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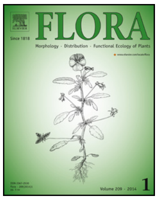

Variation of inbreeding depression in *Centaurea cyanus* L., a self-incompatible species

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ABSTRACT

The evolutionary trajectory of isolated or small populations of self-incompatible species depends on genetic variation in the capacity to transition to self-compatibility and the impact of inbreeding on plant fitness. *Centaurea cyanus* is an example of a self-incompatible, arable field-dependent weed species that is in regression in western Europe due to agricultural intensification. However, some cases of self-fertilization have been observed. Here, we investigated how inbreeding depression impacts the plants by studying two generations of self-fertilized families. As early as at the stage of the first selfing generation, we observed reduction in the number of florets per capitulum. By the second selfing generation, we observed reductions in germination rate, plant height, above-ground plant biomass, seed set and the final number and mass of seeds per plant. In particular, dramatically lower germination rate and reduced seed set contributed to a low cumulative fitness index of the generations that resulted from selfing. Nevertheless, although high average inbreeding depression was observed, large variation in cumulative fitness among families would allow some of these selfed families to persist and trigger evolution of selfing in small, isolated populations.

Introduction

Increased exploitation of agro-ecosystems is responsible for marked losses in biodiversity (Millennium Ecosystem Assessment, 2005). In particular, agriculture intensification has led to recent decreases in the frequency and abundance of arable weeds (Fried et al., 2009), which in turn reduces trophic resources for pollinators, phytophagous insects and granivorous animals on farmlands (Marshall et al., 2003; Storkey, 2006). The decrease in both the number of locations where a species is present and the number of plants in each population also has consequences for the biology of weed species.

There is a risk of reduced fecundity, especially in small and isolated populations of self-incompatible species, because of the reduced probability of finding compatible mates (Busch and Schoen, 2008). The ability to recognize and reject pollen that has at least one self-incompatibility allele in common with the maternal plant (Takayama and Isogai, 2005) enforces outcrossing and promotes gene exchange. Self-incompatible species are generally adapted to outcrossing to such an extent that any departure from outcrossing would cause inbreeding depression (ID), because of increased homozygosity of accumulative recessive lethal and/or mildly deleterious mutations, a feature less likely to occur in autogamous species (Charlesworth and Charlesworth, 1999).

High ID will allow for counter-selection of homozygous plants and genetic factors favoring selfing, thus maintaining the outcrossing regime strongly based on self-incompatibility (e.g., in small populations of the rare *Brassica insularis* (Glémin et al., 2005)), which in turn has dramatic impact on maintenance of populations in a context of pollinator decline (Eckert et al., 2010). However, a review of 150 plant species has shown that evolution toward selfing has occurred in many species under high ID (Goodwillie et al., 2005), either after purging genetic load or selection for flower morphology adaptation, etc. (Goodwillie et al., 2010; Thomann et al., 2013), thus opening the way for sustainable self-compatibility and mixed mating systems (Goodwillie et al., 2005; Porcher and Lande, 2005). Low ID also would open the way to selfing. Therefore, estimating potential ID and its variability has major importance of anticipating the fate of populations and the evolution of mating strategies.

One segetal arable weed that is markedly in decline in western Europe (Sutcliffe and Kay, 2000) is *Centaurea cyanus* L. (or the new scientific name *Cyanus segetum*, Hill), cornflower, though its true

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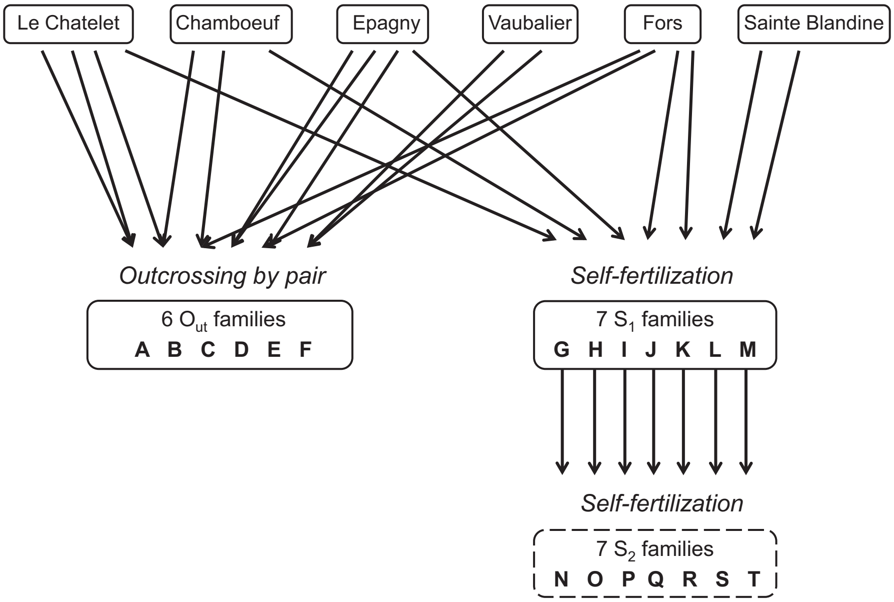

Fig. 1. Origin of the plant material used in the experiment. Field sampled populations are characterized by the name of the village where the seeds were sampled in France. Every arrow indicates the reproduction treatment of one single plant that resulted in the production of a seed family. The family letter code is arranged as the arrow order (e.g., P belongs to I which belongs to Epagny). O_{ut}: families obtained through outcrossing. S_1 and S_2: families obtained after one and two generations of self-pollination, respectively.

distribution has been obscured by widespread introductions and colonization from garden escapees and sowings from wildflower seed-mixes (Wilson, 2007). *Centaurea cyanus* is considered a self-incompatible and insect-pollinated annual Asteraceae species that depends largely on arable land in western Europe (Svensson and Wigren (1984); Bellanger et al., 2012). However, a recent study was at variance with this view as showing an unexpected high rate of autonomous autogamy in a *C. cyanus* population (up to 93% of capitula set on average 0.21 seed per floret; Penet et al., 2012). Our own study also showed plants producing several seeds through pollinator-mediated self-fertilization, but they were 12% only (they produced on average 0.11 seed per floret) while 88% produced less than 0.01 “accidental” seed per floret (Bellanger et al., 2014).

Thus, not all populations of *C. cyanus* are exclusively self-incompatible. Therefore, it is possible that the frequency of homozygous plants would increase if ID is not too detrimental, especially in regions where *C. cyanus* populations are small and threatened, like in western France (Bellanger et al., 2012), and in locations where the presence of pollinators is declining. Thus, the survival of endangered cornflower populations would depend on whether or not (1) inbreeding depression in this species is strong or not strong enough to allow sufficiently vigorous selfed plants to grow and reproduce, (2) continuous self-fertilization regime makes fitness costs worse, and (3) genetic variation provides room for the development of self-compatible subpopulations. To quantify the effects of inbreeding in *C. cyanus* populations, we compared the performance of outcrossed and self-pollinated progeny at five life stages: seed maturation, seed germination, juvenile survival, vegetative growth and reproduction. A second generation of self-pollinated seeds was also included in the study in order to exacerbate the effects of homozygosity on the potential ID.

Materials and methods

Seed materials

We collected *Centaurea cyanus* seeds in six fields in France (three fields in Burgundy and three in Poitou–Charentes), each having large populations (>100 individuals), in order to avoid phenomena related to biparental inbreeding and to achieve random representation of the species’ potentialities rather than properties of a given single population. These seeds were used to produce offspring, either isolated in individual insect-proof growth cages to obtain spontaneous selfed progeny, or randomly paired to produce outcrossed progeny as described in Bellanger et al. (2014). Only the central disc florets of the capitulum are hermaphrodite and have one single ovule each. Seeds produced by paired plants formed outcrossed O_{ut} seed families (seed set under selfing in absence of foreign pollen competition was, on average, lower than 2% (Bellanger et al., 2014), so that it is likely that O_{ut} seeds mostly belong to crosses). Seeds produced by isolated plants formed the S_1 self-pollinated seed families (Fig. 1).

We grew a sub-sample of every S_1 seed family, and again allowed them to self-fertilize; these seeds formed the S_2 seed families (Fig. 1) (Bellanger et al., 2014). The seeds were individually weighed, and those weighing less than 2.1 mg were considered aborted because the mean mass of empty achene envelope left after seed germination was 2.12 mg (with 0.04 the standard error of the mean). Seven S_1 families and seven S_2 families contained enough seeds (range from 10 to 30) and were used in the subsequent experiment.

Experimental design

Seeds of the three treatments, O_{ut}, S_1 and $S_{2,}$ were set to germinate in 19 cm × 13 cm × 4-cm polystyrene dishes (Caubère, Yèbles, France) lined with pleated strip paper in a temperature-regulated growth chamber at 15–25 °C (night–day) and a photoperiod of 12 h–12 h (provided by Osram L18W/640 fluorescent tubes). Newly germinated seedlings (from 3 to 27, according to families, see Section Result) were transplanted into 0.3 L pots of loam soil in a frost-free greenhouse (maximum 10 °C) for two months with 10 h day length to mimic the overwintering period. Then, plants were individually transplanted to 5 L pots of loam soil and randomly placed at 18–25 °C (night–day) under a photoperiod of 16 h day to initiate flowering in a 3 m × 9 m insect-proof cage in a regulated greenhouse.