

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 can be negative due to competition (refs) or positive due to facilitation Carvalheiro et al. (2014). Moreover, once the pollinator has landed on the stigma some other issues for the species fitness may arise, the arrival of foreign pollen and conspecific pollen loss (Morales & Traveset 2008)

Paragraph 2

The effect of heterospecific pollen has been widely studied (Morales & Traveset 2008). 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

Paragraph 3

Paragraph 4

The great difficulty of working with pollen in a coflowering community make the understanding of heterospecific 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 following 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?

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

54 calculate this) – check with liam about potentially using missing values analyses for the species we
55 don't have?

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

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

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

67 Result Effect size of Seed set ~ phylogenetic distance relationship We found that the variation ?/ mean
68 effect size of seed set is positively related to phylogenetic distance. This means the more unrelated the
69 species are, the greater the negative impact of heterospecific pollen (give stats effect size i.e. Procrustes,
70 $X = 0.35$; $P = 0.03$)

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

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

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

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

79 comment finishes

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

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

90 **Hand-pollination**

91 Foreign pollen effect was studied through two different treatments, one with 50% conspecific pollen and
92 50% heterospecific pollen and a second one with 100% foreign pollen (N=10). Seed set was the proxy of
93 effect (see Brown and Mitchell 2001, for differences in effect between seed set and fruit set) and “pollen
94 tubes”. Moreover, hand cross pollination, hand self pollination, apomixis (bagged emasculated flowers)
95 and natural selfing were tested (N=10). Flowers were emasculated the day prior anthesis and hand
96 pollinated next day with a toothpick. Hand-pollination was realized with 3-4 gentle touches on the
97 surface of the stigma. The mixes of pollen were performed on an eppendorf based on the pollen counts
98 made with Neubauer chamber (each anther was counted 4 times for 20 different anthers per species).

99 **Evolutionary distance**

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

102 **Traits**

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

106 We used the statistical language **R** (R Core Team 2018) for all our analyses. These were implemented in
107 dynamic **rmarkdown** documents using **knitr** (Xie 2014, 2015, 2018) and **rmarkdown** (Allaire et al.
108 2018) packages. All the multilevel models were fitted with **lme4** (Bates et al. 2015).

109 **RESULTS**

110 **DISCUSSION**

111 Discussion

- 112 1. What are the implications of the findings?

113 **CONCLUSIONS**

114 **ACKNOWLEDGEMENTS**

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