

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

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## INTRODUCTION

### Paragraph 1

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 therefore ovule fertilization. An increasing number of visits generally correlates with higher chances of fertilization (Refs). However this is not always the case, among these possible flower visitors we find also nectar robbers and pollen thieves (Refs) and the quality of pollen that is deposited on the stigma is also highly relevant to the pollination succes (Refs). Moreover, other less studies issues in the pollination process are conspecific pollen loss and the arrival of foreign pollen which can have important detrimental effects for the species fitness Morales and Traveset (2008).

### Paragraph 2

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

43 Explain traits. Put examples

### 44 **Paragraph 3**

### 45 **Paragraph 4**

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

## 52 **METHODS**

53 comment starts Glasshouse trial • Species selected and why – how you made them co-flower • Give  
54 details of sources and planting seeds, growth medium in pots, temperature and light details • Hand

55 crosses and how you did them, how you measured seed set over time. • Analyses of data –  
56 standardization, means, matrices etc.

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

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

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

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

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

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

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

81 Need to provide correlation matrix for all traits just for 10 species Show both ways to present this.

82 Which particular traits do you find significant effects for? Show this and give stats. Present plot for  
83 each trait and effect size

84 comment finishes

85 The study was conducted in a glasshouse at University of New England (Armidale, Australia) from  
86 November 2017 to March 2018. Rooms were temperature controlled depending on the requirements of  
87 the species with day and night temperature differences. The species selected (Table 1) belonged to  
88 three different families, Solanaceae, Brassicaceae and Convolvulaceae. The criteria of species/family  
89 selection was based on close/distant related species (see phylogenetic tree for relatedness fig 1),  
90 heterogeneous traits, low structural flower complexity and fast life cycle. For the purpose of the  
91 experiment all the species were considered as pollen recipient and as pollen donor (see interaction  
92 matrix, fig 2). Species were watered once or twice per day and fertilized weekly (NPK 23: 3.95: 14).  
93 Brown and Mitchell 2001 could be a good paper to explain why we pick seed set as a proxy and not  
94 fruit set. We cannot see changes on it, losing information with it.

## 95 **Hand-pollination**

96 Foreign pollen effect was studied through two different treatments, one with 50% conspecific pollen and  
97 50% heterospecific pollen and a second one with 100% foreign pollen (N=10). Seed set was the proxy of  
98 effect (see Brown and Mitchell 2001, for differences in effect between seed set and fruit set) and “pollen  
99 tubes”. Moreover, hand cross pollination, hand self pollination, apomixis (bagged emasculated flowers)  
100 and natural selfing were tested (N=10). Flowers were emasculated the day prior anthesis and hand  
101 pollinated next day with a toothpick. Hand-pollination was realized with 3-4 gentle touches on the  
102 surface of the stigma. The mixes of pollen were performed on an eppendorf based on the pollen counts  
103 made with Neubauer chamber (each anther was counted 4 times for 20 different anthers per species).

## 104 **Evolutionary distance**

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

## 107 **Traits**

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

111 We used the statistical language **R** (R Core Team 2018) for all our analyses. These were implemented in  
112 dynamic **rmarkdown** documents using **knitr** (Xie 2014, 2015, 2018) and **rmarkdown** (Allaire et al.  
113 2018) packages. All the multilevel models were fitted with **lme4** (Bates et al. 2015).

## 114 RESULTS

## 115 DISCUSSION

116 Discussion

117 1. What are the implications of the findings?

## 118 CONCLUSIONS

## 119 ACKNOWLEDGEMENTS

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