

The pollination trade-off

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Abstract

TBC

Introduction

Pollination systems play a disproportionately 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.

Second year

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

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

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

57 Methods

58 Data collection

What is a "community" as used later?

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 entomophilous 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.

←marked?

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 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 fit ~~four~~ 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.

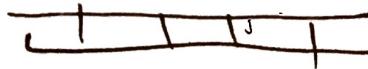
115 As predictors, we included the species/degree (number of animal partners) as well as other factors that
116 could influence the trade-off. Specifically, we included the plants' relative abundance and the temporal
117 niche overlap. To calculate the plant's relative abundance we simply aggregated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

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

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

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

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

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

156 Results

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

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

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

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

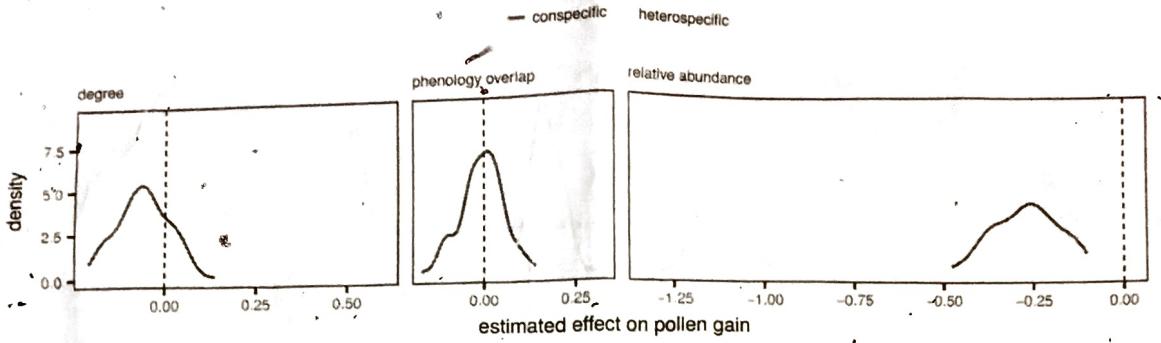


Figure 1: Distribution of effect estimates for models of conspecific and heterospecific pollen density gain. Results are shown for models with explanatory var

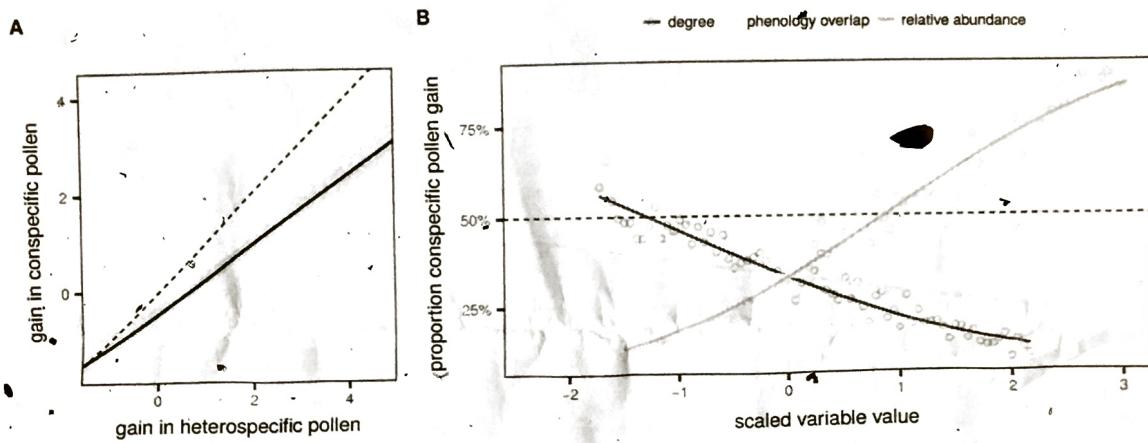


Figure 2: The interplay between the gain on heterospecific and conspecific pollen. (A) Overall and species-level relationship. Grey lines indicate the relationship in each of the samples, the median is indicated with a black solid line. Dotted lines show the relationship for each species. Invasive species 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.

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

Discussion

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

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

204 References

- 205 Bosch, J., Martín González, A.M., Rodrigo, A. & Navarro, D. (2009). Plant-pollinator networks: Adding the
206 pollinator's perspective. *Ecology Letters*, 12, 409–419.
- 207 Kalisz, S. & Vogler, D.W. (2003). Benefits of autonomous selfing under unpredictable pollinator environments.
208 *Ecology*, 84, 2928–2942.
- 209 Marrero, H.J., Medan, D., Zarlavsky, G.E. & Torretta, J.P. (2016). Agricultural land management negatively
210 affects pollination service in Pampean agro-ecosystems. *Agriculture, Ecosystems and Environment*, 218,
211 28–32.
- 212 Marrero, H.J., Torretta, J.P. & Medan, D. (2014). Effect of land use intensification on specialization in plant-
213 floral visitor interaction networks in the Pampas of Argentina. *Agriculture, Ecosystems and Environment*,
214 188, 63–71.
- 215 Marrero, H.J., Torretta, J.P., Vázquez, D.P., Hodara, K. & Medan, D. (2017). Exotic plants promote