How phylogenetic relatedness and floral traits are involved in heterospecifc pollen effect in an artificial co-flowering community

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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 perpective can be negative due to competition (refs) or positive due to facilitation (refs). 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 quatities 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 relatednes is involve 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 heterospecifc 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 folowing 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

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

matter, 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 thow kinds of markers: 1) Internal transcribed spacer (ITS) and 2) ribulose-bisphosphate carboxylase (RBCL)

Traits

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

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

Discuss.

CONCLUSIONS

ACKNOWLEDGEMENTS

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