The pollination trade-off

Fernando Cagua[1], Hugo Marrero[2], Jason Tylianakis[1], Daniel Stouffer[1]

[1] Centre for Integrative Ecology, School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch 8041, New Zealand

[2] Hugo's address

Author for correspondence: E. Fernando Cagua (efc29@uclive.ac.nz) - Centre for Integrative Ecology, School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand

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To do

- References to the introduction
- Settle on analyses
- Nail down discussion
- Abstract

Questions

- Should I separate the "phenology originality" from the "functional originality"?
- Gain in pollen vs. absolute amount of pollen?

Abstract

TBC

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 facilition—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 cummulative costs have a negative impact on fitness, species also start competing for mutualistic partners. The possible negative effects of competition for pollinators have been long recognised and widely documented. Based mainly on two-species systems, 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 diferentiation. However, these competitive interactions between species pairs do not occur in isolation. Plants often ocurr in communities in which multiple competitive interactions operate simultaneusly and lead to emergent phenomena not observed at smaller scales.

Perhaps the most obvious ecological factor influencing the cost of sharing pollinators is the species degree—the number of interaction partners. Yet, evidence at the community scale of **the mutualistic trade-off**—the extent to which species degree affects the benefit—is scarce, to say the least. Furthermore, although the relationship between degree and the costs of the pollination service is relatively intuitive, elucidating the magnitude of the trade-off at the community scale has been elusive because multiple other factors like traits, abundance, and the share of the pollen pool might be involved. For instance, recent empirical evidence suggest that species with original traits (far from the community centroid) generally have fewer interaction partners. This evidence is aligned with 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. If evolutionary specialisation occur by changing traits to focus on fewer but better partners, we should expect a reduction of competition for pollination in species with original traits. Alternatively, it might also be the case that density is the dominant force driving pollen transfer. Abundant species might experience a dilution of the available pollinators but might also comprise a greater component of the pollen source pool being transported by pollinators. In this case, a potential reduction in the quantity of pollination could be compensated by an increase in the quality of pollination.

Here, we explore the signature of the mutualistic trade-off in diverse natural communities. To tease appart the multiple factors that operate at the community scale we use data that describes both the structure of the interactions and the pollen flows that ocurr within it, all while accounting for the traits of the species that integrate the community. Specifically, we first estimate the gain on pollen that can be attributed to the mutualistic interactions. Second, we investigate wether there is a relationship between this gain (of both conspecific and heterospecific pollen and by extension the quantity and quality of pollination) and the species degree, functional originality, abundance, and contribution to the pollen pool, which together describe the role of the plant species and the ecological context. Third, and finaly, we explore how these variables shape the quantity and quality of pollination and ultimately the competition for pollinators. We hypothezise that XX?? **TODO: Mini conclusion here**

Methods

Elucidating how the mechanisms and drivers of facilitation scales to the community level and its theoretical concequences 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, as we have described, teasing appart competition for pollinators also requires data that describes the structure of the interactions and the pollen flows that ocurr 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.

Data collection

We performed sampling in three locations, each located in a distinct phytogeograohic 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.

Abbundance was estimated using two 50 m randomly located transects in each fragment. In a first sampling we counted all units of floral attraction taht 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 clasified approx. 50% of pollen and total pollen counts were extrapollated (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 analised 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 samplimg 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 inflorecense on the next day. A quarter of these buds were bagged to prevent animal pollination. Two days after inflorecense, 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).

Data analysis

Conspeficic and heterospecific pollen gain

First, we examine wether there is a relationship between the gain of pollen induced by animal pollination and several ecological variables that can affect the mutualistic trade-off. To do so, we used a combination of multi-model inferencewe and bootstrap resampling of the data sets. In summary we fitted two sets of linear mixed models (using the R package `nlme 3.1-131`, Pinheiro *et al.* 2018) which differed on whether the pollen deposited was conspecific or heterospecific. In both set of models, our response variable was the difference between pollen density (pollen counts per stigma) between open and bagged flowers. Each model set was based on the same one hundred bootstrap resamples of the deposition data.

We used bootstrap resampling because, as open and bagged flowers were not paired, this allowed us to directly

model the *gain* in pollen density rather than density itself. This strategy, not only simplified the model specification and intepretation, but most importantly it also allowed us to easily evaluate the uncertainity of the model results. In each data replicate we randomly sampled with replacement a number of open flowers within each community-species combination and an equal number of closed flowers. We then log-transformed the pollen densities to improve normality and minimise the impact of outliers (Figure S1 and S2), this generally offered better results that a generalised linear model with poisson (or quasiposson) error structure. Finally, we calculated the difference between the pollen density among treatmemts. This difference was the response variable in all our models.

As predictors, we included species degree, the plant relative abbundance, the plant species' share on the pollen pool, and the species' functional originality. Species degree was calculated from combining the quantitative and qualitative visitation networks and corresponds to the number of animal species that were observed to visit the plant species in the community where it was sampled. The plant's relative abbundance we simply aggregated the flower counts for each plant species in the community. As with the deposition data, we found that log-transforming the abundance counts drastically improved normality (Figure S3). The species' share of the pollen pool was calculated as the proportion of pollen of that species in the pollen counts being carried by pollinators from the community.

Functional originality is defined as the distance of a species from the community trait average–the centroid of functional space of the community (Laliberté & Legendre 2010; Coux *et al.* 2016). Specifically, we recorded morphological traits that relate to plant type (herb, shrub, climber), life cycle (annual, perenial), flower coloration, and whether the species is native in the study region or not. As phenology has been shown to be a key mechanisms of niche diferentiation among plants, we also included the plant abundance in each of the survey months (November to March) as an additional trait in our analysis. This approach allowed us to integrate the originality in phenological niche alongside the originality on other functional traits. All traits were scaled to have a mean of zero and a standard deviation of one prior to calculating the centroid of the functional space. The species-specific functional coordinates were calculated using the R package `FD 1.0-12` (Laliberté & Legendre 2010; Laliberté *et al.* 2014). To faciliate comparison across model estimates, we scaled all independent variables to have a mean of zero and a standard deviation of one.

In both 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 for random intercepts that assumed that the plant species grouping was hierachically 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. After determining the best random

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structure, we then focused on the model's fixed structure composed by our explanatory variables. We used multi-model inference to calculate the relative importance of each variable and its mean effect on pollen gain. First we fitted all possible combinations of explanatory variables to the same bootstrap resamples used previously. This yielded one hundred models for each "candidate formula"—a particular combination of explanatory variables. We then determined the likelyhood of each candidate formula and its relative wheight by calculating the median ΔAIC of the one hundred models that constitute it. As AIC is only useful to compare models with the same response variable, the ΔAIC of each candidate formula was calculated indepenently for each bootstrap resample. Calculating the median value of each candidate formula allowed us to estimate the relative importance of the explanatory variables using the standard methods of adding the median weight of the candidate models that included the variable.

To calculate the effect that each variable had on the gain of conspecific and heterospecific pollen we sampled from the estimated coefficients in each candidate model proportional to the median likelyhood of each set. In other words, the estimated coefficients from more likely candidate constitute a larger share of the distribution of coefficients. When a variable was not included in a model set we setted the estimate of its coefficient in the model to zero. We expect some of our predictors to be correlated to each other. Estimating the models using multiple combinations of explanatory variables also allowed us to evaluate the potential collinearity between them. To do so we inspect how the effect of the explanatory variables changed across candidate formulaes, when one or more of them were removed in turns.

Quantity and quality of pollination

At the community scale, sharing mutualistic partners might induce competition for pollination mainly trough two mechanisms. The first is competition for visits, where plants with more atractive flowers reduce the number of visits for those less atractive. Competition for visits affects the *quantity* of the pollination service as it induces a reduction in the amount of conspecific pollen received by flowers. The second, more subtle, mechanism is interspecific pollen transfer, where even receiving a visit might not translate into net facilitation. Interspecific pollen transfer affects the *quality* of the pollination service because a focal plant might receive pollen from a different species, or conversely pollen from the focal plant might be lost to different species. Generally speaking the larger the proportion of conspecific relative to heterospecific pollen the higher the quality of the pollination service.

To evaluate the quantity of pollination we first, we explored wether animal pollination resulted in a quantitative increase in the amount of conspeific pollen. To do that, we compared the amount of conspecific pollen

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on stigmas from flowers open to animal pollination and that from flowers that were bagged, where only autogamus self-pollination ocurred. The difference between these two counts is not directly equivalent to the pollen deposited by animals because self-pollination is an adaptive trait (Kalisz & Vogler 2003). Nevertheless, this difference still provides valuable indication of what the animal contribution to reproductive output. We performed this comparisons using a set of non parametric Mann-Whitney tests both at the community and the the species level (grouping species across communities). Second, we calculated the relationship between the ecological variables included in the models of pollen gain and the quantity of pollination. To do so, we simply calculated the mean value of the distribution of the effects of conspecific pollen gain. Again, we used bootstrap resampling from the coefficient distributions to calculate the confidence intervals of the mean.

The quality of pollination can be estimated by the relationship that exists between the gain in conspecific and heterospecific pollen. First, we calculated an overal 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 then followed a similar procedure to calculate a relationship per species in a community but instead of fitting a SMA per model pair, we did this across the 100 models for each random intercept independently. This allowed us to estimate the extent of facilitation for each plant species in its community. We also calculated the relationship between the ecological variables and the quality of pollination. To do so we calculated the mean difference between a sample of the distribution of the effects of conspecific pollen gain and a sample of those of heterospecific pollen gain. Similar to the mean effects on the quantity of pollination, we used bootstrap resamplong to calculate confidence intervals of the means.

Results

Conspeficic and heterospecific pollen gain

We found that, for both the model sets of conspecific and heterospecific pollen, the random structure that was best supported the data was the one that included plant species nested in community as the grouping factors (Table S3). In addition, AIC scores of the candidate model sets indicated that the species' share in the pollen pool and the functional originality were the most important variables determining the gain in both conspecific and heterospecific pollen (Figure 1). In addition to these two variables, the species relative abundance was also important for predicting the gain in heterospecific pollen (Figure 1). Surprisingly, the
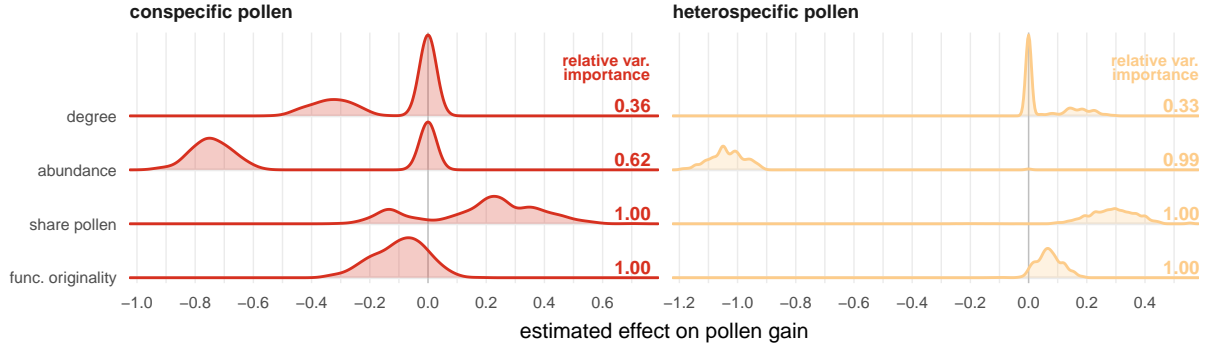
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Figure 1: Distribution of effect estimates for models of conspecific and heterospecific pollen density gain. Results are shown for models with explanatory var

species degree was comparatively less important as it was only included in models with relatively large AICc values (Table S4).

Overall we observed that species degree, abundance, and share of the pollen pool had a neggative effect on the gain of conspecific pollen, while functional originality had a positive effect. This These, three explanatory variables were positively correlated (Figure S5). Neverthelsess, the effect that each of them had on conspecific pollen was relatively similar among models that included all or just some of these three explanatory variables (Figure S6). The effects of the explanatory variables on the heterospecific pollen was more nuanced. When inspecting the most likely models, those with the lowest AIC scores, we observed that the gain in heterospecific pollen was neggatively related to the species abundance but positively correlated the species' share in the pollen pool, functional originality, and to a lesser extent, degree. We, however observe that when not accounting for a species abundance, both a species' share in the pollen pool and its functional originality have a negative relationship with the gain in heterospecific pollen (Figure S6).

Quantity and quality of pollination

Over all study sites, we found that 41% of species observed a significant increasse (at the $\alpha = 0.05$ level) in conspecific 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). In addition, using the results from the models of conspecific pollen gain, we found that from all the explanatory variables examined, only the plant's functional originality has a positive effect on the quantity of pollination (Figure 2).

We then examined the quality of pollination, which is given by the relationship between conspecific and heterospecific pollen. We found that, overall, the gain in conspecific pollen is positively correlated with the

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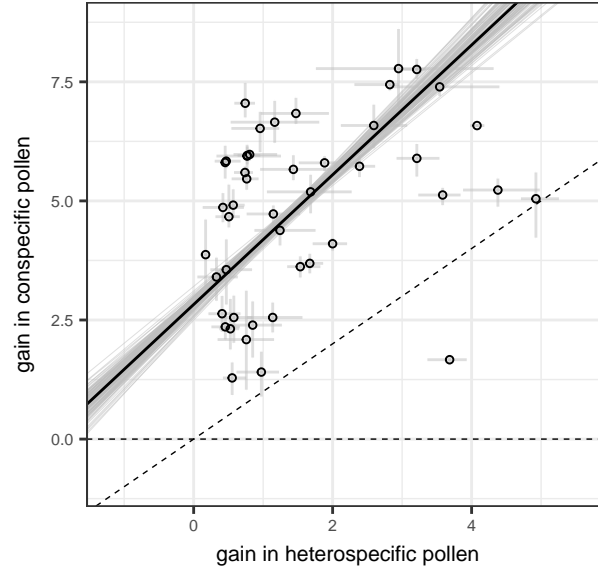| mean effect				
	degree	abundance	func. originality	share pollen
quantity of pollination	**−0.12** [−0.147, −0.093]	**−0.464** [−0.516, −0.406]	**−0.096** [−0.113, −0.082]	**0.181** [0.148, 0.209]
quality of pollination	**−0.174** [−0.202, −0.142]	**0.565** [0.503, 0.623]	**−0.168** [−0.186, −0.151]	**−0.105** [−0.143, −0.067]

Figure 2: asd

Figure 3: 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.

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 3A). At the species level, we found that XXX (Figure 3B). Furthermore, by examining the difference between the conspecific and heterospecific pollen gain, we found that the species abundance has an important positive effect in the quality of pollination, while the share in the pollen pool and, to an smaller extent, the species degree have a negative effect.

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 than net

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₂₂₈ facilitation is the dominant outcome. This agrees with previous findings. However the amount of added

₂₂₉ conspecific pollen is in average smaller than the amount of added heterospecific pollen.

₂₃₀ • Despite finding that facilitation is the most common outcome. We also were able to find clear signature

₂₃₁ of competition for pollinators at the community level.

₂₃₂ • We found that, as assumed in pollination models, the mutualistic benefit is modulated by the species

₂₃₃ degree and therefore there is indeed evidence for a trade-off. Moreover, the trade off does not depend on

₂₃₄ the species or the community. Which is a good thing because we tend to simplify models by assuming

₂₃₅ a common trade-off for the whole community.

₂₃₆ • However the mutualistic benefit was not just modulated by the degree but other factors also played

₂₃₇ significant roles.

₂₃₈ • These factors, in particular the plant relative abundance, was important at explaining the differences

₂₃₉ between plants.

₂₄₀ • We found that models performed better when explanatory variables were constructed so that they take

₂₄₁ into account information across all the communities in which a species was present. This means that

₂₄₂ despite the particularities of each community (random effects acounted for a large proportion of the

₂₄₃ variance) we can still predict general outcomes by knowing some general traits about the plant species

₂₄₄ and how it relates to other plants in their community.

₂₄₅ Understanding what makes species and communities different is important not only because of its ecological

₂₄₆ and evolutionary implications. It is also important because it is fundamentaly related to the way we

₂₄₇ understand and mathematically represent mutualistic systems. Some of the most widely used theoretical

₂₄₈ models of pollination account for the negative effects of mutualisim by adjusting the mutualistic benefit

₂₄₉ of an interaction so that it can be negatively affected by the number of mutualistic partners—the species

₂₅₀ degree. The extent to which species degree affects the benefit is called *mutualistic trade-off*. This approach,

₂₅₁ considerably simplifies the mathematical treatment of competition for pollinators. At the same time it is

₂₅₂ based on the intuitive rationale that a species that interacts with few species benefits strongly from each

₂₅₃ of them, whereas a species that interacts with a large number of species does so comparatively weakly.

₂₅₄ Although, the mutualistic trade-off has been shown to play a determining role on the stability and diversity

₂₅₅ of mutualistic communities, empirical evidence is scarce, to say the least.

₂₅₆ It is based on the intuitive rationale that a species that interacts with few species benefits strongly from each

₂₅₇ of them, whereas a species that interacts with a large number of species does so comparatively weakly.

₂₅₈ At the community scale, understanding the costs of sharing mutualistic partners at the community scale is

₂₅₉ important not only because of its ecological and evolutionary implications. It is also important because it is

fundamentaly 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 mutualisim 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, 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.

At the community scale other factors we would expect

Here, we explore the signatures competition for pollinators in diverse natural communities, where empirical data is more scarce.

Here we examine the signature, at the community scale, that sharing mutualistic partners has in the quality and quantity of pollination. Although seeimgly a simple question, teasing appart the signature of this "trade-off" has been elusive in part because of the large amounts of data required. Most studies that have examined competition for pollinators are based the pollen deposited on flower stigmas. However

All together, existing studies at the community scale show that, as expected, the effective result of sharing mutualistic partners, in most cases, one of facilitation. We hipothezise that Recent empirical evidence suggest that 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. However, they also show a large variability in this result—both across species and across communities. One of the most intuitive drivers of the variability in competition, and therefore widely used in theoretical models of mutualism to account for the neggative effects of mutualism, is the species degree—the number of mutualistic partners. It is based on the intuitive rationale that other factors might also play an important role shaping the competition for pollination.

First, we estimate the gain on pollen that can be attributed to the mutualistic interactions. Second, we investigate wether there is a relationship between this gain and a a suite of variables that together describe the role of the plant species and the ecological context. Third, and finaly, we explore how these variables shape the quantity and quality of pollination and ultimately the competition for pollinators. **TODO: Mini conclusion here**

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