

# 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.

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**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 Bartomeus et al. (2008) Montgomery and Rathcke (2012) Ashman and  
49 Arceo-Gómez (2013) Fang and Huang (2013), being the generalist species the ones that receive greater  
50 loads of heterospecific pollen Fang and Huang (2013). Surprisingly, this low ranges of heterospecific  
51 pollen have been shown to decrease fitness greatly Thomson et al. (1982). Although heterospecific  
52 pollen quantity is fundamental to understand the outcome of the interaction so is the different traits of  
53 both pollen donor and recipient. Ashman and Arceo-Gómez (2013) postulated the first predictive  
54 framework for traits of heterospecific pollen effect, where different traits such as compatibility system  
55 and pollen size among others seems to be crucial to understand foreing pollen effect. Moreover, in Tong  
56 and Huang (2016) an assymetric effect was shown in a crossing experiment between 6 species of the  
57 genus *Pedicularis* where the pollen of long styled species was able to grow the full length of the style on  
58 short styled species but not viceversa. Despite these recent caveats, we still lack empirical evidence to  
59 affirm what are the main traits that drive heterospecific pollen effect for both pollen donor and  
60 recipient at seed production level. Interestingly, to comprehend how these traits interact is also crucial  
61 to look at the phylogenetic relatedness of the species. There is a considerable amount of literature of  
62 crosses between close related species Brown and Mitchell (2001) Arceo-Gómez et al. (2016) Tong and  
63 Huang (2016) but few works focused on heterospecific pollen of far related species. Although the effect

of close related species is predicted to be greater Ashman and Arceo-Gómez (2013) the presence of pollen of non related species on multiple species Arceo-Gómez and Ashman (2016) and the higher chances to coexist with a species that has less niche overlap (Ref) make foreign pollen from far related species also an important subject of study in order to understand the importance of heterospecific pollen in natural systems. Notwithstanding, the effect of heterospecific pollen of far and close related species at community level remains to be explored beyond single pairwise interactions.

### **Paragraph 3** Expanding ideas with examples

Interestingly, incompatibility system seems to play an important role in foreign pollen effect where species that are self incompatible would have stronger barriers towards heterospecific pollen than self compatible species Ashman and Arceo-Gómez (2013). The type of incompatibility, sporophytic or gametophytic is related with the place of pollen recognition where the former take place at the sitgma level and the latter occurs within the style, this last late acting pollen recognition mechanism is associated with greater negative effect (???). Remarkably, there is a great variability in mating systems across populations Whitehead et al. (2018) and therefore predict an effect of foreign pollen is a bit obscured by the variability within species, however species that are strong selfers or strong outcrossers have less variability in mating systems and predictions of effect could be more realistic (see figure 1 from Whitehead et al. (2018)). Moreover, other traits such as number of pollen grains per flower and number of ovules have been tradittionally associated with the type of incompatibility system where species with higher pollen ovule ratios are predicted to be xenogamous and species with low pollen ovule ratios autogamous (REF). Selfer species would have a reduction of herkogamy (REF), less pollen production per ovule (REF) and therefore less likely to contribute in exporting pollen in the community. Other morphological traits, like stigma size can be determinant for the total pollen quatity that a stigma can receive and therefore related to do that pollen size would also play an important role. Example with pollen here.

### **Paragraph 4** Introducing our experiment

The great environmental variability in natural systems and complexity of floral structures make heterospecific pollination studies a daunting task. Moreover, variation in sampling effort have been shown to be determinant to characterize pollen transfer interactions Arceo-Gómez et al. (2018).

92 Although plant-pollinator network and pollen network studies can give a first picture of the importance  
93 of foreign pollen is necessary to address how its effect is shaped with both traits and relatedness of the  
94 species. For this reason, in this study we have created an artificial co-flowering community with 10  
95 species belonging to three different families where we try to test the following questions: 1) Does  
96 heterospecific pollen reduce seed set, if so, 2) Does heterospecific pollen effect depend on the  
97 relatedness of the species, 3) Does heterospecific pollen effect depend on any floral trait?

## 98 METHODS

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

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

### 109 Hand-pollination

110 Foreign pollen effect was studied through two different treatments, one with 50% conspecific pollen and  
111 50% heterospecific pollen and a second one with 100% foreign pollen (N=10). Seed set was the proxy of  
112 effect (see Brown and Mitchell 2001, for differences in effect between seed set and fruit set) and “pollen  
113 tubes”. Moreover, hand cross pollination, hand self pollination, apomixis (bagged emasculated flowers)  
114 and natural selfing were tested (N=10). Flowers were emasculated the day prior anthesis and hand  
115 pollinated next day with a toothpick. Hand-pollination was realized with 3-4 gentle touches on the  
116 surface of the stigma. The mixes of pollen were performed on an eppendorf based on the pollen counts  
117 made with Neubauer chamber (each anther was counted 4 times for 20 different anthers per species).

## 118 **Evolutionary distance**

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

## 121 **Traits**

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

130 We used the statistical language R (R Core Team 2018) for all our analyses. These were implemented in  
131 dynamic rmarkdown documents using `knitr` (Xie 2014, 2015, 2018) and `rmarkdown` (Allaire et al.  
132 2018) packages. All the multilevel models were fitted with `lme4` (Bates et al. 2015).

## 133 **RESULTS**

## 134 **DISCUSSION**

### 135 Discussion

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

## 137 CONCLUSIONS

## 138 ACKNOWLEDGEMENTS

## 139 REFERENCES

- 140 Aizen, M. A., and L. D. Harder. 2007. Expanding the limits of the pollen-limitation concept: Effects of  
141 pollen quantity and quality. *Ecology* 88:271–281.
- 142 Allaire, J., Y. Xie, J. McPherson, J. Luraschi, K. Ushey, A. Atkins, H. Wickham, J. Cheng, and W.  
143 Chang. 2018. Rmarkdown: Dynamic documents for r.
- 144 Arceo-Gómez, G., C. Alonso, T.-L. Ashman, and V. Parra-Tabla. 2018. Variation in sampling effort  
145 affects the observed richness of plant–plant interactions via heterospecific pollen transfer: Implications  
146 for interpretation of pollen transfer networks. *American journal of botany* 105:1601–1608.
- 147 Arceo-Gómez, G., and T.-L. Ashman. 2016. Invasion status and phylogenetic relatedness predict cost  
148 of heterospecific pollen receipt: Implications for native biodiversity decline. *Journal of Ecology*  
149 104:1003–1008.
- 150 Arceo-Gómez, G., R. A. Raguso, and M. A. Geber. 2016. Can plants evolve tolerance mechanisms to  
151 heterospecific pollen effects? An experimental test of the adaptive potential in clarkia species. *Oikos*  
152 125:718–725.
- 153 Ashman, T.-L., and G. Arceo-Gómez. 2013. Toward a predictive understanding of the fitness costs of  
154 heterospecific pollen receipt and its importance in co-flowering communities. *American Journal of*  
155 *Botany* 100:1061–1070.
- 156 Bartomeus, I., J. Bosch, and M. Vilà. 2008. High invasive pollen transfer, yet low deposition on native  
157 stigmas in a carpobrotus-invaded community. *Annals of Botany* 102:417–424.
- 158 Bascompte, J., P. Jordano, C. J. Melián, and J. M. Olesen. 2003. The nested assembly of plant–animal

mutualistic networks. *Proceedings of the National Academy of Sciences* 100:9383–9387.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67:1–48.

Brown, B. J., and R. J. Mitchell. 2001. Competition for pollination: Effects of pollen of an invasive plant on seed set of a native congener. *Oecologia* 129:43–49.

Carvalho, L. G., J. C. Biesmeijer, G. Benadi, J. Fründ, M. Stang, I. Bartomeus, C. N. Kaiser-Bunbury, M. Baude, S. I. Gomes, V. Merckx, and others. 2014. The potential for indirect effects between co-flowering plants via shared pollinators depends on resource abundance, accessibility and relatedness. *Ecology letters* 17:1389–1399.

Engel, E. C., and R. E. Irwin. 2003. Linking pollinator visitation rate and pollen receipt. *American Journal of Botany* 90:1612–1618.

Fang, Q., and S.-Q. Huang. 2013. A directed network analysis of heterospecific pollen transfer in a biodiverse community. *Ecology* 94:1176–1185.

Inouye, D. W. 1980. The terminology of floral larceny. *Ecology* 61:1251–1253.

Montgomery, B. R., and B. J. Rathcke. 2012. Effects of floral restrictiveness and stigma size on heterospecific pollen receipt in a prairie community. *Oecologia* 168:449–458.

Morales, C. L., and A. Traveset. 2008. Interspecific pollen transfer: Magnitude, prevalence and consequences for plant fitness. *Critical Reviews in Plant Sciences* 27:221–238.

Pauw, A. 2013. Can pollination niches facilitate plant coexistence? *Trends in ecology & evolution* 28:30–37.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Thomson, J. D., B. J. Andrews, and R. Plowright. 1982. The effect of a foreign pollen on ovule

- 182 development in *diervilla lonicera* (caprifoliaceae). *New Phytologist* 90:777–783.
- 183 Tong, Z.-Y., and S.-Q. Huang. 2016. Pre-and post-pollination interaction between six co-flowering  
184 *pedicularis* species via heterospecific pollen transfer. *New Phytologist* 211:1452–1461.
- 185 Whitehead, M. R., R. Lanfear, R. J. Mitchell, and J. D. Karron. 2018. Plant mating systems often vary  
186 widely among populations. *Frontiers in Ecology and Evolution* 6:38.
- 187 Xie, Y. 2014. Knitr: A comprehensive tool for reproducible research in R. *in* V. Stodden, F. Leisch,  
188 and R. D. Peng, editors. *Implementing reproducible computational research*. Chapman; Hall/CRC.
- 189 Xie, Y. 2015. *Dynamic documents with R and knitr*. 2nd editions. Chapman; Hall/CRC, Boca Raton,  
190 Florida.
- 191 Xie, Y. 2018. *Knitr: A general-purpose package for dynamic report generation in r*.





