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Erosion of a pollination mutualism along an environmental gradient in a south Andean treelet, *Embothrium coccineum* (Proteaceae)

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The limit of a species' distribution can be determined biotically if an environmental gradient causes the loss of critical mutualists such as pollinators. We assessed this hypothesis for *Embothrium coccineum*, a self-incompatible red-flowered treelet growing along a strong west-east precipitation gradient from rainforest to forest-steppe ecotone in the rain shadow of the southern Andes in northwestern Patagonia. For 16 populations along this gradient, we quantified composition of the pollinator assemblage, pollination efficiency and limitation, and reproductive output. The treelet has a generalized pollination system, but the hummingbird *Sephanoides sephaniodes* was the most effective pollinator. The relative importance of this hummingbird as a flower visitor within populations influenced pollen transfer and fruit set more strongly than local precipitation. As hummingbirds and other pollinators, including passerine birds and nemestrinid flies, were replaced by bees towards the dry eastern range limit, pollen limitation increased and reproduction eventually failed. These results support the hypothesis that pollinators can act as important biotic filters influencing plant distribution, and warn against predictions of geographical range shifts based solely on climatic variables.

A species' range limit can be determined by both abiotic and biotic factors, such as climate and interspecific interactions, respectively, which can influence different stages of the life cycle (MacArthur 1972, Gaston 2009). For plants, decreasing rainfall is often assumed to be the main factor limiting the distribution of species adapted to mesic conditions, because water stress can decrease seed production, impair seedling establishment and increase adult mortality (Primack 1987, Suarez et al. 2004, Engelbrecht et al. 2005). However, changes in plant–animal interactions (e.g. herbivory, pollination, frugivory) can also affect plant performance along environmental gradients and shape the geographical ranges of species (Weiher and Keddy 1999, Ackerly 2003, Baraza et al. 2007, Lambers et al. 2008). In particular, the loss of efficient pollinators can disrupt pollen transfer and greatly reduce or completely eliminate seed production, contributing to a demographic collapse before or along with the impact of any abiotic factor (Ashman 2004, Sargent and Ackerly 2008). Hence, reduced reproductive output in a stressful environment could be caused by pollen rather than resource limitation (Burd 1994), resulting from an erosion of the pollination mutualism.

The possibility of erosion of a pollination mutualism is consistent with the well-supported idea that even for generalist plants, not all flower visitors are equally efficient as pollinators. For example, different pollinator taxa vary in their abundance and intrinsic ability to remove and deposit pollen (which can influence pollination quantity), and in their flight capabilities and foraging behavior (which can affect pollination quality) (Waser et al. 1996, Schemske and Horvitz 1984, Herrera 1987, Sánchez-Lafuente et al. 1999, Aizen and Harder 2007). Within the geographical distribution of a plant species, the likelihood and frequency of interaction with its most efficient pollinator(s) can vary spatially, according to the distributional overlap of the mutualistic partners. Even in areas of overlap, the biological context can induce the pollinator to visit other competing plant species (Morales and Traveset 2009). Thus, superimposed to a hypothetical plant 'reproductive landscape' driven by regional variation in resource availability, there could be another driven by the presence and abundance of one or more effective pollinators. Here we explore the extent to which regional variation in the pollinator assemblage associated with a forest treelet visited by birds and insects relates to changes in pollination efficiency and pollen limitation.

Although both resource and effective pollinator availability can simultaneously limit seed set (Campbell and Halama 1993, Burd 1994), at any given time most plants are more likely to be limited by resources or pollination, but not by both (Ashman et al. 2004, Burd 2008). Thus, across different populations of a given plant species seed output may alternatively be driven by whatever factor becomes more limiting. As a consequence, the existence of a biotic filter determining species distribution can be invoked to the extent that the lack of effective pollinators becomes increasingly important in limiting seed set, and reduced seed production becomes critical to plant demography (Sargent and Ackerly 2008).

The south-Andean Proteaceae, *Embothrium coccineum*, a self-incompatible shrub or small tree endemic to temperate forests of southern South America, is an excellent species for studying the role of biotic pollination in determining range limits. This species is widespread in southern Argentina and Chile, occurring from rainforests with > 4000 mm of precipitation at the west to xeric ecotonal habitats with < 700 mm precipitation at the rain-shadowed eastern slopes of the Patagonian Andes. Furthermore, although this species exhibits characteristics commonly associated with bird pollination (Rovere and Chalcoff 2010), it is actually visited by a diverse assemblage of animals including hummingbirds, passerine birds, and insects (Smith-Ramírez and Armesto 1998, 2003, Smith-Ramírez et al. 2005, Devoto et al. 2006, Rovere and Chalcoff 2010).

In this paper, we used a correlative approach to identify the most effective pollinators of the south-Andean endemic treelet *Embothrium coccineum* and to assess how the spatial distribution of these pollinators along the precipitation gradient from rainforest to Patagonian steppe affects pollination and reproductive output. We specifically evaluated whether 1) the pollinator assemblage of *E. coccineum* varies geographically, 2) pollination efficiency changes with assemblage composition, 3) pollen limitation increases with decreasing rainfall (i.e. towards the eastern range limit of *E. coccineum*), and 4) observed changes in reproductive success along the gradient can be better explained by the loss of the most effective pollinator/s (i.e. a biotic filter hypothesis) than by water stress (i.e. an abiotic filter hypothesis). Although we acknowledge that abiotic factors, particularly water availability, contribute to the demographic performance of this species at its eastern boundary, we propose that reproductive failure caused by the mutualism erosion ultimately determines the distribution boundary of *E. coccineum*.

Material and methods

Study species and sites

Embothrum coccineum is widespread in southern Argentina and Chile, occurring from rainforests with > 4000 mm of precipitation at the west to xeric ecotonal habitats with < 700 mm precipitation at the rain-shadowed eastern slopes of the Patagonian Andes. It inhabits most of the temperate forest of southern Argentina and Chile (Sleumer 1984, Donoso Zegers 1993), a biome that extends as a narrow strip (100–200 km wide), from 35°S to 55°S and from the Pacific Ocean to the eastern slopes of the Patagonian Andes. This biome, dominated by several evergreen or deciduous *Nothofagus* species, is bounded on the east by the arid Patagonian steppe. *Embothrium coccineum* is ecologically important as an early colonizer, regenerating vigorously in degraded and fragmented areas (Rovere and Chalcoff 2010).

Plants flower from October to January in the northern portion of the range (Rovere and Chalcoff 2010). The protandrous, predominantly outcrossing flowers are red, tubular, with four tepals fused with the anthers, secreting abundant nectar that varies in sugar concentration (9–46% sucrose-equivalent units) and composition (69–99% of sucrose as the dominant sugar) among populations (Rovere and Chalcoff 2010). As with other Proteaceae, the anthers dehisce before anthesis and the pollen is deposited onto a swollen part of the style, the 'pollen presenter', from which the pollen is transferred to the pollinators' bodies. After the pollen is deposited on the presenter, the tepals open and curl downwards, exposing the style and stigma. The stigma becomes receptive after pollen removal, thus dichogamy prevents the saturation of the stigma with self pollen. Total flower lifespan varies between seven and 10 days. Because of its attractive red flowers and profuse flowering, *E. coccineum* is widely cultivated as a garden plant ('Chilean firebush') (Rovere and Chalcoff 2010).

Although flowers exhibit characteristics commonly associated with bird pollination, it is visited by a diverse assemblage of floral visitors (from eight to 32 species), including hummingbirds, passerine birds, nemestrinid flies and several species of bees (Smith-Ramírez and Armesto 1998, 2003, Smith-Ramírez et al. 2005, Devoto et al. 2006, Rovere and Chalcoff 2010). Some geographical patterning of the pollinator assemblage is evident from previous studies (Devoto et al. 2006, Smith-Ramírez and Armesto 1998, 2003, Smith-Ramírez et al. 2005, Rovere and Chalcoff 2010). Whereas the passerine *Elaenia albiceps* is the most frequent and important pollinator of *Embothrium* in some localities in Chile (Smith-Ramírez and Armesto 1998), the hummingbird *Sephanoides sephaniodes* and some insects, such as the dipterans *Trichophthalma* sp., commonly visit flowers in Argentinean populations. No evidence of pollination by *E. albiceps* or any other passerine exists for populations of *E. coccineum* on the eastern side of the Andes (Fraga et al. 1997, Devoto et al. 2006, Rovere and Chalcoff 2010).

Geographic variation in flower visitors

During the 2005–2006 flowering season, we surveyed 16 populations of *E. coccineum* on both sides of the Andes in the northern part of its distribution (Supplementary material Appendix A1 Table A1, Fig. 1A). Five populations were located on the western side of the Andes (i.e. Chile), and 11 on the eastern side (i.e. Argentina). In each population, we recorded flower visitors to determine the composition, relative abundance and visitation frequency of the pollinator assemblage. In each population we conducted six 30-min observations per sampling day, three for birds, in which the observer remained 3–5 m from focal plants so as not to disturb these visitors, and three for insects, in which the observer stood close enough to focal plants to identify these smaller visitors and capture them as needed for identification. Insect and bird observations were conducted alternately between 8:00 and 18:00 p.m. At the beginning of an observation, we chose a flowering focal individual of *E. coccineum* randomly, without replacement during a sampling day. We first counted the flowers on the whole shrub or branch observed, and then recorded the number of flowers visited by each visitor that contacted the stigma/pollen presenter. From these data and for each pollinator group, we computed visitation frequency (i.e. no. of visits flower^{-1} 30 min^{-1}). Visitors were surveyed at the beginning, middle and end of the flowering season in each population, resulting in 288 observation periods totalling 144 h (16 populations × 18 periods per population × 0.5 h per period). Because of logistic constraints, different populations were sampled on different days.

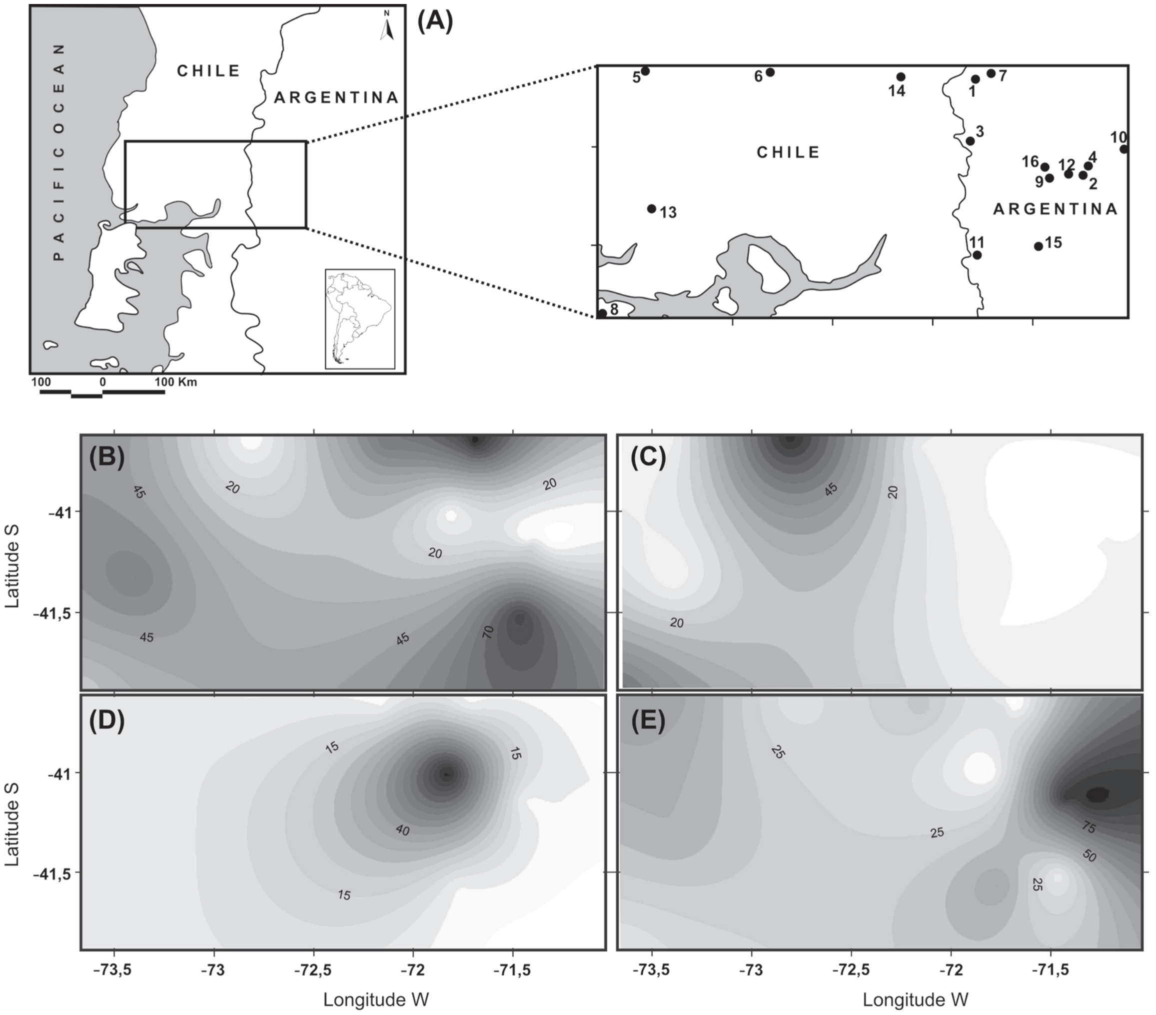

Figure 1. Geographic location of study sites (A: see Supplementary material Appendix A1 Table A1 for names), and isolines maps for the percentage of all visits to 16 populations of *Embothrium coccineum* flowers made by: (B) Apodiformes, (C) Passeriformes, (D) Diptera and (E) Hymenoptera.

Insect visitors were identified to the lowest taxonomic level possible with the aid of a reference collection (Laboratorio Ecotono, INIBIOMA, CONICET-UN Comahue), previous studies (Smith-Ramírez and Armesto 2002, Smith-Ramírez et al. 2005, Devoto et al. 2006, Aizen et al. 2008), and identification keys (Edwards 1930, Shannon and Aubertin 1933, Moure 1964, Goulet and Huber 1993). However, because of the diverse animals that visit *E. coccineum* flowers and known major differences in pollination efficiency associated with higher taxonomic levels, we characterized variation in the pollinator assemblage at the taxonomic rank of order rather than species (Feinsinger 1983).

Pollination efficiency

We estimated pollen transfer for all 16 populations during peak flowering, based on 10 randomly selected plants. From each plant, we collected 10 pollen presenters (i.e. pistils) from unvisited flowers at the beginning of the male phase (i.e. recently opened flowers with abundant fresh orange pollen on an undisturbed presenter) and 20 pistils from flowers that had been open for several days and had started wilting. These structures were placed immediately in individual plastic microcentrifuge tubes with FAA (formalin + alcohol + acetic acid, 1:1:8).

In the laboratory, we estimated pollen production from the samples of fresh pollen presenters. After increasing the volume of FAA in each microcentrifuge tube to 0.5 ml, we vortexed them for 60 s, and then counted pollen grains in four subsamples of 10 µl using a hemocytometer (Kearns and Inouye 1993). We used pistils from wilting flowers to quantify pollen deposition and the effective number of microgametophytes. We cleared the pistils in 10 mol l^{-1} NaOH, stained them with 0.1% aniline blue in 0.1 mol l^{-1} K_3PO_4 (Martin 1959), squashed and then examined them using an epifluorescence microscope. In each style, we counted the ungerminated pollen grains on the stigma and the pollen tubes in the style right below the stigma. Total pollen deposition per flower was estimated by combining these two counts.

Reproductive success and pollen limitation

In *E. coccineum,* total seed output varies primarily with fruit set (i.e. no. of fruits/ no. of flowers) rather than with number of seeds per fruit (Aizen and Feinsinger 2003), so we estimated fruit set as a measure of reproductive success. Because flowers last 7–10 d, we estimated total flower production in 3–4 individuals per population by summing flower counts conducted every 15 days during the flowering season, including senescent flowers to account for flowers that opened between sampling days. Total fruit production was counted for the same individuals at the end of the fruiting season based on the lignified fruits that are retained on branches for months after the release of the wind-dispersed seeds.

We assessed the extent of pollen limitation along the W–E precipitation gradient by supplementing the pollen on stigmas of plants from seven populations (Aeropuerto, Circunvalación, Gutierrez, Manso, Paraguay, Puyehue and Suiza; Supplementary material Appendix A1 Table A1). In each population, we marked 20 receptive flowers in each of 3–4 individuals per population during peak flowering. For each individual, we randomly selected 10 of these flowers at the peak of stigma receptivity and supplemented their stigmas to saturation with a mixture of fresh cross pollen from 3–5 plants. The remaining 10 flowers were left as open-pollinated controls. Fruit set was quantified for supplemented and naturally pollinated flowers at the end of the fruiting season.

Statistical analyses

We constructed isoline maps to illustrate spatial (geographical) variation in the pollinator assemblage across the study area. We generated a continuous surface of relative visitation frequency for each of the four main taxonomic orders (i.e. Apodiformes, Passeriformes, Hymenoptera and Diptera) based on a geostatistical technique of interpolation using the kriging method (Matheron 1963). We did not include the orders Lepidoptera and Coleoptera in this analysis because these groups were extremely rare; in both cases we observed a single species of each of these groups in a single population. Estimates of visitation frequency and geographical coordinates (i.e. latitude and longitude) for each of the 16 study populations were used as input data, yielding contour lines that indicate the expected relevance of each taxon as an *Embothrium* flower visitor in each point of the study area.

Using a regression approach including data from all populations, we estimated the effectiveness of each of the four main orders of flower visitors (i.e. Apodiformes, Passeriformes, Hymenoptera and Diptera) by relating total pollen deposition to visitation rate. We first estimated the relative quantitative importance of each order of visitors in a given plant population based on the proportion of all observed flower visits that it contributed during the flowering season. Using hierarchical regression (Gelman and Hill 2007), with trees nested within populations, we related mean pollen receipt per flower to the relative proportion of visits by each the four orders, with mean total pollen production per flower as a covariate. We considered relative rather than absolute visitation frequency because all the pollen produced is removed from the flowers at the end of the male phase, and thus efficiency of a given pollinator in depositing pollen and eventually eliciting fruit set would depend on the presence and number of visits of other flower visitors. We then used the partial regression coefficient of pollen receipt on relative visitation frequency as an estimate of pollinator effectiveness. For each of the four main orders of flower visitors, this coefficient estimates the effect of a unit increase in relative visitation frequency on pollen receipt.

We evaluated whether reproductive success decreased along the gradient of decreasing precipitation and whether any trend was associated with increasing pollen limitation. Taking into account the hierarchical structure of the data (i.e. trees nested within populations), we conducted, using mixed-effects models, a sequence of analyses in which we first regressed (arcsine root-square transformed) fruit set and mean pollen receipt of the 16 populations against precipitation, then compared fruit set from pollen-supplemented (FS_{sup}) versus open-pollinated (FS_{nat}) flowers, and finally assessed changes in the extent of pollen limitation along the precipitation gradient. The extent of pollen limitation was evaluated as ln (FS_{sup}/ FS_{nat}) (Ashman et al. 2004).

To evaluate whether the biotic-filter hypothesis ‘explained’ the data adequately, we tested a confirmatory causal scheme model with a generalized mixed hierarchical structure following Shipley (2009). We chose this analysis because although the classical SEM (structural equation modeling) also allows testing of direct and indirect effects, it assumes linear responses and normally-distributed residuals while not considering the hierarchical structure of the data (e.g. individual plants within populations). The specific model we tested proposes the loss of the most efficient pollinator(s) as the main factor causing changes in plant reproductive success along the precipitation gradient (Fig. 2). We hypothesized that female reproductive success (i.e. fruit set) is mainly determined by the (relative) visitation frequency of one or more effective pollinators via pollen receipt. Pollen receipt could also be (positively) affected by pollen production which can in turn be (positively) related to increasing productivity associated with higher rainfall. Rainfall, however, also might negatively affect pollination by dislodging pollen from stigmas (Aizen 2003). Finally, we expected an association of efficient pollinators with rainfall because of their requirement of forest habitat. Since we demonstrated that the relationship of fruit set to precipitation could be explained by the association of fruit set with pollen receipt, we excluded this causal effect in our model. Following Shipley (2009) we generated all possible independence

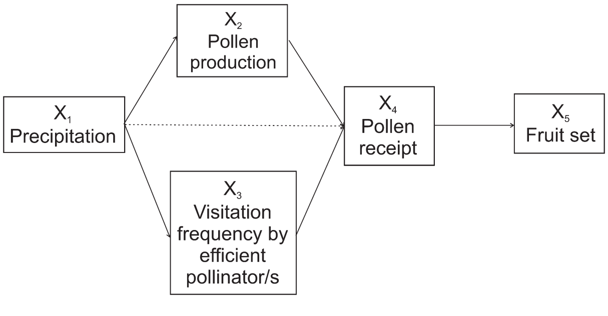

Figure 2. Causal scheme for the effects of precipitation and the frequency of more efficient pollinator visits on pollen deposited and its direct effect in fruits production in *Embothrium coccineum*. Solid arrows indicate expected positive effects and dashed arrows indicate expected negative effects of the independent variable on the dependent variable.

claims (i.e. directional separations or d-sep's) based on the path diagram depicted in Fig. 2 (Supplementary material Appendix A1 Table A2). A d-sep consists of a pair of variables that, according to a given path model, are expected to be statistically independent after accounting for the effect of other variables (Shipley 2009). Considering the hierarchical structure of the data (i.e. 5–10 individuals within each of the 16 populations), each d-sep was tested separately using a general mixed model, then combining the null probabilities (p_i) for each conditional path according to:

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

Model fit was evaluated by comparing C to a χ^2-square distribution with $2k$ degrees of freedom (where k = number of d-sep) (Shipley 2009). All variables were standarized as (x-mean)/SD to allow comparisons of the effect sizes of the different paths.

All statistical analyses were conducted with R ver. 2.11.1 (R Development Core Team 2010). Specifically, we used the lme and lmer functions of nlme and lme4 packages to fit the hierarchical models involved in the analysis of pollinator effectiveness and confirmatory path analysis (Shipley 2009).

Results

Geographic variation in flower visitors

We recorded a variety of visitors to the flowers of *Embothrium coccineum*, including two orders of birds, Passeriformes (four species) and Apodiformes (one species), and four orders of insects, Hymenoptera (10 species), Diptera (five species), Lepidoptera (one species) and Coleoptera (one species). Hymenoptera and Apodiformes accounted for the largest proportions of visitors (48.5% and 29.2%, respectively; Supplementary material Appendix A1 Table A3). The families observed most frequently were Trochilidae, Colletidae, Halictidae and Nemestrinidae (Supplementary material Appendix A1, Table A3), and the most frequent species were the hummingbird *Sephanoides sephaniodes*, the bees *Diphaglossa gayi* and *Corynura* sp., and the fly *Trichophthalma philippii*. The hummingbird was the most ubiquitous single flower visitor being recorded in 10 of the 16 populations (Supplementary material Appendix A1 Table A3). Pollinator species richness varied greatly among populations. We recorded the highest number at Estación (nine species) and the smallest numbers at Espejo, Manso and Steffen (two species per population; Supplementary material Appendix A1 Table A3). At the taxonomic rank of order, populations with the richest assemblages were Cordillera, Estación, and Puyehue with four different orders, whereas populations with the poorest assemblages were Aeropuerto, Circunvalación, Cerro Otto and Suiza with one order (Hymenoptera only; Supplementary material Appendix A1 Table A3, Fig 3).

Both birds and insects interacted frequently with *E. coccineum* flowers. The most interactive orders were Apodiformes, Hymenoptera and Diptera (44.9%, 26.03% and 23.02% of all visits respectively, Supplementary material Appendix A1 Table A3, Fig. 3). Families that frequently visited flowers were Trochilidae, Nemestrinidae, Apidae and Colletidae, and the most interactive species were the hummingbird *Sephanoides sephaniodes*, the fly *Trichophthalma philippii,* and the bees *Apis mellifera* and *Diphaglossa gayi* (Supplementary material Appendix A1 Table A3).

Flower visitation by the main animal groups was patterned geographically. Hummingbird visitation was highest in rainforest populations east and west of the Andes, except for one Chilean population (Entrelagos), then decreased in importance and even disappeared in ecotonal dry habitats to the east, where only hymenopterans visited flowers. Dipterans (i.e. *T. philippii*) were particularly frequent visitors in one wet-forest population, Blest, whereas Passeriformes were particularly frequent in humid southwestern forests in Chile (Fig. 1).

Pollination efficiency

Pollen receipt varied greatly among 16 populations. On average, stigmas of *E. coccineum* received (mean ± SD) 25.4 ± 35.9 pollen grains ranging from 4.8 ± 10.0 (Juncos) to 43.8 ± 39.8 (Aduana). Pollen production per flower was also highly variable among populations, averaging 54709 ± 43427 grains and ranging from 26141 ± 22097 to 108862 ± 49647 grains.

The relations of pollen receipt to the relative abundance of different pollinator groups indicate considerable dissimilarity in their effectiveness as pollinators. Regression analysis revealed that pollen receipt increased most strongly with visitation by *Sephanoides sephaniodes* (Fig. 4), suggesting that it is the most effective pollinator of *E. coccineum*. Several aspects of this relation support this conclusion. First, populations from which *S. sephaniodes* was absent had the lowest mean pollen receipt (i.e. the intercept for *S. sephaniodes* is smaller than that for other groups). Second, mean pollen receipt was highest when *S. sephaniodes* was very abundant.

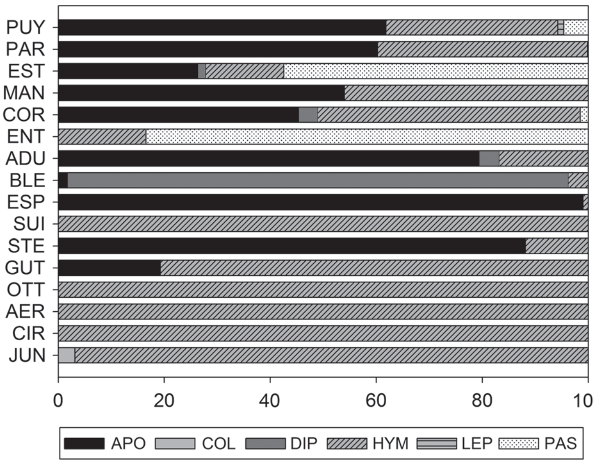

Figure 3. Relative frequency of visits by floral visitors (x-axis) to the 16 study populations of *E. coccineum.* APO = Apodiformes, COL = Coleoptera, DIP = Diptera, HYM = Hymenoptera, LEP = Lepidoptera and PAS = Passeriformes. Populations are indicating by acronyms (full names in Appendix A1 Table A1) and are ordered according to their average annual precipitation from the wettest, top, to the driest, bottom.

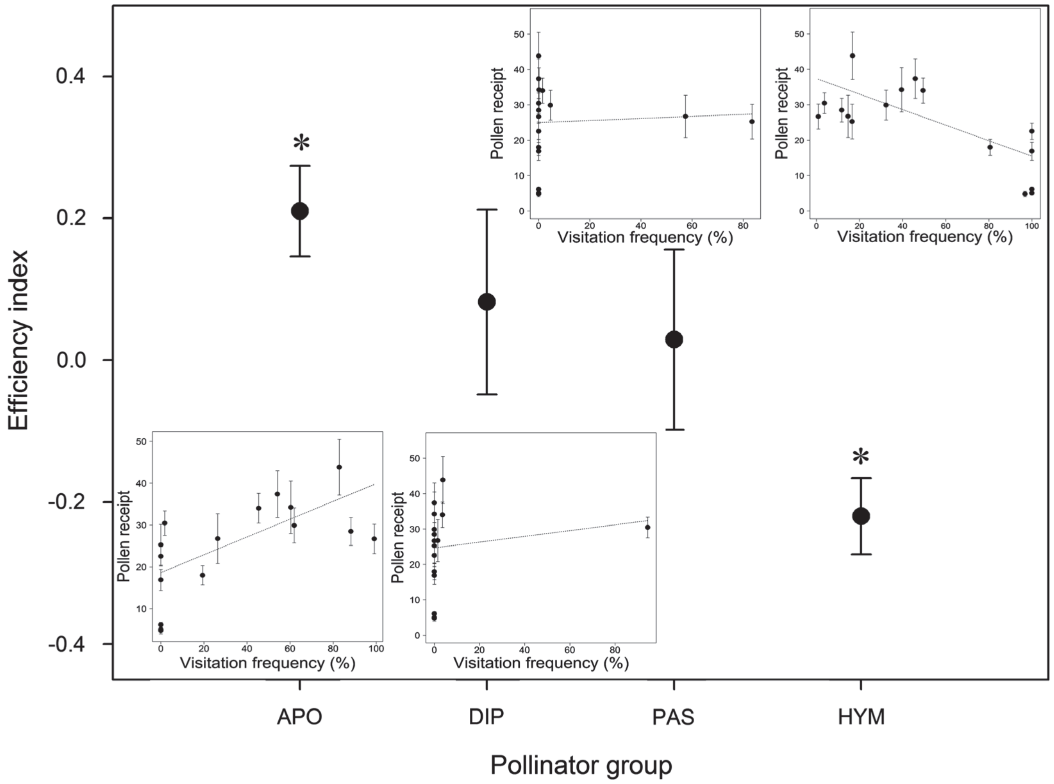

Figure 4. Relations of pollen receipt in 16 populations to the proportion of visits to flowers by Apodiformes (APO), Diptera (DIP), Passeriformes (PAS), and Hymenoptera (HYM). The means (± SE) in the main plot depict the estimated partial regression coefficients for the relations depicted in the inset plots; these coefficients are estimates of pollinator effectiveness (asterisks indicate statistically significant regression coefficients). Each embedded graph depicts the data (± SE) and the fitted partial regression for one taxon.

Third, and as a corollary to the preceding two observations, *S. sephaniodes* had the largest partial regression coefficient, indicating that a similar increase in its relative abundance caused the greatest improvement in pollen receipt. The relations of pollen deposition to the relative abundance of Diptera and Passeriformes were not statistically significant (Fig. 4), perhaps because they were rare components of the visiting faunas in most populations. Nevertheless, the results suggest that these two orders had a limited positive impact on the pollination of *E. coccineum*. Finally, pollen receipt declined with increasing abundance of Hymenoptera and was lowest in populations with bee-dominated assemblages (Fig. 4).

Reproductive success and pollen limitation

Reproductive success varied greatly among the16 populations (Fig. 5). On average (± SD), 6.4 ± 5.8% of flowers did set fruit, with a range among populations from 0% (Juncos) to 17.0 ± 6.42% (Blest). Pollen receipt and fruit set decreased strongly in populations occurring towards the dry end of the precipitation gradient (Fig. 5A–B), whereas there was no evidence that pollen production was related to precipitation ($t = 1.08$, $p = 0.3$). Even though fruit set was significantly related to precipitation, this result could be explained by the association of fruit set with pollen receipt. After including pollen receipt in a multiple regression along with precipitation, this latter variable became non-significant (slope = 0.00002, $p = 0.79$). Overall, pollen-supplemented flowers produced almost three times as many fruits as open-pollinated flowers, on average (mean + SE = 0.25 ± 0.04 vs 0.09 ± 0.02 fruit set (fruits/flowers), respectively; $t = 4.08$, $p = 0.0002$, based on arcsine square-root transformed data). However, the intensity of pollen limitation increased as precipitation decreased (Fig. 5C). Specifically, open-pollinated and pollen-supplemented flowers had similar fruit set in wet-forest populations, whereas in the ecotone populations, open-pollinated flowers produced 90% fewer fruits than supplemented flowers. Populations exhibiting the lowest fruit set and highest pollination limitation also had pollinator assemblages dominated by bees (Fig. 5, solid squares).

Pollination model

Data fitted the pollination model we proposed when considering flower visitation by the most efficient pollinator, the hummingbird ($C = 5.43$, $k = 8$, $p = 0.08$). According to this model, precipitation was significantly related to relative visitation frequency by hummingbirds, which in turn promoted fruit set by elevating pollen receipt. In this model, rainfall had an unexpected direct positive effect on pollen receipt (Fig. 6A) that would imply some form of water-mediated pollen transfer, which we consider most unlikely given the marked intrafloral dichogamy presented by *Embotrhrium coccineum*. However, this effect decreased in importance when considering visitation by dipterans and passerines, in addition to hummingbirds (Fig. 6B; $C = 12.6$, $k = 8$, $p = 0.06$), suggesting that any effect of precipitation on fruit set is indirect and mediated by visitation of several taxa of effective pollinators. Neither of these models, i.e. excluding or including dipterans and passerines, provides evidence that pollen production increased significantly with rainfall or that pollen production influenced pollen receipt.

Discussion

The geographical relationship between fecundity of *E. coccineum* and abundance of its most effective pollinator supports a mutualism version of the biotic filter hypothesis. Strong decreases in reproductive success towards the eastern range limit might relate directly to water stress. However, the reproductive collapse of marginal populations appears to be a consequence of decreasing pollen receipt and increasing pollination limitation associated with the loss of effective pollinators. These results suggest that the disruption of plant-mutualism interactions can contribute significantly to determine range limits, even under an increasingly stressful abiotic environment.

The fauna visiting the flowers of *E. coccineum* is taxonomically and morphologically diverse. We observed a total of 22 species of flower visitors, which is consistent with previous reports of a generalist pollination system (eight

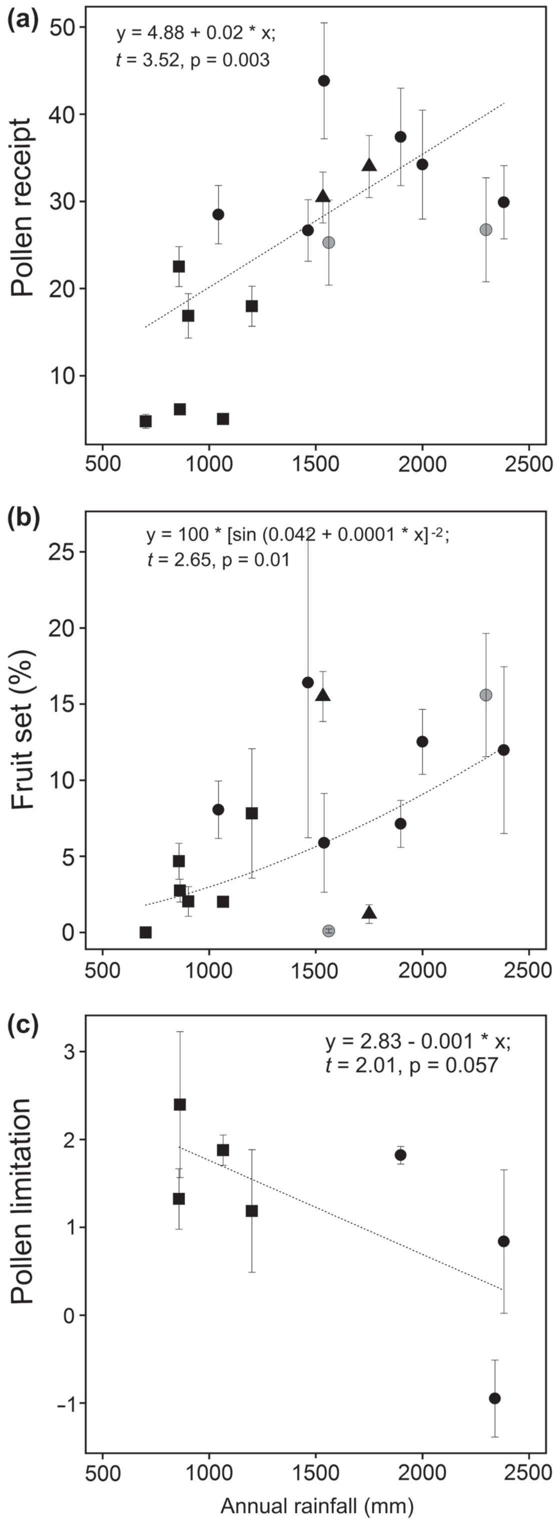

Figure 5. Changes in mean (± SE) (A) pollen receipt, (B) proportion of flowers setting fruit in 16 populations, and (C) pollen limitation (fruit set from pollen-supplemented versus open-pollinated flowers) for seven populations along the precipitation gradient. Symbols identify the principal (>50% of visits) pollinator group in each population: circles = hummingbirds; squares = hymenopterans; gray circles = passerines; and triangles = mixed visitors.

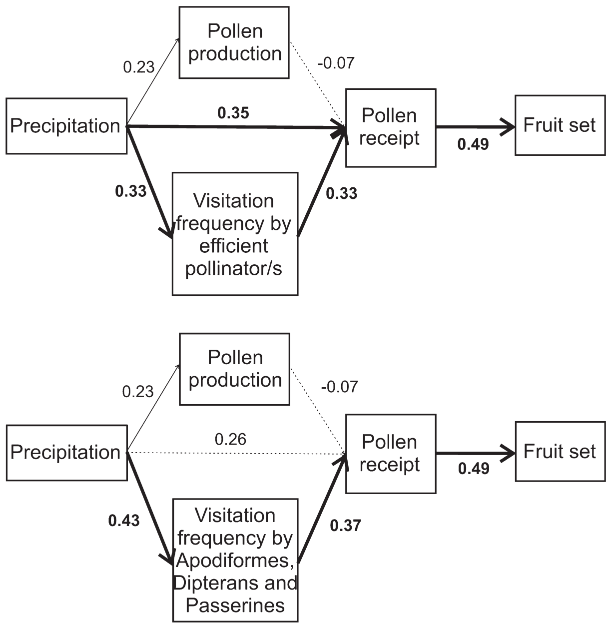

Figure 6. Causal schemes evaluating the indirect effects of precipitation on fruit set via pollination in *Embothrium coccineum* considering (A) the proportion of visits by hummingbirds, and (B) the additional contribution of flies and passerines. Numbers in arrows are the path coefficients, and with bold we indicate which of them have significant effects of the independent variable on the dependent variable.

species, Smith-Ramírez et al. 2005; nine, Smith-Ramírez and Armesto 2003; 32, Devoto et al. 2006; and 11, Aizen et al. 2008). Probably more relevant than species richness is the relatively large number of taxonomic orders represented by the visitors, which shows that flowers of this plant are attractive to morphologically and functionally different groups, from small insects to relatively large passerine birds. Despite the tubular corollas of *Embothrium* flowers, the relatively voluminous nectar produced by the basal nectary is readily accessible to many animals, because, as it is typical of the Proteaceae, the tepals are not completely fused (Sleumer 1984, Heywood et al. 2007). In addition, a variety of insects, including bees and beetles, harvest pollen from their flowers. Thus, the diversity and disparate nature of the assemblage of flower visitors associated with *E. coccineum*, a truly 'cornucopian' species in the temperate forest of South America, could be explained by the production of accessible and plentiful floral resources.

Beyond this overall high diversity, the composition of local visitor faunas varied geographically. These differences in assemblage composition can be in part attributed to spatial changes in species abundances. For instance, decreasing relative visitation of the forest hummingbird *S. sephaniodes* in more xeric populations reflects its drastic decline in density at the forest-steppe transition (G. A. Amico unpublished). On the other hand, changes in visitation of other taxa seem to be context-dependent and independent of changes in their local abundance. For example, the passerine *Elaenia albiceps* is the main frugivore and seed disperser in the temperate forest of South America (Amico and Aizen 2005). This migrant forest bird is abundant on both sides of the Andes and was present at all populations surveyed (V. R. Chalcoff pers. obs.). However, we observed it feeding on *E. coccineum* nectar only in some Chilean populations (Fig. 2, Smith-Ramírez and Armesto 1998). This geographically-patterned dietary shift might be induced by stronger resource competition with a more diverse assemblage of frugivores on the western side of the Andes (Aizen et al. 2002). Similarly, the long-tongued nemestrinid flies (*Tricophtalma* spp.) are also abundant in forest habitats throughout the region, but we recorded them as frequent flower visitors of *E. coccineum* in only one population (Blest). Smith Ramírez and Armesto (1998) did not observe any visits of these flies in populations on Chiloe Island, Chile, although they were important flower visitors and presumed pollinators of *Embothrium* flowers near its northern range limit (Devoto et al. 2006). Similar context-dependence is evident for hymenopterans. Whereas several plant species in the Andean temperate forest are mainly visited and pollinated by bees (Aizen et al. 2002), they dominated the local visitor faunas of *Embothrium* at the forest-steppe transition near its eastern range limit (see also Devoto et al. 2005). In this case, bees could be acting as opportunistic exploiters in the absence of more efficient foragers. However, bees could be more efficient than birds and nemestrinid flies in extracting the more concentrated and viscous nectar found in these more xeric populations (Chalcoff unpubl.). Whatever the factors determining geographical variation in assemblage composition, this spatially-structured variability seems to shape the regional pollination and reproductive landscape of *E. coccineum*.

Pollination efficiency can be affected by total visitation frequency (pollinator quantity) and the differential efficacy of visitors at delivering pollen to the stigmas (pollinator quality) (Vázquez et al 2005). In the specific case of *E. coccineum*, quality seems to be more important than quantity. In all populations surveyed, we found barely any pollen grains remaining on the pollen presenter at the end of the male phase, indicating that visit frequency did not limit pollen removal. Under this scenario, the fraction of pollen production dispersed to receptive stigmas, rather than lost during transport, governs stigmatic pollen loads (Harder and Wilson 1994). In turn, this fraction will be largely influenced by pollinator morphology and behavior (Lebuhn and Holsinger 2010). Consistent with this view, the proportion of all visits made by each of the main four taxa (rather than their absolute visitation frequency) was predictive of pollination success. Thus, *E. coccineum* in forest populations predominantly pollinated by hummingbirds imported the most pollen, whereas those in eastern ecotonal populations served primarily by bees imported the least. Field observations corroborated that *S. sephaniodes* visited flowers legitimately, carried abundant *Embothrium* pollen on the forehead, and touched the pollen presenter and stigma during most visits (Fraga et al. 1997, Smith-Ramírez and Armesto 1998). On the other hand, small and medium-sized bees harvested nectar by entering the flower from the side and rarely touched stigmas. Also, most bees, including alien *Apis mellifera*, collected pollen actively during the male phase, and thus they can be considered mostly pollen thieves or nectar robbers that provide at best a poor pollination service (Hargreaves et al. 2009). In addition, these animal taxa can differentially affect pollination quality given the outcrossing breeding system of *E. coccineum*, because whereas the hummingbird visited a few flowers of each plant and moved frequently between plants, most bees tended to focus their foraging on single shrubs. Finally, it has been shown that in some bird-pollinated Proteaceae bees can be poor pollinators (Paton 2000) and in some cases that they even repel birds (Geerts and Pauw 2010).

Indirect evidence provided by other studies also indicates that flies, particularly long-tongued nemestrinids, and passerines, mainly the tyrannid *E. albiceps*, can pollinate *E. coccineum* (Smith-Ramírez and Armesto 1998, Devoto et al. 2006). Nevertheless, our ranking of pollinator effectiveness suggests that the quality of the service provided by these two taxa is second to the one provided by the hummingbirds, although more information from populations with intermediate visitation from these two groups will be needed to fill the gaps in our data. Agreeing with the results of our confimatory path analysis (Fig. 6), for this generalist plant species nemestrinid flies and passerine birds can be viewed as backup pollinators that can still account for substantial reproductive success in case of absence of the hummingbird but only in humid areas, where they are found. Pollination and resources are two of the most important factors limiting plant sexual reproduction (Campbell and Halama 1993, Burd 1994, Ashman et al. 2004, Aizen and Harder 2007). Decreasing pollination associated with increasing bee dominance may be responsible for the strong decline in fruit set towards the xeric, eastern range boundary of *E. coccineum*. In addition to the correlative evidence summarized in the causal model diagrams, the pollen-supplementation results demonstrate intensified pollen limitation at the easternmost, bee-visited populations. Interestingly, estimates of fruit set and the severity of pollen limitation in a planted urban population of *E. coccineum* visited almost exclusively by honey bees was similar to that in these eastern populations (Aizen and Feinsinger 2003). In contrast, fruit set was higher and pollination limitation lower in two natural populations visited by hummingbirds and nemestrinid flies to the west (Aizen and Feinsinger 2003). Decreases in reproductive success associated with declines in pollination quantity and quality have been also found in other Australian Proteaceae (Vaughton and Ramsey 1991, Vaughton 1992). Thus, our results support the proposed biotic-filter hypothesis; more specifically that disruption of a pollination mutualism can represent a primary factor in determining a reproductive collapse under conditions of increasing environmental stress.

It is of course plausible that environmental stress could still affect different post-dispersal phases, including seed germination, seedling establishment, and plant growth and survivorship (Primack 1987, Suarez et al. 2004, Engelbrecht et al. 2005), all processes that can add to a demographic breakdown and contribute to shape distribution boundaries. However, disruption of a plant–pollinator mutualism can be considered a primary factor shaping range limits when it leads to a complete reproductive failure because this factor alone would avoid plant recruitment (Groom 1998). Indeed, the very existence and temporal persistence of some isolated *E. coccineum* populations at the eastern distribution boundary (i.e. where we found none or almost no seed production) would depend on the relaxation of the pollination filter, perhaps associated with sporadic eastwards expansions of the hummingbird geographical range. It is perhaps in years following those in which there is some seed production that other factors, particularly water stress, may play a role as a cause of seedling and sapling mortality. However, in those self-maintaining frontier populations this abiotic filter cannot be so stringent as to inhibit plant recruitment completely.

As a general implication, our study warns against the unquestioned application of niche-based models that predict changes in species distribution considering abiotic variables only (Valiente Banuet et al. 2006). In an era of rapid global climate change, this has been the frequent goal of an increasing number of studies (Hughes 2000, Parmesan 1996, 2006). In our specific example, a drier climate in Patagonia could predict a longitudinal, westwards shrinkage of the distribution of *E. coccineum* which might be unwarranted given the role that mutualisms can have in setting distribution boundaries. Indeed, the current potential geographical range of *E. coccineum* based solely on climatic factors is expected to extend 50/60 km to the east of its present boundary (R. Vidal-Russell unpubl.). Thus, an understanding of the spatial relations of the focal species and its key interactors, and more generally of the role of different biotic filters, should be required for reliable predictions of future geographical ranges.

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Supplementary material (available online as Appendix o19663 at<www.oikosoffice.lu. se/appendix>). Appendix A1