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Original article

Is rapid evolution of reproductive traits in *Adonis annua* consistent with pollinator decline?

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ABSTRACT

Growing human footprint on the environment rapidly modifies the living conditions of natural populations. This could lead to phenotypic changes through both plasticity and evolution. Therefore, distinguishing the role of evolution in the phenotypic response to global change is a major challenge. In this study, we benefited from past and recent seeds from a population of the annual self-compatible weed *Adonis annua*. Seeds were sampled from the same locality at an 18 years interval and close to a region where reduction of bee pollinators' density has been reported. We used a common garden experiment to investigate evolutionary changes, between the old (1992) and the recent (2010) sample, for some reproductive traits expected to be under selection in the context of climate warming and pollinator decline. Plants of the recent sample flowered earlier, had larger flowers, but also evolved a shorter floral longevity. The capacity of plants to reproduce autonomously (autonomous selfing) was similar in the two samples. These results are consistent with adaptation of flowering phenology to climate warming and in part consistent with the evolution of increased pollinator attraction under pollinator decline. Together with other recent studies, this study provides evidence that short-term evolution is a frequent phenomenon accompanying global change.

1. Introduction

Human activities rapidly modify the environment of numerous species through land-use intensification, climate warming (Vitousek, 1994), and changes in community composition such as biological invasion (Vitousek et al., 1996) or pollinator decline (Potts et al., 2010). Species traits may partly change in response to new environmental conditions because of phenotypic plasticity, but recent reviews have additionally highlighted that global changes can be strong drivers of genetic evolution (Stockwell et al., 2003; Eckert et al., 2010; Hoffmann and Sgro, 2011; Hansen et al., 2012; Thomann et al., 2013). For example, the evolution of higher frequency of freezing sensitive morphs in populations of Thymus vulgaris was associated with climate warming (Thompson et al., 2013). However a major challenge when documenting phenotypic changes in the context of the global change is to differentiate genetic response from plasticity (Merilä and Hendry, 2014; Franks et al., 2014).

Wakening dormant propagules in common gardens (e.g. seed, cysts) and growing ancestors and descendants side-by-side to document phenotypic evolution (Franks et al., 2008; see also Orsini et al., 2013) has recently been developed in various groups of organism such as crustaceans (e.g. Kerfoot et al., 1999) or plants (Franks et al., 2007). This approach allows to attribute phenotypic differences between past and recent samples to evolution rather than plasticity. For example, in plants, such an approach was used to demonstrate rapid evolution of earlier flowering phenology in response to a drought event after only 7 years (Franks et al., 2007). Because adequate sources of stored seeds are currently rare, only few studies have been able to implement this approach in plants, but they did provide original empirical contributions to our knowledge of the short-term evolutionary processes accompanying different environmental changes, such as climate change (Franks et al., 2007; Vigouroux et al., 2011; Van Dijk and Hautekèete, 2014), biological invasion (Sultan et al., 2013) or pollinator decline (Thomann et al., 2015).

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Much attention has been given to the effect of climate change on plant phenology. Advance of flowering phenology with climate warming have been observed repeatedly (eg. Menzel et al., 2006; Calinger et al., 2013). Although there is growing evidence that evolution likely play a role in these phenotypic shifts, the role of evolution versus plasticity is still an active research question (Franks et al., 2014). The direct comparison of ancestors and descendants from population experiencing climate warming could provide important insight on this question.

Pollinator decline is another documented component of human-mediated environmental changes that is expected to affect plant populations (Potts et al., 2010). Most flowering plant species depend on pollinators for producing seeds (Ollerton et al., 2011). Accordingly, a large reduction in seed production is often detected when pollinators are locally rare (e.g. Gomez et al., 2010), particularly when plants could not insure reproduction through spontaneous selfing (Aguilar et al., 2006). Until now, studies have mainly focused on the possibility that pollinator decline endangers plant populations if increased pollen limitation also increase seed limitation (Lennartsson, 2002; Anderson et al., 2011). Pollinator decline may indeed ultimately lead to population extinction as suggested by documented cases of parallel decline of plant and their pollinators (e.g. Biesmeijer et al., 2006; Pauw and Hawkins, 2011; Anderson et al., 2011).

Beside its ecological consequences, pollen limitation is a major evolutionary factor on plant mating systems and floral traits (Lloyd, 1992). Increased pollen limitation can increase selection on floral traits involved in autonomous selfing ability (Fishman and Willis, 2008) or on pollinator attraction (Ashman and Morgan, 2004). Accordingly, theoretical works in plant mating system evolution have long shown that pollen limitation facilitates the evolution of selfing (Lloyd, 1979). Alternatively, it has also been predicted that pollen limitation could select for increased allocation to attractive traits because it increases the probability of pollen import (Haig and Westoby, 1988). Therefore, increased pollen limitation associated with pollinator decline could induce the evolution of one of two opposed reproductive strategy: increased autonomous selfing or increased pollinator attraction (discussed in Thomann et al., 2013).

So far, the evidence that pollinator decline can induce short-term evolution of plant reproductive strategies rely on few studies. One ex-situ experiment (Bodbyl Roels and Kelly, 2011) and one spatial comparison along an environmental gradient mimicking pollinator decline through time (Brys and Jacquemyn, 2012) have provided strong support for the possibility that plants adapt to pollinator decline through evolution of selfing ability. Additionally, a study of a natural population of the self-incompatible cornflower (*Centaurea cyanus*) reported the exaggeration of several floral traits involved in pollinator attraction after less than 20 years of evolution, in a context of pollinator decline (Thomann et al., 2015). These studies confirmed that both increased selfing and increased attractiveness are possible evolutionary trajectories under pollinator decline. A question emerging from these works is whether self-compatible plant species would preferentially evolved increased selfing or if they can also evolve increased pollinator attraction. A more comprehensive picture of the evolutionary consequences of rapid environmental changes for plant reproductive strategies needs new studies in wild populations.

Here we report an evaluation of the contemporary evolution of some reproductive traits in a plant population experiencing recent climate warming and pollinator decline. In a common garden experiment, we germinated seeds of the annual crop weed *Adonis annua* from two temporal samples (18 years apart) of the same locality. We studied whether the old and the recent samples differed in reproductive traits related to flowering phenology, pollinator attraction, reproductive investment and ability to self autonomously. We aimed to test whether evolution of floral traits occurred, and whether it is consistent with the evolution of autonomous selfing or the evolution of increased attractiveness toward pollinators.

2. Material and methods

A. annua (Ranunculaceae) is a self-compatible hermaphroditic annual crop weed. Flowers have deep red petals and are produced singly at the ends of the stems. The seed-head usually bears 25 to 35 achenes (hereafter seeds). At seed maturity, fertilized seeds are usually large, weighing between 8 and 14 mg and can easily be distinguished from the much smaller unfertilized or aborted seeds.

The studied population of *A. annua* was sampled in 1992 and in 2010 near Amiens in the north of France, 100 km away from a region where strong decline of Apoidae pollinators has been documented (Rasmont et al., 2005). Moreover, an increase in spring temperature during the 1990–2010 period has been recorded in the sampling region (see Suppl. Fig. A-1). In 1992, seeds from 50 randomly chosen plants were sampled in a cultivated field supporting approximately 300 *A. annua* plants (49°55′18″N; 2° 9′44.56″E). Because *A. annua* was not found in this particular field in 2010, the nearest field containing *A. annua* was sampled that year (1 km away; 49°55′33″N; 2° 9′3.85″E). In this second field, seeds were collected from 30 plants among approximately 500 plants. All seeds were sampled by the National Botanical Conservatory of Bailleul and then stored at 5 °C and under low humidity conditions. The botanical conservatory provided us with a sample of 100 seeds for each of the two samples.

The common garden experiment was conducted in the "Plateforme des Terrains d'Expériences du LabEx CeMEB"(43°38′19″N, 3°51′43″E) in Montpellier, south of France. Seeds were individually weighed and sown in January 2011 in a greenhouse under natural photoperiod without temperature control. Germination was recorded daily. Because spontaneous germination was low one month after sowing, a cold treatment (4 °C overnight) was conducted during one week. This treatment successfully improved germination. In early April 2011, seedlings of both temporal samplings were then transferred in 0.7 L pots and grown outside in a common garden until flowering.

At flowering (late spring 2011), and throughout the flowering period, all plants were placed in an insect-proof greenhouse. For each flower of each plant, we recorded: the date of anthesis, the date of senescence and we measured the flower diameter. From these data, we determined the following phenological traits for each plant: the onset date of flowering, the mean flowering date (i.e. the within-plant mean date of anthesis of its flowers) and the duration of the flowering period. We also determined the following floral traits for each plant: the mean flower size, the mean floral longevity, the mean floral display (i.e. the mean number of open flower per day over the flowering duration of a plant) and the total number of flowers produced. Finally, we estimated the capacity for autonomous selfing by calculating the ratio of the number of fertilized seeds to the number of ovules (seed: ovule ratio) in absence of pollinators (i.e. in the insect-proof greenhouse) for the first flower of each plant.

Seed mass was compared between populations with a Welch t-test. Germination success was compared with a Generalized Linear Model (GLM) using a binomial distribution and including seed mass as a covariate. The difference in the germination dynamic was tested with a Kolmogorov–Smirnov test. The difference in phenological traits (onset of flowering, mean flowering date, and flowering duration), floral traits (mean flower size, mean floral longevity, mean floral display and number of flowers produced)