

working title Compatibility system and stigma 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

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 0 and 20 percent
48 of the total pollen load, being the generalist species the ones that receive greater loads of heterospecific
49 pollen Bartomeus et al. (2008) Montgomery and Rathcke (2012) Ashman and Arceo-Gómez (2013)
50 Fang and Huang (2013). Although heterospecific pollen quantity is fundamental to understand the
51 outcome of the interaction so is the different traits of both pollen donor and recipient. Ashman and
52 Arceo-Gómez (2013) postulated the first predictive framework for traits of heterospecific pollen effect,
53 where different traits such as compatibility system and pollen size among others seems to be crucial to
54 understand foreing pollen effect. Moreover, in Tong and Huang (2016) an assymetric effect was shown
55 in a crossing experiment between 6 species of the genus *Pedicularis* where the pollen of long styled
56 species was able to grow the full length of the style on short styled species but not viceversa. Despite
57 these recent caveats, we still lack empirical evidence to affirm what are the main traits that drive
58 heterospecific pollen effect for both pollen donor and recipient at seed production level. Interestingly, to
59 comprehend how these traits interact is also crucial to look at the phylogenetic relatedness of the
60 species. There is a considerable amount of literature of crosses between close related species Brown and
61 Mitchell (2001) Arceo-Gómez et al. (2016) Tong and Huang (2016) but few works focused on
62 heterospecific pollen of far related species which is a more realistic feature in natural systems due to a
63 less likely niche overlap (REF). Although close related species seems to affect greatly species fitness

Arceo-Gómez and Ashman (2016) the importance of heterospecific pollen effect of far and close related species remains to be explored at community level.

Paragraph 3 Expanding ideas with examples

Traditionally,

. For this reason, controlled pollination experiments where hand pollination is applied instead of using pollinators as pollen vectors have simplified this task. However, other issues may arise such as overestimation of the effect (REFs), different methods across studies or lack of descriptive methodology for reproducible work (REFs). One of the most common ways to estimate heterospecific pollen effect is through hand pollination with mixes of 50% conspecific pollen and 50% heterospecific pollen (REF).

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 highly 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

The great environmental variability in natural systems and complexity of floral structures make heterospecific pollination studies a daunting task. Although plant-pollinator network and pollen network studies can give a first picture of the importance of foreign pollen is necessary to address how its effect is shaped with both traits and relatedness of the species. For this reason, in this study we have created an artificial co-flowering community with 10 species belonging to three different families where we try to test the following questions: 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

90 depend on any floral trait?

91 **METHODS**

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

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

102 **Hand-pollination**

103 Foreign pollen effect was studied through two different treatments, one with 50% conspecific pollen and
104 50% heterospecific pollen and a second one with 100% foreign pollen (N=10). Seed set was the proxy of
105 effect (see Brown and Mitchell 2001, for differences in effect between seed set and fruit set) and “pollen
106 tubes”. Moreover, hand cross pollination, hand self pollination, apomixis (bagged emasculated flowers)
107 and natural selfing were tested (N=10). Flowers were emasculated the day prior anthesis and hand
108 pollinated next day with a toothpick. Hand-pollination was realized with 3-4 gentle touches on the
109 surface of the stigma. The mixes of pollen were performed on an eppendorf based on the pollen counts
110 made with Neubauer chamber (each anther was counted 4 times for 20 different anthers per species).

111 **Evolutionary distance**

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

114 **Traits**

115 The traits measured for each species were pollen per anther, number of ovules, stigma width and length
116 and stigmatic area, style width and length, ovary width and length. Moreover stigma type was tested.
117 Pollen was counted for 20 anthers of each species with 4 replicates per sample with an hemocytometer.
118 Previously anthers were squashed on a known solution with the pippete tip and homogeneize with a
119 vortex for 30 seconds. Ovule number was counted with the help of an stereomicroscope and a small
120 grid over a petri dish from 15 randomly selected flowers. The different morphometrical traits were
121 measured with XXXX. Levels of self incompatibility were estimated by dividing the the fruit set of
122 hand self pollination by hand cross pollination

123 We used the statistical language **R** (R Core Team 2018) for all our analyses. These were implemented in
124 dynamic **rmarkdown** documents using **knitr** (Xie 2014, 2015, 2018) and **rmarkdown** (Allaire et al.
125 2018) packages. All the multilevel models were fitted with **lme4** (Bates et al. 2015).

126 **RESULTS**

127 **DISCUSSION**

128 Discussion

129 1. What are the implications of the findings?

130 CONCLUSIONS

131 ACKNOWLEDGEMENTS

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