working title Compatibility system and stygma size of pollen recipient as main predictors of heterospecific pollen effect

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Pollinator sharing can have negative consequences for species fitness with the arrival of foreign pollen. However, the costs of heterospecific pollen are not yet well understood. For this reason, we have conducted a glasshouse experiment where we try to understand how phylogenetic relatedness and the different traits of these species are involved in this process. We experimentally crossed 10 species belonging to three different families: Brassicaceae, Solanaceae and Convolvulaceae. Overall, more than 4000 crosses were done and seed set and pollen tubes were considered as proxy of effect. We found that for all species foreign pollen (50% or less) reduced seed set. Moreover, the seed set reduction is not dependent on the degree of relatedness of the pollen donor. However, the effect is governed by the degree of relatedness and the traits of the species recipient. Our results show that the outcome of heterospecific pollen deposition is determined in greater degree by the traits of the pollen recipient than the pollen donor and that certain traits such as compatibility system are crucial to understand the costs of heterospecific pollen.

Keywords: heterospecific pollen, plant reproduction, fitness, interspecific competition, phylogenetic distance.

INTRODUCTION

Paragraph 1 General idea to our concept

In natural systems plant species normally coexist and share their floral visitors with other species Bascompte et al. (2003). This pollinator sharing from the plant perspective at the pre-pollination stage can be negative due to competition Pauw (2013) or positive due to facilitation Carvalheiro et al. (2014). Once the floral visitor has arrived to the flower, pollen deposition on the stigma can take place and hence ovule fertilization. An increasing number of visits generally correlates with higher chances of fertilization Engel and Irwin (2003). However this is not always the case, among these possible flower visitors we find also nectar robbers and pollen thiefs Inouye (1980) and the quality of pollen that is deposit on the stigma is also highly relevant to the pollination succes Aizen and Harder (2007). Moreover, other less study issues in the pollination process are conspecific pollen loss and the arrival of foreign pollen which can have important detrimental effects on species fitness Morales and Traveset (2008) Ashman and Arceo-Gómez (2013).

Paragraph 2 Introducing topic and knowledge gap

Recent studies have advanced in the ecological understanding of heterospecific pollen effect Morales and Traveset (2008) Ashman and Arceo-Gómez (2013) Arceo-Gómez and Ashman (2016). A general overview of foreign pollen arrival is that it can play an important role on species fitness but seems to be context dependent and not always produce a decrease in fitness Morales and Traveset (2008). Part of this unpredictability is due to the enormous variability of foreing pollen transferred in nature, where levels between 0 and 75 percent are seen, but most commonly values ranges between 0 and 20 percent of the total pollen load, being the generalist species the ones that receive greater loads of heterospecific pollen Bartomeus et al. (2008) Montgomery and Rathcke (2012) Ashman and Arceo-Gómez (2013) Fang and Huang (2013). Although heterospecific pollen quantity is fundamental to understand the outcome of the interaction so is the different traits of both pollen donor and recipient. Ashman and Arceo-Gómez (2013) postulated the first predictive framework for traits of heterospecific pollen effect, where different traits such as compatibility system and pollen size among others seems to be crucial to understand foreing pollen effect. Moreover, in Tong and Huang (2016) an assymetric effect was shown in a crossing experiment between 6 species of the genus *Pedicularis* where the pollen of long styled species was able to grow the full length of the style on short styled species but not viceversa. Despite these recent caveats, we still lack empirical evidence to affirm what are the main traits that drive heterospecific pollen effect for both pollen donor and recipient at seed production level. Interestingly, to comprehend how these traits interact is also crucial to look at the phylogenetic relatedness of the species. There is a considerable amount of literature of crosses between close related species Brown and Mitchell (2001) Arceo-Gómez et al. (2016) Tong and Huang (2016) but few works focused on heterospecific pollen of far related species which is a more realistic feature in natural systems due to a less likely niche overlap (REF). Although close related species seems to affect greatly species fitness

Arceo-Gómez and Ashman (2016) the effect of heterospecific pollen of far and close related species remains to be tested explicitly.

Paragraph 3 Expanding ideas with examples

The great environmental variability in natural systems and complexity of floral structures make heterospecific pollination studies a daunting task. For this reason, controlled pollination experiments where hand pollination is applied instead of pollinators as pollen vectors have simplified this task. However, other issues may arise such as overestimation of the effect (REFs), standarized methods across studies or lack of descriptive methodology for reproducible work (REFs). One of the most common ways to estimate heterospecific pollen effect through hand pollination treatments is by using 50% pollen mixes of conspecific and heterospecific pollen which a realistic proportion (REF) but challenging because of the different pollen properties such as size or hydratation levels (REFS).

I would like to add that the experiments focus on two proxies of effect prezygotic and postzygotic. Why focus on postzygotic? Is the final stage where we can see the effect. Further studies should also study germination rates.

Traditionally heterospecific pollen effect has focused its attention on different pollen donors as a main driver of different effect. However in this article we want to emphasize that this is true for the cases that the species are higly close related where pollen recognition can take place (eg hybridization) but not when this pollen is from less closely related species which the main driver of effect is determined by the reproductive biology of the female part of the plant(compatibility system, stigma type, stigma area and number of ovules).

Paragraph 4 Introducing our experiment

Sell well our work: We are the first empirical experiment testig the effect of heterospecific pollen with phylogenetic distance

The great difficulty of working with pollen in a coflowering community make the understanding of heterospecifc pollen effect a real challenge. For this reason we have created an artificial co-flowering community in a glasshouse to test the effect with all the possible combinations among them. Where we test the folowing hypothesis: 1) Does heterospecific pollen reduce seed set, if so, 2) Does heterospecific pollen effect depend on the relatedness of the species, 3) Does heterospecific pollen effect depend on any floral trait?

Maybe another possible hypothesis to test is the reciprocity of the effect of heterospecifc pollen????

Use the sterile species as a proof of the mechanical interference. Was a mistake but seems cool proof!!!

METHODS

comment starts Glasshouse trial • Species selected and why – how you made them co-flower • Give details of sources and planting seeds, growth medium in pots, temperature and light details • Hand crosses and how you did them, how you measured seed set over time. • Analyses of data – standardization, means, matrices etc.

• Analyses and technical difficulties: We calculated effect size by subtracting the mean of the cross pollinated seed set by the mean effect of the HP pollen (explain exactly what figures you used to calculate this) – check with liam about potentially using missing values analyses for the species we don't have?

Check that the method is working well to prove that your crosses were close to 50% results in SI i.e not all mixes were 50/50% and we have now counted all the pollen to make this a quantitative variable. We also need to factor in the point that we have different total abundances of pollen across our treatments, irrespective of ratios. To what extent are differences in the ratios of pollen applied by hand across different plant families influenced by plant traits such as pollen size, morphology and stigma surface type?

Results – may need to include amount of pollen in models as random factor- prefill matrix with missing value analyse for the species you don't have.
Question 1: how do different pollination treatments (100% HP, 50% HP, self and cross) impact HP pollen across different plant families? Even with 100% HP one (or more species?) still produced seed set.

Result Effect size of Seed set ~ phylogenetic distance relationship We found that the variation ?/ mean effect size of seed set is positively related to phylogenetic distance. This means the more unrelated the species are, the greater the negative impact of heterospecific pollen (give stats effect size i.e. Procrustes, X = 0.35; P = 0.03)

Question 2 : what are the main traits impacting HP impacts? (compatibility system, pollen size, stigma surface, wet/dry stigma, length of style etc.

Effect size of seed set ~ floral traits/ reproductive plant traits We found that the three best terms to explain the variation in seed set is pollen/ovule ratio, stigma width and style length (Stats effect size i.e. X = 0.39, P = 0.02).
Need to provide correlation matrix for all traits just for 10 species Show both ways to present this. Which particular traits do you find significant effects for? Show this and give stats. Present plot for each trait and effect size

comment finishes

The study was conducted in a glasshouse at University of New England (Armidale, Australia) from November 2017 to March 2018. Rooms were temperature controlled depending on the requirements of the species with day and night temperature differences. The species selected (Table 1) belonged to three different families, Solanaceae, Brassicaceae and Convolvulaceae. The criteria of species/family selection was based on close/distant related species (see phylogenetic tree for relatedness fig 1), heterogeneous traits, low structural flower complexity and fast life cycle. For the purpose of the experiment all the species where considered as pollen recipient and as pollen donor (see interaction matrix, fig 2). Species were watered once or twice per day and fertilized weekly (NPK 23: 3.95: 14).

Brown and Mitchell 2001 could be a good paper to explain why we pick seed set as a proxy and not fruit set. We cannot see changes on it, losing information with it.

Hand-pollination

Foreign pollen effect was studied through two different treatments, one with 50% conspecific pollen and 50% heterospecific pollen and a second one with 100% foreign pollen (N=10). Seed set was the proxy of effect (see Brown and Mitchell 2001, for differences in effect between seed set and fruit set) and "pollen tubes". Moreover, hand cross pollination, hand self pollination, apomixis (bagged emasculated flowers) and natural selfing were tested (N=10). Flowers were emasculated the day prior anthesis and hand pollinated next day with a toothpick. Had-pollination was realized with 3-4 gentle touches on the surface of the stigma. The mixes of pollen were performed on an eppendorf based on the pollen counts maded with Neubaeur chamber (each anther was counted 4 times for 20 different anthers per species).

Evolutive distance

Two types of evolutive distances were calculated with MEGA7 thow kinds of markers: 1) Internal transcribed spacer (ITS) and 2) ribulose-bisphosphate carboxylase (RBCL)

Traits

Several traits of the ten species were measured. Pollen per anther was counted, number of ovules, stigma width and length and stigmatic area, style width and length, ovary width and length. Moreover stigma type was tested. Self-incompatibility was

We used the statistical language `R` (R Core Team 2018) for all our analyses. These were implemented in dynamic rmarkdown documents using `knitr` (Xie 2014, 2015, 2018) and `rmarkdown` (Allaire et al. 2018) packages. All the multilevel models were fitted with `lme4` (Bates et al. 2015).

RESULTS

DISCUSSION

Discussion

1. What are the implications of the findings?

CONCLUSIONS

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