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Additional Information

Mating system of *Centaurea aspera* (asteraceae) polyploid relatives - Short communication

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ABSTRACT

Centaurea aspera polyploid complex represents a comprehensive model. The aim of this short communication was to study the mating system of the three main species. The results showed that allotetraploid *C. seridis* was self-compatible (SC), while autotetraploid *C. gentilii* was self-incompatible (SI) as the diploid parental *C. aspera* (SI).

Keywords: Compositae ; *Centaurea aspera* ; *Centaurea seridis* ; *Centaurea gentilii* ; allotetraploid ; autotetraploid ; self-incompatible ; self-compatible

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Introduction

Polyploidy is one of the most important processes in the evolution of flowering plants (Jiao et al. 2011). Around 35% of current species are considered to be polyploid, and genomic data even indicates ubiquity among angiosperms (Soltis et al. 2009; Levin 2019). *Centaurea aspera* L. polyploid complex is composed of natural populations with basic chromosome number $n = 11$ including the parental diploid (*C. aspera*), an allotetraploid (*C. seridis* L.) and an autotetraploid (*C. gentilii* Braun-Blanq. & Maire) (Ferriol et al. 2015). *Centaurea seridis* phenotype clearly differs from that of *C. aspera* and in a genetic analysis 44.4% of loci showed fixed or nearly fixed heterozygosity, which suggested an allotetraploid origin (Ferriol et al. 2014). In addition, there are several contact zones with triploid and tetraploid sterile hybrids (*C. ×subdecurrens* Pau 3x and *C. ×paucispina* (Ferriol, Merle & Garmendia) P.P.Ferrer 4x (Ferriol et al. 2012; Ferrer-Gallego et al. 2018). Tetraploid hybrid *C. ×paucispina* is completely sterile probably due to the odd number of homologous chromosomes (AAAB) (Garmendia et al. 2015). Such a comprehensive complex offers a great model that can be useful to better understand polyploid establishment and interactions (Garmendia et al. 2020). The aim of this short communication was to study the mating system of the three main species, *C. aspera*, *C. seridis*, and *C. gentilii*, to determine if they are self-compatible or self-incompatible and the ploidy level of progeny.

Materials and methods

Individuals of *C. aspera*, *C. seridis*, and *C. gentilii* were grown in the CIEF (Centro para la Investigación y Experimentación Forestal, Quart de Poblet, Spain) greenhouse. Cypselae were sampled from two natural populations of each species. *Centaurea aspera* populations came from Spain, while *C. seridis* and *C. gentilii* populations came from Morocco. The sampled capitula from the natural populations were stored at 4 °C for 2 months. In all cases, the sampled capitula came from 4 to 5 mothers under open pollination conditions. One hundred cypselae were randomly extracted from the mixture of capitula sampled from each population and were germinated. At least 50 plants of each population were grown in pots for the experiments.

Three controlled pollination treatments were carried out: (i) self-pollination; (ii) cross-pollination between individuals of the same species but different populations; and (iii) bagged treatment. Treatments were conducted during the flowering period, from June to August 2018 and 2019. Pollinations were performed with the newly open capitula bagged in semi-permeable nylon bags prior to anthesis. Upon anthesis, capitula were brushed gently against one another once a day on two consecutive days. In the bagged treatment, capitula were only bagged and not brushed. After pollinations, capitula were re-bagged for 6 weeks until fruit set. For each treatment, the total cypselae per capitulum were counted and the ploidy level of all the resulting cypselae was analyzed by flow cytometry, as described by Garmendia et al. (2015).

Results

In *C. aspera*, 50 self-pollinated capitula yielded only two cypselae (0.04 ± 0.03 cypselae per capitulum), while 98 intraspecific cross-pollinated capitula produced 257 cypselae (2.62 ± 0.35 cypselae per capitulum). Significant differences appeared between treatments (Kruskal-Wallis test: p -value $< 2.2 \times 10^{-16}$, SM 1). The bagged treatment yielded no cypselae (SM 1). In *C. seridis*, 30 self-pollinated capitula yielded 150 cypselae (5.00 ± 0.79 cypselae per capitulum), while 48 intraspecific cross-pollinations produced 234 cypselae (4.88 ± 0.64 cypselae per capitulum). The bagged treatment yielded fewer cypselae (3.11 ± 0.67 cypselae per capitulum), however, no significant differences were found with the other two treatments (SM 2). In *C. gentilii*, 36 self-pollinations yielded no cypselae, while 82 intraspecific cross-pollinations produced 192 cypselae (2.34 ± 0.41) with significant differences between treatments (KW test: $p = 7.2 \times 10^{-8}$, SM 3). The bagged treatment yielded no cypselae (SM 3).

As expected, the embryos that originated from the self-pollinations and intraspecific pollination crosses in *C. aspera* individuals were diploid as parental individuals. The embryos that originated from the self-pollinations and intraspecific pollination crosses of the *C. seridis* and *C. gentilii* individuals were all tetraploid as parental individuals.

Discussion

The results showed that the diploid *C. aspera* is self-incompatible (SI). The selfing treatment yielded only two cypselae on 50 capitula. These two cypselae could be due to either pollen contamination or a very small percentage of pseudo-self-compatibility (PSC). PSC is frequent in other *Asteraceae* (Bellanger et al. 2014). This result is in accordance with a previous study carried out in the field (Ferriol et al. 2015), confirming the self-incompatibility of *C. aspera*. *Centaurea seridis* from Morocco was self-compatible (SC) as self and bagged treatment produced a high number of cypselae. Spanish populations were previously determined in the field as being self-compatible (Ferriol et al. 2015), but the mating system of the isolated Moroccan populations was unknown until now. In both experiments (with Spanish and Moroccan populations), the bagged treatment yielded less cypselae than the self or cross treatments. This probably means that brushing (pollinator work) is important for pollen arrival, as *C. seridis* has not yet modified the floral structure for a better selfing benefits. The results showed that *C. gentilii* is self-incompatible, as the self and bagged treatments yielded no cypselae. Thus, the allotetraploid was able to break the self-incompatibility system, while the autotetraploid was not.

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Disclosure statement

The authors declare no conflict of interests.

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