working title Compatibility system and stygma 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.

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

fertilization Engel and Irwin (2003). However this is not always the case, among these possible flower visitors we find also nectar robbers and pollen thiefs Inouye (1980) and the quality of pollen that is deposit on the stigma is also highly relevant to the pollination succes Aizen and Harder (2007). Moreover, other less study issues in the pollination process are conspecific pollen loss and the arrival of foreign pollen which can have important detrimental effects on species fitness Morales and Traveset (2008) Ashman and Arceo-Gómez (2013).

Paragraph 2 Introducing topic and knowledge gap

Recent studies have advanced in the ecological understanding of heterospecific pollen effect Morales and Traveset (2008) Ashman and Arceo-Gómez (2013) Arceo-Gómez and Ashman (2016). A general overview of foreign pollen arrival is that it can play an important role on species fitness but seems to be context dependent and not always produce a decrease in fitness Morales and Traveset (2008). Part of this unpredictability is due to the enormous variability of foreing pollen transferred in nature, where levels between 0 and 75 percent are seen, but most commonly values ranges between 0 and 20 percent of the total pollen load Bartomeus et al. (2008) Montgomery and Rathcke (2012) Ashman and Arceo-Gómez (2013) Fang and Huang (2013), being the generalist species the ones that receive greater loads of heterospecific pollen Fang and Huang (2013). Surprisingly, this low ranges of heterospecific pollen have been shown to decrease fitness greatly Thomson et al. (1982). Although heterospecific pollen quantity is fundamental to understand the outcome of the interaction so is the different traits of both pollen donor and recipient. Ashman and Arceo-Gómez (2013) postulated the first predictive framework for traits of heterospecific pollen effect, where different traits such as compatibility system and pollen size among others seems to be crucial to understand foreing pollen effect. Moreover, in Tong and Huang (2016) an assymetric effect was shown in a crossing experiment between 6 species of the genus *Pedicularis* where the pollen of long styled species was able to grow the full length of the style on short styled species but not viceversa. Despite these recent caveats, we still lack empirical evidence to affirm what are the main traits that drive heterospecific pollen effect for both pollen donor and recipient at seed production level. Interestingly, to comprehend how these traits interact is also crucial to look at the phylogenetic relatedness of the species. There is a considerable amount of literature of crosses between close related species Brown and Mitchell (2001) Arceo-Gómez et al. (2016) Tong and 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 variablity 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).

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 depend on any floral trait?

METHODS

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 where 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. Had-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 maded with Neubaeur chamber (each anther was counted 4 times for 20 different anthers per species).

Evolutive distance

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

Traits

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

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

RESULTS

DISCUSSION

Discussion

1. What are the implications of the findings?

CONCLUSIONS

ACKNOWLEDGEMENTS

REFERENCES

Aizen, M. A., and L. D. Harder. 2007. Expanding the limits of the pollen-limitation concept: Effects of pollen quantity and quality. Ecology 88:271–281.

Allaire, J., Y. Xie, J. McPherson, J. Luraschi, K. Ushey, A. Atkins, H. Wickham, J. Cheng, and W. Chang. 2018. Rmarkdown: Dynamic documents for r.

Arceo-Gómez, G., C. Alonso, T.-L. Ashman, and V. Parra-Tabla. 2018. Variation in sampling effort affects the observed richness of plant–plant interactions via heterospecific pollen transfer: Implications for interpretation of pollen transfer networks. American journal of botany 105:1601–1608.

Arceo-Gómez, G., and T.-L. Ashman. 2016. Invasion status and phylogenetic relatedness predict cost of heterospecific pollen receipt: Implications for native biodiversity decline. Journal of Ecology 104:1003–1008.

Arceo-Gómez, G., R. A. Raguso, and M. A. Geber. 2016. Can plants evolve tolerance mechanisms to heterospecific pollen effects? An experimental test of the adaptive potential in clarkia species. Oikos 125:718–725.

Ashman, T.-L., and G. Arceo-Gómez. 2013. Toward a predictive understanding of the fitness costs of heterospecific pollen receipt and its importance in co-flowering communities. American Journal of Botany 100:1061–1070.

Bartomeus, I., J. Bosch, and M. Vilà. 2008. High invasive pollen transfer, yet low deposition on native stigmas in a carpobrotus-invaded community. Annals of Botany 102:417–424.

Bascompte, J., P. Jordano, C. J. Melián, and J. M. Olesen. 2003. The nested assembly of plant–animal

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

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

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

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

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

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

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

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

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

177 Pauw, A. 2013. Can pollination niches facilitate plant coexistence? Trends in ecology & evolution
178 28:30–37.

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

181 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.

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