversely correlated with its own relative ($P < 0.05$, $\rho = -0.79$) and absolute ($P < 0.05$, $\rho = -0.76$) abundances in the subsequent year in the time series, as well as positively related with the relative abundance of *Apis* in the subsequent year ($P < 0.05$, $\rho = 0.79$). Thus, both species had abundances that peaked in alternate years. Likewise, the absolute abundance of *B. dahbomii* in the preceding year was inversely associated with its own relative abundance ($P < 0.05$, $\rho = -0.8333$). On the other hand, the absolute abundance of *Apis* was inversely associated with the total species richness of coleopterans in the sample from the preceding year ($P < 0.05$, $\rho = -0.78$).

Nestedness analysis

The abundances of the two dominant species of hymenopterans in the pollinator assemblage, as well as the variations in the maximum and minimum temperatures and the precipitation, led to a nested temporal structure of pollinator species composition during the study period (Table 2). The composition of the full species assemblage of pollinators of *E. cordifolia* in any particular year was found to be a subset of the insect species present during years with lower relative abundances of honeybees. The same was true for dipterans as a separate taxonomic group. Conversely, the hymenopteran species found in years with lower (absolute) bumblebee abundance (and higher honeybee abundance) were a subset of the hymenopteran sets found in years of higher bumblebee—and lower honeybee—abundance. Higher maximal temperatures during the flowering season of *E. cordifolia* were linked to more inclusive sets of pollinator species, specifically coleopteran species. Higher minimal temperatures during the favorable season (austral spring to early summer) also favored pollinator emergence from winter dormancy. For both the full pollinator assemblage and the hymenopteran group, the species composition of pollinators that visited *E. cordifolia* during the flowering seasons preceded by higher spring precipitation were subsets of the sets of pollinator species that visited during the flowering seasons preceded by lower spring precipitation. Likewise, hymenopteran species found during years of higher rainfall during the peak flowering period of *E. cordifolia* (February) were subsets of those found during years of lower rainfall (Table 2). The nested patterns detected for the full pollinator assemblage (Table 2) held regardless of the incorporation or exclusion of the two dominant bee species (*B. dahlbomii* and *Apis*) in the analyses.

Multivariate analysis

The total abundance of pollinator species in the annual sample, excluding the two dominant bees, was negatively affected by the absolute abundance of *Apis* in the sample (MANOVA absolute abundance *F* model = 2.04, $r^2 = 0.19$, $P < 0.01$; MANOVA relative abundance *F* model = 0.95, $r^2 = 0.10$, $P = 0.55$; Fig. 4a). In turn, we found that the relative or absolute abundance of the native bumblebee, *B. dahlbomii*, had no effect (MANOVA relative abundance *F* model = 1.43, $r^2 = 0.15$, $P = 0.10$; MANOVA absolute abundance *F* model = 1.41, $r^2 = 0.13$, $P = 0.11$). When the analyses were conducted by grouping species according to taxonomic order, the absolute abundances of non-dominant hymenopterans were negatively affected by

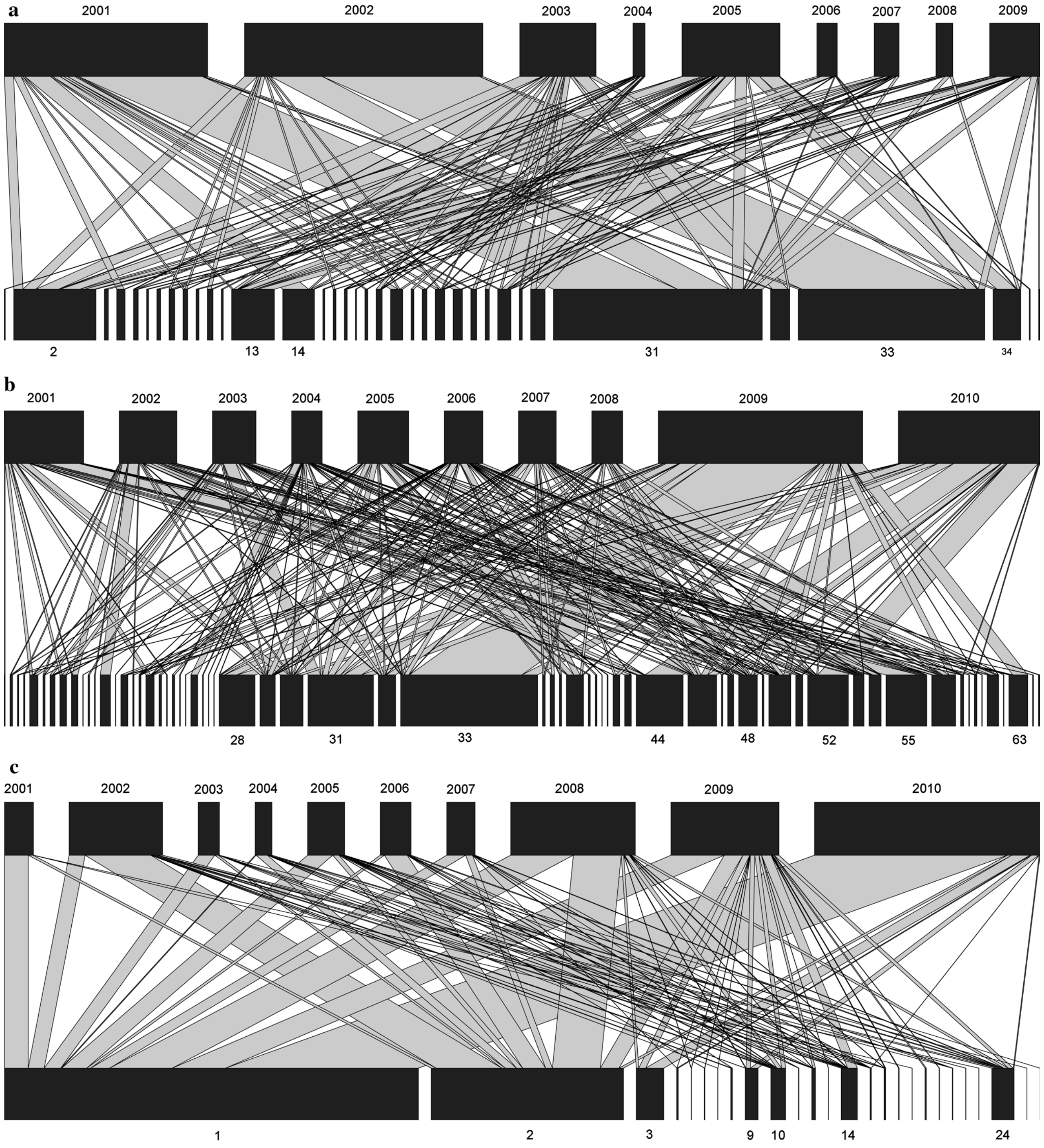

Fig. 2 Bipartite graphs of the pollinator assemblage of *Eucryphia cordifolia* through the 10 years of the study. *Upper bars* represent successive years (2001–2010), *lower bars* represent pollinator species of a given order: Coleoptera (**a**, with no records during 2010), Diptera (**b**), and Hymenoptera (**c**). *Linkage width* indicates the frequency of visits to *Eucryphia cordifolia* by a given pollinator species during a given year. *Widths of the upper and lower bars* indicate the frequency of visits of the whole group (order) of pollinators during a given year and the frequency of visits of a given pollinator species during the entire period of the study, respectively

the absolute abundance of *Apis* honeybees (MANOVA absolute abundance F model $= 6.84$, $r^2 = 0.29$, $P < 0.01$; MANOVA relative abundance F model $= 0.90$, $r^2 = 0.07$, $P = 0.49$; Fig. 4b). In contrast, the relative and absolute abundances of *B. dahlbomii* had positive effects on the abundances of other hymenopterans in the sample

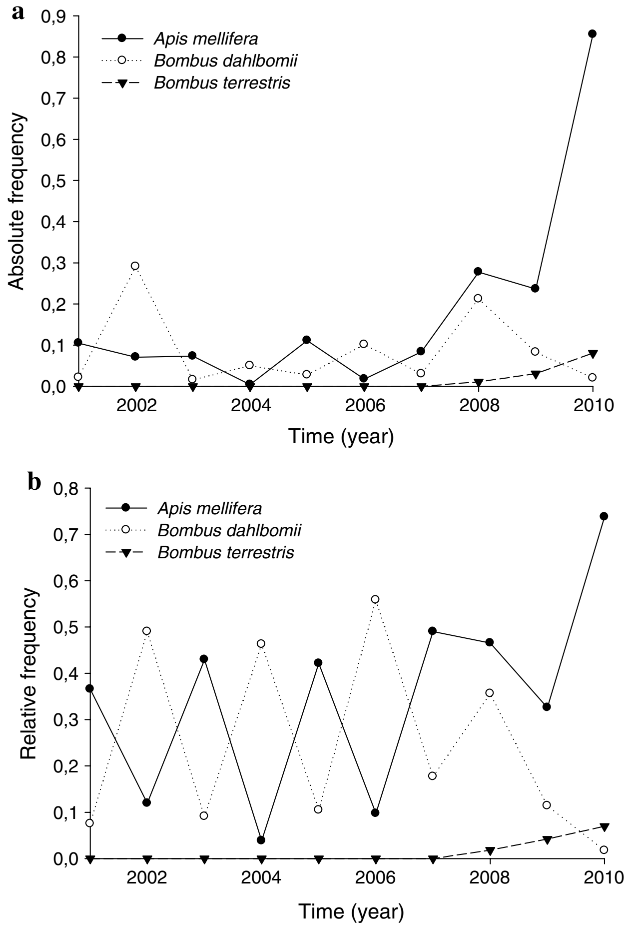

Fig. 3 Time series of flower visit frequency for the two dominant pollinators, *Apis* and *B. dahlbomii*, as well as the invader bumblebee *B. terrestris* over the study period. **a** Absolute frequency and **b** relative frequency of visits are shown (frequency values are the number of visits/minute/flower)

Table 2 Summary of nestedness analysis for adjacency matrices that include the full pollinator assemblage ("Full"), only coleopterans ("C"), only dipterans ("D"), and only hymenopterans ("H")

Matrix	NODF	*z*-score	Sorting variable	Effect	Season
Full	40.2	1.95	A. m. rel	−	Current year
Full	44.79	2.61	$T_{\max}$	+	*E. cordifolia* flowering
Full	41.72	2.18	$T_{\min}$	+	Pollinator emergence
Full	38.24	1.66	PP	−	Pollinator emergence
C	36.23	1.87	$T_{\max}$	+	*E. cordifolia* flowering
C	35.5	1.89	$T_{\min}$	+	Pollinator emergence
D	39.08	1.74	A. m. rel	−	Current year
H	32.85	1.65	B. d. abs	+	Current year
H	34.18	1.88	PP	−	*E. cordifolia* flowering
H	38.94	2.64	PP	−	Pollinator emergence

Sorting variables are absolute ("abs") and relative ("rel") abundances of *Apis* ("A. m.") and *B. dalhbomii* ("B. d."); total annual precipitation ("PP") and annual average maximum ($T_{\max}$) and minimum ($T_{\min}$) temperatures. The sign of the relationship between the sorting variable and nestedness ranking is show in the column labeled "Effect." For example, a (−) effect means that in years with higher values of the sorting variable, species richness tended to be lower and the species composition was a subset of the set of species recorded in years with higher richness (and lower values of the sorting variable)

(MANOVA relative abundance *F* model = 3.11, $r^2 = 0.25$, $P = 0.04$; MANOVA absolute abundance *F* model = 4.47, $r^2 = 0.19$, $P = 0.01$; Fig. 4c, d). During spring and summer, mean maximum temperatures above 17–18 °C had positive effects on the absolute abundances of the less frequent hymenopteran species (MANOVA *F* model = 2.67, $r^2 = 0.12$, $P = 0.03$; Fig. 4e). Furthermore, an average precipitation exceeding 400 mm during the spring and summer months negatively affected the absolute abundances of non-dominant hymenopterans that visited flowers of *E. cordifolia* (MANOVA *F* model = 3.75, $r^2 = 0.16$, $P = 0.02$; Fig. 4f). For the 10 years of the study, during the spring and summer months, precipitation ranged from 200 to 1,000 mm (see Online Resource 1 of the Electronic supplementary material, ESM). We did not find a significant relationship between temperature or rainfall and dipteran abundance in our analysis. The limited non-zero abundance data available precluded this type of analysis for the Coleoptera. Analyses of the entire pollinator assemblage before and after the first occurrence of the invasive *B. terrestris* in the pollinator assemblage (it was present during the last 3 years) did not highlight any changes in tendency from those reported above.

Discussion

The results of this long-term analysis of a plant–pollinator system reveal major and repeated interannual shifts in the composition of the pollinator assemblage of *E. cordifolia* over a decade of study. Only three insect species, the two most frequent hymenopterans plus the syrphid dipteran *Eristalis tenax* (an introduced species), were present during all 10 years of the study (Online Resource 1 of the ESM). The two dominant (most frequent) pollinator species in the assemblage, the native bumblebee *B. dahlbomii* and the introduced honeybee *Apis*, exhibited oscillating dynamics with a period of 2 years, with opposing trends observed in alternate years. Years when the abundance of *B. dahlbomii* was high were followed by years of dominance of the assemblage by *Apis*. This oscillating tendency was maintained for 7 years, before the invasive exotic bumblebee *B. terrestris* became a new member of the pollinator assemblage. After that, the abundance of *B. dahlbomii* decreased and the abundances of *B. terrestris* and *Apis* increased.

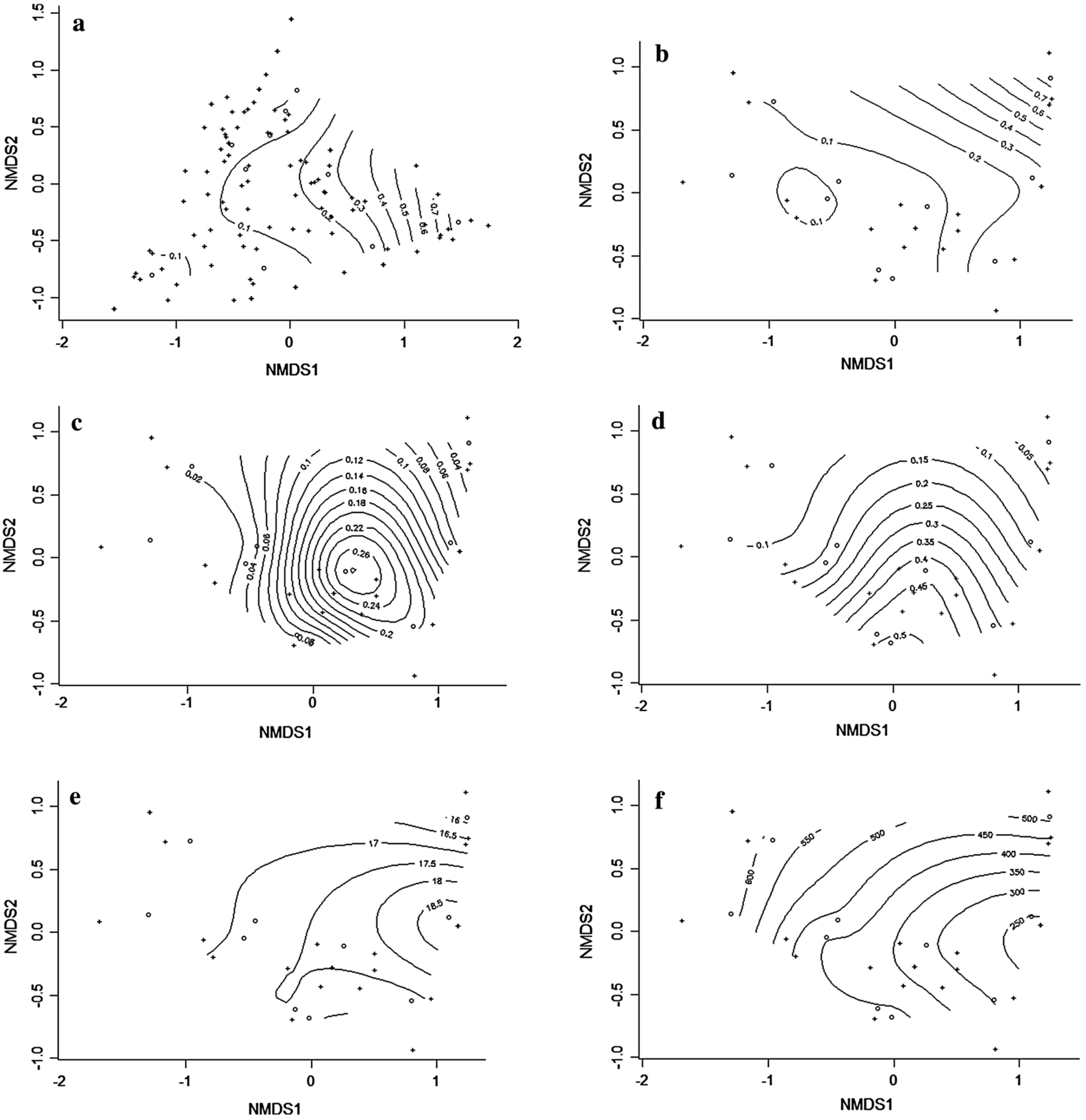

Fig. 4 Non-metric multidimensional scaling (NMDS) joint plots showing pollinator species abundance across 10 years (*circles* years, *crosses* species). Biotic and environmental variables are overlaid as thin plate splines onto the plot region in the ordination. **a** NMDS of non-dominant pollinator species abundance for the absolute abundance of *Apis*. **b–f** NMDS of non-dominant hymenopteran species abundance for the following variables: **b** absolute abundance of *Apis*, **c** absolute abundance of *B. dahlbomii*, **d** relative abundance of *B. dahlbomii*, **e** spring and summer maximum temperatures, and **f** spring and summer precipitation

The results of the two main types of analysis that we conducted in this study supported each other. Multivariate and nestedness analyses suggested that the absolute (and relative) abundance of the honeybee *Apis* would negatively affect the abundances (frequency of flower visits) and species richness of the less frequent species of insect pollinators of *E. cordifolia*. For hymenopteran pollinators, the two analyses together indicated that the abundances and species richness of the non-dominant hymenopterans were positively related to the absolute abundance of the native bumblebee *B. dahlbomii*; only the multivariate analysis suggested an inverse relationship between the absolute abundance of *Apis* and those of the non-dominant hymenopterans. Thus, a higher abundance of *Apis* in the flowers of *E. cordifolia* would negatively affect the abundances of other hymenopterans in the flowers but not their specific composition.

With respect to the influences of abiotic factors, the two analyses jointly indicated that the total amount of precipitation during the flowering periods of *E. cordifolia* and pollinator emergence in spring was inversely related to the absolute abundances and the specific composition of the non-dominant, less frequent hymenopterans. Conversely, higher maximum temperatures during those seasons were positively related only to the absolute abundances of the less frequent hymenopterans, not to their specific composition. In addition, nestedness analysis indicated that during the season of pollinator emergence, the species richness of the whole assemblage was negatively affected by rainfall and positively affected by minimum temperature, while the maximum temperature during the flowering period of *E. cordifolia* positively affected the total species richness of the pollinator assemblage.

Although pollinator diversity per tree stabilized after 60–100 min of field records based on the sampling period, additional pollinator species do continue to appear during the entire flowering period of each tree, although with very small visitation frequencies. Considering our 10-year-long sample, we found an average similarity between years for the pollinator assemblages of *E. cordifolia* of 2.3 %, which is the lowest similarity observed among various interannual studies of pollinator assemblages (Herrera 1988; Olesen et al. 2008; Dupont et al. 2009). These studies also reported a remarkable turnover of pollinator species among years, indicating that strong temporal dynamics may be a general property of insect pollination networks (Wolda 1988). In contrast, in the same study area, the more specialized tubular red flowers of *Embothrium coccineum* J.R. et G. Foster (Proteaceae) maintained exactly the same pollinator assemblage over three consecutive years of study (Smith-Ramírez and Armesto 1998; Smith-Ramírez et al. 2007). We think that the lower pollinator turnover among years of specialized flowers (such as the tubular red flower of *Embothrium*) in comparison to a generalist flower type with a rich pollinator assemblage can be explained by the reduced number of flower visitors of specialized flowers as compared to generalist flowers. Generalist flowers with a rich pollinator assemblage include *E. cordifolia*, *Lavandula latifolia* (Herrera 1988), and many of the plants studied by Olesen et al. (2008) and Dupont et al. (2009).

Concerning the density-dependent inverse dynamics that involved the native *B. dahlbomii* and the introduced *Apis*, we suggest that this pattern may be related to parasitoid transmission between these two hymenopterans (Ruíz-Gonzalez and Brown 2006). Previous studies have reported pathogen transmission from introduced honeybees to native bumblebees (Schmid-Hempel and Loosli 1998; Goka et al. 2001, 2006; Whittington and Winston 2004; Velthius and van Dorn 2006). These pathogens would be transmitted between individuals during flower visits (Durrer and Schmid-Hempel 1994). Inverse oscillatory patterns are typical of predator–prey interactions and of interactions between parasitoids and their host species. Such a pattern has been described, for instance, for two bruchid hosts and the parasitoid *Lariophagus distinguendus* (Bellows and Hassell 1988). It is possible that the arrival of a new invasive bumblebee, *B. terrestris*, could introduce additional diseases into the plant–pollinator system, and particularly to the native *B. dahlbomii*, similar to phenomena described in Argentina by Arbetman et al. (2012) and Schmid-Hempel et al. (2014). Arbetman et al. (2012) found a parasitic protozoa, *Apicystis bombi*, in *B. dahlbomii* after the introduction of a commercial *Bombus* to Argentinian Patagonia. The population collapse of *B. dalhbomii* that took place in Argentinian Patagonia, as reported by Morales (2007), has now been related to this novel parasite. Even though the population size of *B. terrestris* has been comparatively low in relation to that of native *Bombus*, it was nevertheless steady during the last years of this study. Diseases can be transmitted between pollinators quickly, since they are transmitted by flower contact, which multiplies the possibility of transmission. Based on the present study performed in southern Chile and the trend found in Argentinian Patagonia, we believe that populations of the native *B. dahlbomii* in Chiloé Island will show further declines in response to the arrival at this island of the introduced *Bombus*.

We believe that, in addition to the parasitic protozoan *A. bombi*, which is shared by the introduced *Bombus* and *B. dalhbomii* in Argentinian Patagonia, another unknown parasite is shared by *Apis* and *B. dahlbomii*. This might explain the oscillatory population trend observed during the first 7 years of this study. The unicellular parasite *Nosema ceranae* (Microsporidiae), common in *Apis*, has been found in three *Bombus* native to Argentina, but not in *B. dahlbomii* (Plischuk et al. 2009; Schmid-Hempel et al. 2014). However, Schmid-Hempel et al. (2014) found another parasite transmitted by *B. terrestris* and *ruderatus* to *B. dahlbomii*: the protozoan *Crithidia bombi*. Those authors concluded that the decline in the *B. dahlbomii* population was due to the introduction and expansion of introduced *Bombus* species, especially *B. terrestris*. Less plausible is the idea that the oscillatory patterns found between *Bombus* and *Apis* originate in resource competition, which may occur if *E. cordifolia* has fewer flowers or less nectar in one year than in the following year. However, every year we chose to study trees that flowered abundantly in order to avoid this bias, although we did not study nectar production. On the other hand, competition could take place in other floral resources as well as *E. cordifolia* flowers. However, unpublished data from the present authors show the same tendency for oscillating populations of *B. dahlbomii* and *Apis* in three Myrtaceae species studied during three summer seasons (the years 2000, 2001, and 2002) 15 km west of the *E. cordifolia* study area. This tendency agrees with that found in the present study. Myrtaceae trees bloom before *E. cordifolia*, which means that this tendency is not associated with a particular species or month, but happens each blooming season. In addition, Morales (2007) observed the same oscillatory tendency in a study of *B. dahlbomii* visiting *Alstroemeria aurea* flowers in Patagonia across three summer seasons. After the introduction of *B. terrestris*, this population pattern almost disappeared. Another reason for the oscillatory population pattern could be nesting competition between bees. However, this does not occur among introduced and native *Bombus* and *Apis*. On Chiloé Island, *B. dahlbomii* nests in Sphagnum, rotten trunks and branches, and holes in the soil—it does not build nests, in contrast with *Apis* and *B. terrestris*, which do. Finally, based on these observations, we believe that the pollinator patterns found do not apply exclusively to *E. cordifolia*, or to plants that flower during February or to the north of Chiloé Island. We think that this is the same tendency found in neighboring areas of Argentina, which is related to the spillover of diseases transmitted by introduced bees to the natives.

In the temperate forest studied, more diverse and abundant pollinator assemblages were observed during years with higher summer temperatures and lower precipitation, in accord with other studies of the influence of local climate on pollinator diversity. Roubik (2001), González et al. (2009), and others have documented the same trend across a climate gradient from wet to dry conditions in the Andes. In this context, pollinator assemblages from South American temperate forests tend to be more complex and diverse in warmer and drier periods and probably sites. This pattern of co-occurrence matches the well-known spatial pattern of species richness of Hymenoptera and Coleoptera, which increases towards central Chile, north of the study area. In this zone, the Mediterranean-climate influence is stronger, with drier and warmer summers (Michener 1979; Peña 1996). In contrast, dipterans are more frequent in humid than in dry habitats (Kearns 1992), and for this reason their visits to *E. cordifolia* flowers were more frequent in years with wet springs and summers.

Patterns of richness and abundance for non-dominant pollinators in the assemblage during years of high abundance of introduced honeybees are in agreement with earlier results of Sugden et al. (1996), Butz (1997), Kearns et al. (1998), Dupont et al. (2004), and others. Those authors showed that honeybees have negative impacts on native pollinator abundances. We think that competition between *Apis* and other non-dominant pollinators was primarily for nectar and pollen resources, as has been reported by Dupont et al. (2004) in *Echium wildpretti* H. Pearson ex Hook.f. in Tenerife, Spain. Regarding the declining abundance of the native *B. dahlbomii*, presumably in response to the recent arrival of the exotic *B. terrestris*, we predict that the abundances of *Apis* and *B. terrestris* in the pollinator assemblage of *E. cordifolia* will increase, leading to further declines in the abundances and richness of non-dominant native pollinators.

In conclusion, we present evidence for strong interannual variability of the species-rich pollinator assemblage of the generalist canopy tree *E. cordifolia*, driven mainly by changes in the non-dominant flower visitors. In this tree, the two dominant pollinator species—particularly the introduced honeybee *Apis*—were good indicators of the species richness of non-dominant species and of the overall abundance of pollinators, especially native hymenopterans. Furthermore, warmer and drier conditions during the period of insect emergence (spring and summer) were good indicators of higher pollinator species diversity and abundance. Although temperature and precipitation during the flowering period of *E. cordifolia* influenced the diversity of pollinators, it did not enhance the frequency of visits to flowers.

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