

The pollination trade-off

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Abstract

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Introduction

Pollination systems play a disproportionally important role in food production and maintenance of global biodiversity. In the context of animal pollination, the support to biodiversity is fundamentally achieved by virtue of intra-species facilitation—the positive feedback loops that exist between plants that share pollinators or pollinators that share plants. Facilitation is able to promote species coexistence because it offsets the effects of direct competition for resources. Indeed, studies that are fundamental to our current knowledge of mutualism predict that an upper limit to biodiversity is achieved when the number of mutualistic partners is maximised in a community. And yet, fully connected natural pollination communities do not exist.

This is so, because sharing a mutualistic partner also has costs. The end result is that, when the cumulative costs have negative impact on fitness, species also start competing for mutualistic partners. For the 88% of flowering plants that rely on animal pollination, there are two mechanisms through which competition for pollinators might arise. The first is competition for visits, where plants with more attractive flowers reduce the pollination service for those less attractive. The second is interspecific pollen transfer, where even receiving a visit might not translate into net facilitation. A focal plant might receive pollen from a different species, or conversely pollen from the focal plant might be lost to different species. Here we focus on this second mechanism.

The potential negative effects of competition for pollinators have been long recognised and widely documented. It is currently clear that they have the potential to drive the evolution of flower phenologies, morphologies and reproductive strategies as well as driving ecological differentiation. Indeed there is considerable evidence demonstrating the effects that interspecific pollen transfer in two-species systems. However, these competitive interactions between species pairs do not occur in isolation. Instead, plants often occur in communities in which multiple competitive interactions operate simultaneously and lead to emergent phenomena not observed at smaller scales.

Nevertheless, only a handful of studies have examined interspecific pollen transfer at the community level, and therefore we still understand little about how competition for pollinators operates at this scale. All together, existing studies show that, as expected, the effective result of mutualistic interactions is, in most cases, one of facilitation. Importantly, they also show a large variability in this result—both across species and across communities. However, the driving factors behind this variability are currently not understood.

Understanding what makes species and communities different is important not only because of its ecological and evolutionary implications. It is also important because it is fundamentally related to the way we understand and mathematically represent mutualistic systems. Some of the most widely used theoretical models of pollination account for the negative effects of mutualism by adjusting the mutualistic benefit of an interaction so that it can be negatively affected by the number of mutualistic partners—the species degree. The extent to which species degree affects the benefit is called *mutualistic trade-off*. This approach, considerably simplifies the mathematical treatment of competition for pollinators. At the same time it is based on the intuitive rationale that a species that interacts with few species benefits strongly from each of them, whereas a species that interacts with a large number of species does so comparatively weakly. Although, the mutualistic trade-off has been shown to play a determining role on the stability and diversity of mutualistic communities, empirical evidence is scarce, to say the least.

Elucidating how the mechanisms and drivers of facilitation scales to the community level and its theoretical consequences has been elusive in part because of the large amounts of data required. Previous studies focus on the final picture of interspecific pollen transfer that is obtained by examining the pollen deposited on flower stigmas. However, teasing apart the competition for pollinators also requires data that describes the structure of the interactions and the pollen flows that occur within it, while accounting for the traits of the species that integrate the community. Here, we examine a comprehensive data set that includes information about pollen deposition, transfer, visitation, species abundance, and species traits across diverse communities in the Argentinean Pampas. Specifically, we estimate the gain on pollen that can be attributed to the mutualistic interactions. Second, we investigate whether there is a relationship between this gain and a suite of variables that describe both the role of the plant species and the ecological context. Here, we pay special attention to the role of species degree because of its ramifications to theoretical ecology. At the same time, we examine whether the plant role and ecological context is best inferred from dynamics occurring at the community level, or whether it can be well approximated using information available from all sites in which a species occurs. Third, and finally, we explore how plant species differ on the extent to which competition for pollinators shapes facilitation.

Methods

Data collection

We performed sampling in three locations, each located in a distinct phytogeographic region of the Argentinean Pampas. The sampling locations are distributed across a gradient of precipitation with a humid temperate climate in the eastward Flooding Pampas and an drier as we move inland and westward towards the Pampean Grasslands. Across all sites, annual mean temperatures range between 14.8 and 15.8 Celsius.

In each location, we sampled two restored and two agricultural fragments. However, due to the lack of available sites in the Flooding Pampas, it was only possible to sample one restored site. We visited each fragment in November 2010 and February 2011. In each of these visits we gathered information related to abundance, plant-pollinator visitation, pollen transfer, and pollen deposition.

Abundance was estimated using two 50 m randomly located transects in each fragment. In a first sampling we counted all units of floral attraction that were found in a 2 m wide strip. In a second sampling we counted and collected all floral visitors while walking at a pace of 10 m per minute (Memmott 1999; Marrero *et al.* 2014). We constructed quantitative visitation networks using information about the floral visitors collected during abundance transects (Marrero *et al.* 2014). In addition, we also constructed qualitative visitation networks during independent two hour observations of floral visits across each fragment. We estimated pollen transfer by examining the pollen loads present on the collected floral visitors (Marrero *et al.* 2017). Where the pollen count on an individual was estimated to be less than 2,000 grains, we identified every grain to the species level whenever possible and to pollen complexes when it was not. When the pollen count was above 2,000 grains, we classified approx. 50% of pollen and total pollen counts were extrapolated (Bosch *et al.* 2009). If more than 10 pollen grains from a plant species were identified, we assumed that pollination service between the plant and the pollinator existed. Finally, we analysed pollen deposition in a subset of the plant community (Marrero *et al.* 2016). This subset comprised between three and nine of the most common entomophilus species that were flowering during the sampling period while ensuring that the chosen species covered a wide range on a specialization-generalization gradient. In the selected plants we removed all flowers except buds that were expected to go into inflorescence on the next day. A quarter of these buds were bagged to prevent animal pollination. Two days after inflorescence, we analysed the pollen grains in the flowers' pistils and classified them between conspecific and heterospecific pollen.

More details about the study sites, data collection, and laboratory protocols can be found in Marrero *et al.* (2014, 2016, and 2017).

87 Data analysis

88 First, we explored whether animal pollination resulted in a quantitative increase in the pollination service. To
89 do that, we compared the amount of conspecific pollen on stigmas from flowers open to animal pollination and
90 that from flowers that were bagged, where only autogamous self-pollination occurred. The difference between
91 these two counts is not directly equivalent to the pollen deposited by animals because self-pollination is an
92 adaptive trait (Kalisz & Vogler 2003). Nevertheless, this difference still provides valuable indication of what
93 the animal contribution to reproductive output. We performed this comparisons using a set of non parametric
94 Mann-Whitney tests both at the community and the the species level (grouping species across communities).

95 Our main aim was to examine the extent to which the pollination service was influenced by the number
96 of pollination partners while accounting for other metrics that describe the plant's pollination niche and
97 community composition. To do so, we fitted four sets of linear mixed models (using the R package nlme
98 3.1-131, Pinheiro *et al.* 2017) which differed on whether the pollen deposited was conspecific or heterospecific
99 and on whether independent variables were calculated across communities or independently within each
100 community. In all set of models, our response variable was the difference between pollen density (pollen
101 counts per stigma) between open and bagged flowers. All together the results from each model set—and the
102 relationship between them—provide with two important pieces of information. First, they provide indication
103 of both the quantity and quality of animal pollination, and the potential trade-off between these two. Second,
104 it allowed us to examine whether indeed community level information is necessary to explain the observed
105 patterns or whether less granular data is sufficient.

106 All model sets were based on the same one hundred bootstrap resamples of the deposition data. We used
107 bootstrap resampling because, as open and bagged flowers were not paired, this allowed us to directly model
108 the *gain* in pollen density rather than density itself. This strategy, not only simplified the model specification
109 and interpretation, but also allowed us to easily evaluate the uncertainty of the model results.

110 In each replicate we randomly sampled with replacement a number of open flowers within each community-
111 species combination and an equal number of closed flowers. We then log-transformed the pollen densities
112 to improve normality and minimise the impact of outliers (Figure S1 and S2). Finally, we calculated the
113 difference between the pollen density among treatments. This difference was the response variable in all our
114 models.

115 As predictors, we included the species/degree (number of animal partners) as well as other factors that
116 could influence the trade-off. Specifically, we included the plants' relative abundance and the temporal
117 niche overlap. To calculate the plant's relative abundance we simply aggregated the flower counts over

the study period—either across communities or for each community depending on the model set. As with the deposition data, we found that log-transforming the abundance counts drastically improved normality (Figure S3). We then used the log-transformed abundance counts for each month between November 2010 and March 2011 to calculate the temporal niche overlap between plants. Specifically, we used the Pianka’s niche overlap index (Pianka 1973; `spaa` 0.2.2 R package Zhang 2016) which calculates an overlap index for each pair of species—again, across and within communities depending on the model set. To calculate an overlap metric per species, we simply averaged the pairwise indices for a species. To facilitate comparison across model estimates, we scaled all independent variables to have a mean of zero and a standard deviation of one.

In all set of models we first evaluated a set of candidate random effects which included random intercepts for plant species as well as random slopes for the relationship between degree and pollen gain. In addition we also tested random intercepts assuming that the plant species grouping was hierarchically nested in either their community, the land use, and the locality. We selected the best random structure by comparing the median Akaike Information Criterion (AIC) of evaluated models fitted using restricted maximum likelihood. After determining the best random structure, we then focused on the model’s fixed structure where we fitted the fixed effect coefficients using maximum likelihood. Because our purpose was to perform inter-model comparisons across multiple data resamples, we did not perform model selection. Instead, in all model sets, we obtained the estimates for all the fixed effects we considered and then evaluated their relevance by examining their distribution.

We compared the performance of the within and across communities model sets using the root-mean-square error normalised by the interquartile range (NRMSE). We tested the significance of this difference using a two sample paired Wilcoxon signed rank test for conspecific and heterospecific pollen models. We chose NRMSE because it provides an easily interpretable metric related to the quality of the predictions while being fairly insensitive to sample size. This insensitivity was desirable because the across communities models had often slightly larger sample sizes owing to the fact of some species not having all data necessary to calculate the independent variables at the community level.

Finally, we focused on how the relationship between conspecific and heterospecific pollen gain. First we examined whether the pollination trade-off was related to our explanatory variables. To do so, we harnessed the results from the conspecific and heterospecific pollen models that performed best (across or within communities). To examine the relationship between these model sets, we sampled 100 estimates from the distribution of coefficients in each set. For each of the samples, we then calculated the difference between the gain in conspecific and heterospecific pollen that would be predicted assuming mean values for the random intercepts and the other predictors.

Second, we calculated the the extent of facilitation. For this purpose, as in Tur *et al.* (2016), we used the relationship between heterospecific and conspecific pollen. We calculated an overall relationship by sampling the predictions from 100 conspecific/heterospecific model pairs and estimated the relationship by fitting an standardised major axis (SMA) to each pair (we used the R package `smatr` 3.4-3, Warton *et al.* 2012). We followed a similar procedure to calculate a relationship per species but instead of fitting a SMA per model pair, we did so across the 100 models for each species independently.

Results

Over all study sites, we found that 41% of species observed a significant increase (at the $\alpha = 0.05$ level) in pollen density when comparing open and bagged flowers (Table S1). When looking at the differences within sites, we found that only 44% of the species that were present in more than one site had the same response across community (Table S2). Despite these differences, the community had a comparatively small effect on whether animal pollination increased the pollen density or not (Table S3).

We found that the random structure that was best supported the data was consistently the one that included plant species nested in community as the grouping factors (Table S4). When comparing the performance of the models constructed at the community or study-wide level we found that the gain in conspecific pollen was better approximated by the model sets using predictors calculated across communities (Table S5). This was so despite the fact that the model predictors calculated at the community level were highly correlated to those at the study wide level (Figure S4). Nevertheless, results from the within community models are similar both qualitatively and quantitatively (with the exception of phenology overlap, but that might change once I use a better metric, Figure S5).

We found that the gain in conspecific pollen is negatively affected by the plants relative abundance and to a smaller extent by the species' degree (Figure 1). The gain in heterospecific pollen was instead positively affected by degree and the plant's phenology overlap, but was also strongly negatively affected by the plant's relative abundance.

The gain in conspecific pollen is positively correlated with the gain in heterospecific pollen. The slope of the relationship is such that it is more likely that the gain on heterospecific pollen increases faster than the gain on conspecific pollen (Figure 2A). In addition, we found that from all the explanatory variables examined, only the plant's relative abundance has a positive effect on the proportion of conspecific pollen gained (Figure 2B). For most of the parameter space plants obtain a larger proportion of heterospecific than conspecific pollen. However, plants can obtain a larger proportion of conspecific pollen when they have high

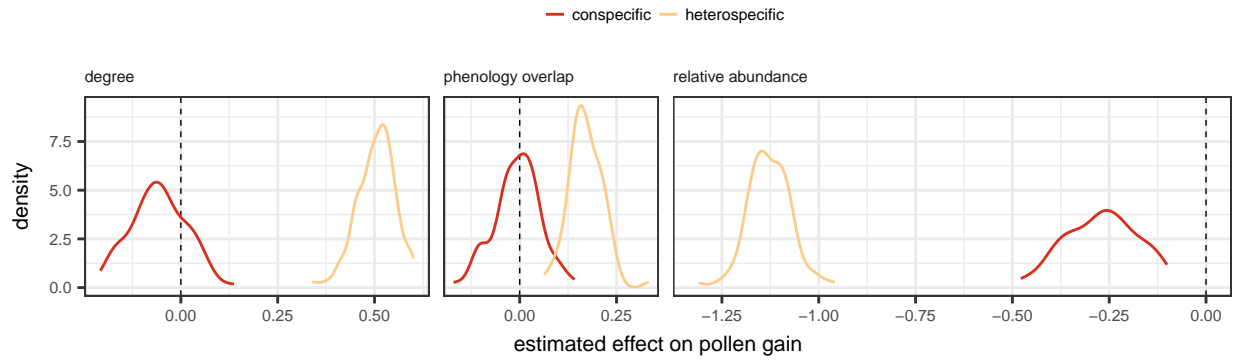


Figure 1: Distribution of effect estimates for models of conspecific and heterospecific pollen density gain. Results are shown for models with explanatory var

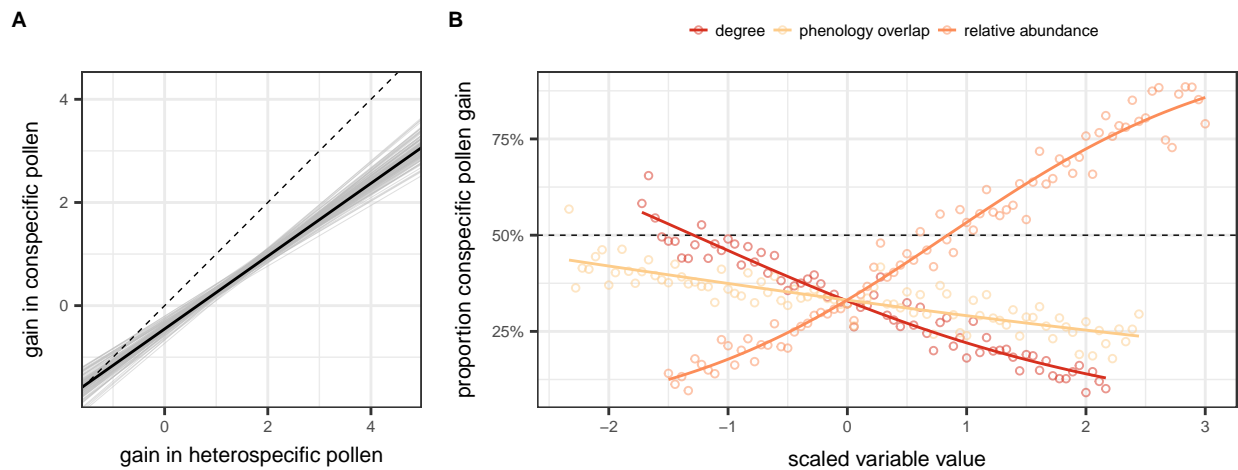


Figure 2: The interplay between the gain on heterospecific and conspecific pollen. (A) Overall and species-level relationship. Grey lines indicate the relationship in each of the samples, the median is indicated with a black solid line. Dotted lines show the relationship for each species. Invasive species are shown in red. (B) The proportion of conspecific pollen relative to the total amount of pollen gained through animal pollination and its relationship to our set of explanatory variables.

180 abundance and to a smaller extent when they have a low degree. When examining the extent of facilitation
181 at the species level, we found that XXX (Figure 2B).

182 Discussion

- 183 • We found that animal pollination only brings a small amount of extra conspecific pollen (compared to
184 selfing) if any. This relatively small difference is probably very important though because crossing has
185 been shown to be evolutionary better than cloning the long term.
- 186 • The relationship between conspecific and heterospecific pollen is positive which indicates that net
187 facilitation is the dominant outcome. This agrees with previous findings. However the amount of added

conspecific pollen is in average smaller than the amount of added heterospecific pollen.

- Despite finding that facilitation is the most common outcome. We also were able to find clear signature of competition for pollinators at the community level.
- We found that, as assumed in pollination models, the mutualistic benefit is modulated by the species degree and therefore there is indeed evidence for a trade-off. Moreover, the trade off does not depend on the species or the community. Which is a good thing because we tend to simplify models by assuming a common trade-off for the whole community.
- However the mutualistic benefit was not just modulated by the degree but other factors also played significant roles.
- These factors, in particular the plant relative abundance, was important at explaining the differences between plants.
- We found that models performed better when explanatory variables were constructed so that they take into account information across all the communities in which a species was present. This means that despite the particularities of each community (random effects accounted for a large proportion of the variance) we can still predict general outcomes by knowing some general traits about the plant species and how it relates to other plants in their community.

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