

Working title, The impact of heterospecific pollen on plant reproductive success is mediated by phylogenetic distance and floral reproductive traits

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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

34 hence ovule fertilization. An increasing number of visits generally correlates with higher chances of
35 fertilization Engel and Irwin (2003). However this is not always the case, among these possible flower
36 visitors we find also nectar robbers and pollen thieves Inouye (1980) and the quality of pollen that is
37 deposit on the stigma is also highly relevant to the pollination succes Aizen and Harder (2007).
38 Moreover, other less study issues in the pollination process are conspecific pollen loss and the arrival of
39 foreign pollen which can have important detrimental effects on species fitness Morales and Traveset
40 (2008) Ashman and Arceo-Gómez (2013).

41 **Paragraph 2** Introducing topic and knowledge gap

42 Recent studies have advanced in the ecological understanding of heterospecific pollen effect Morales and
43 Traveset (2008) Ashman and Arceo-Gómez (2013) Arceo-Gómez and Ashman (2016). A general
44 overview of foreign pollen arrival is that it can play an important role on species fitness but seems to be
45 context dependent and not always produce a decrease in fitness Morales and Traveset (2008). Part of
46 this unpredictability is due to the enormous variability of foreing pollen transferred in nature, where
47 levels between 0 and 75 percent are seen, but most commonly values ranges between 10 and 20 percent
48 of the total pollen load, being the generalist species the ones that receive greater loads of heterospecific
49 pollen Montgomery and Rathcke (2012) Fang and Huang (2013). Although heterospecific pollen
50 quantity is fundamental to understand the outcome of the interaction so is the different traits of both
51 pollen donor and recipient. Ashman and Arceo-Gómez (2013) postulated the first predictive framework
52 for traits of heterospecific pollen effect where traits such as compatibility system and pollen size among
53 others should be crucial to understand foreing pollen effect. Moreover, in Tong and Huang (2016) a
54 assymetric effect was shown in a crossing experiment between 6 species of the genus *Pedicularis* where
55 the pollen of long styled species was able to grow the ful length of the style on short styled species but
56 not viceversa. Despite these recent caveats, we still lack empirical evidence to affirm what are the main
57 traits that drive heterospecific pollen effect for both pollen donor and recipient at seed production level.
58 Interestingly, this trait based question of effect between a pair of species cannot be solved without
59 consider the phylogenetic relatedness of them. A general view is that close related species will have
60 greater negative impact than far related species (REFS) but few studies test this fact. The interaction
61 between close related species have been tradionally overlooked (Refs) but not always the effect of
62 congeners or confamilias can be seen due to these other species are capable of fertilization (Refs.)

Combining both traits and phylogenetic relatedness could give a better picture of how both parts interact in the heterospecific pollen process.

Paragraph 3 Expanding ideas with examples

Examples of articles...

can reduce species fitness (REFS) but seems to be highly context-dependent. There are hypothesized that some traits can play a crucial role in this species interaction such as stigma type, pollen size,

Mention invasive species in this paragraph

Few studies have tried to understand how relatedness is involved in the hp effect but generally Until our knowledge

Rescue from here the useful things:

Invasive species are supposed to have greater negative effect than native ones Arceo-Gómez and Ashman (2016). Although when non-natives species don't have greater negative effect we still don't know why. For this reason, this ecological question is non a native non native one is a trait based issue that is still to be solved. Moreover, the quantity of pollen that integrates in the network can be quite variable ranging from low quantities Bartomeus et al. (2008) to intermediate (ref) to high (ref). Moreover, closely related species are supposed to reduce fitness in greater effect but the evidence is scarce and based on independent studies with different methodologies (Arceo-gomez & Ashman 2016) or studies that just check it with a pair of species that are highly related with the aim to understand hybridization costs (refs). There is a need to deepen into how relatedness is involved in the costs of heterospecific pollen effect. Furthermore, following the conceptual trait framework of Ashman and Arceo-Gomez on heterospecific pollen there are good theoretical basis for trait effect. Notwithstanding, non empirical work has tested how really these traits are involved in heterospecific pollen effect.

Explain traits. Put examples

what is closely related? same genus? Just that right, the rest is far related?

I would like to add that the experiments focus on two proxies of effect prezygotic and postzygotic. Why

88 focus on postzygotic? Is the final stage where we can see the effect. Further studies should also study
89 germination rates.

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

96 **Paragraph 4** Introducing our experiment

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

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

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

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

107 **METHODS**

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

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

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

122 Results – may need to include amount of pollen in models as random factor- prefill matrix with missing
123 value analyse for the species you don't have.

124 Question 1: how do different pollination treatments (100% HP, 50% HP, self and cross) impact HP
125 pollen across different plant families? Even with 100% HP one (or more species?) still produced seed
126 set.

127 Result Effect size of Seed set ~ phylogenetic distance relationship We found that the variation ?/ mean
128 effect size of seed set is positively related to phylogenetic distance. This means the more unrelated the
129 species are, the greater the negative impact of heterospecific pollen (give stats effect size i.e. Procrustes,
130 $X = 0.35$; $P = 0.03$)

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

133 Effect size of seed set ~ floral traits/ reproductive plant traits We found that the three best terms to
134 explain the variation in seed set is pollen/ovule ratio, stigma width and style length (Stats effect size
135 i.e. $X = 0.39$, $P = 0.02$).

136 Need to provide correlation matrix for all traits just for 10 species Show both ways to present this.
137 Which particular traits do you find significant effects for? Show this and give stats. Present plot for
138 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 were 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. Hand-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 made with Neubauer chamber (each anther was counted 4 times for 20 different anthers per species).

Evolutionary distance

Two types of evolutionary distances were calculated with MEGA7 through 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

165 stigma type was tested. Self-incompatibility was

166 We used the statistical language **R** (R Core Team 2018) for all our analyses. These were implemented in
167 dynamic **rmarkdown** documents using **knitr** (Xie 2014, 2015, 2018) and **rmarkdown** (Allaire et al.
168 2018) packages. All the multilevel models were fitted with **lme4** (Bates et al. 2015).

169 **RESULTS**

170 **DISCUSSION**

171 Discussion

172 1. What are the implications of the findings?

173 **CONCLUSIONS**

174 **ACKNOWLEDGEMENTS**

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