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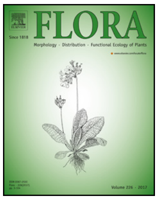

Prolonged stigma and flower lifespan in females of the gynodioecious plant *Geranium sylvaticum*

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ABSTRACT

In gynodioecious plants females need a reproductive advantage over hermaphrodites to be maintained in the same population. Generally, three main proximate causes for a female advantage are considered: inbreeding avoidance, different resource allocation patterns, and differences in ecological interactions. A mechanism potentially causing a female advantage that is rarely discussed is a difference in floral longevity between the genders. Females may have a longer stigma lifespan than hermaphrodites, which can affect pollination. Stigma and flower lifespan are rarely documented in gynodioecious species, although it is a common observation in dioecious species that female plants flower longer than males. Here we focus on the stigma and flower lifespan of gynodioecious *Geranium sylvaticum*, which could potentially contribute to the female advantage observed in this and other closely related species. We measured the stigma and flower lifespan in unpollinated flowers of female, hermaphrodite and plants with an intermediate gender expression. Our results show that stigma lifespan is almost twice as long in females as in hermaphrodites and intermediate plants. Also flower lifespan is longest in females. We discuss the potential mechanisms through which an increased floral lifespan can lead to a female advantage despite the generally lower pollinator visitation rates observed in females by reviewing available studies. Our study shows that increased floral persistence in females can be an important aspect in the maintenance of females in gynodioecious plants and should be taken into account as a potential proximate cause for a female advantage.

1. Introduction

Most flowering plant species are hermaphroditic, with individual flowers having both the male and the female function (Yampolsky and Yampolsky, 1922; Silvertown and Charlesworth, 2001). If a new genotype arises that produces flowers that have lost one of these functions, a reproductive advantage over the hermaphrodites is needed to invade and maintain itself in the population (Charlesworth and Charlesworth, 1978). The most common example of such polymorphic breeding system, found in approximately 2% of angiosperm genera (Dufay et al., 2014; Renner, 2014), are gynodioecious plants, where populations consist of a mixture of individuals with either hermaphrodite or female (i.e. male sterile or pistillate) flowers. To be maintained in the population, females that only contribute genes to the next generation through ovules, need to have a higher seed output than the hermaphrodites. If male sterility is solely determined by nuclear genes, the seed output needs to be at least double that of the hermaphrodites (Charlesworth and Charlesworth, 1978). A smaller advantage is enough if male sterility is determined by nucleo-cytoplasmic hereditary elements (Bailey et al., 2003).

Most studies on gynodioecious plants have indeed showed that total reproductive output is higher in females than in hermaphrodites. Females usually have a higher seed output, seeds that germinate better and/or offspring of a higher quality (reviewed in Shykoff et al., 2003; Dufay and Billard, 2012). Such female reproductive advantage has generally been attributed to three main, non-exclusive, proximate causes (Shykoff et al., 2003; Dufay and Billard, 2012). First, females may avoid inbreeding, since they obligatorily need a hermaphrodite for pollination. Evidence for potential inbreeding depression in gynodioecious plants has been found (reviewed in Dufay and Billard, 2012). However, many plants, including many gynodioecious ones, have evolved mechanisms to avoid self-pollination. Flowers can be dichogamous with intrafloral protandry as the most common pattern (Lloyd et al., 1986), where the male function is active first and the female function later, or flowers can have complete or partial self-incompatibility.

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Second, females investing relatively less in male reproductive tissue or floral attraction may allocate more resources towards higher seed output (Caruso et al., 2003). In low resource conditions, hermaphrodites may not be able to allocate enough resources to the female function and it is expected that females then have a relatively greater reproductive advantage (Ashman, 2007). In at least 14 gynodioecious species female frequency tended to be higher in populations where resource availability was lower (Ashman, 2007; Cuevas and López, 2011).

And third, the sex-specific ecological interaction hypothesis suggests that females may achieve higher seed production due to differences in ecological interactions with enemies or mutualists (Ashman, 2007; Vega-Frutis et al., 2013). Theoretically, females could be more attractive to pollinators if they offered more nectar than hermaphrodites, leading to higher pollination and fertilization rates. However, we are unaware of any study on gynodioecious plants showing pollination discrimination in favor of female plants. In fact, female flowers are almost always smaller than hermaphrodite flowers (reviewed in Delph, 1996 and Shykoff et al., 2003; e.g. Bai et al., 2011; Barr and Fishman, 2011; Cuevas and López, 2011; Griffin and Byers, 2012; Blank et al., 2014; Cuevas et al., 2014) and/or they produce less nectar (Varga et al., 2013) and pollen, leading to lower pollinator visitation rates (Delph, 1996; Ashman, 2000; Bai et al., 2011). On the other hand, the smaller size and number of flowers of female plants may also render them less attractive to enemies like florivores or seed herbivores, leading to a relatively higher seed output in females than hermaphrodites (Marshall and Ganders, 2001; Ashman, 2002; Asikainen and Mutikainen, 2005a; Collin and Shykoff, 2009; Clarke and Brody, 2015).

Whereas these three mechanisms leading to a female advantage have received a lot of attention in recent studies and reviews (Shykoff et al., 2003; Ashman, 2007; Dufay and Billard, 2012; Vega-Frutis et al., 2013), a mechanism that is rarely discussed is the potential difference in flower and stigma lifespan, which can affect pollination levels. In dioecious and monoecious plants female flowers generally remain open for a longer time than male flowers (Primack, 1985). For several protandrous gynodioecious plants it has been suggested that while overall flower lifespan may not differ between the sexes, female flowers become receptive earlier for pollen and have a longer stigma lifespan than hermaphrodite flowers (Shykoff, 1992; Williams et al., 2000; Rodriguez-Riano and Dafni, 2007; Molano-Flores and Faivre, 2015).

Only four studies have actually measured and compared floral lifespan in gynodioecious plants and all showed a longer floral lifespan in females. However, just one study compared floral lifespan between hermaphrodite and female flowers in the absence of pollinators (Arnan et al., 2014). In the other three studies (Ashman and Stanton, 1991; Pettersson, 1992; Yao and Luo, 2011) all flowers were allowed to be naturally pollinated and early floral or stigma senescence in the hermaphrodites may have been induced by faster pollination due to higher insect visitation, as floral persistence may depend on pollination (e.g. Niu et al., 2011).

Here we focus on the stigma and flower lifespan of gynodioecious *Geranium sylvaticum* (Geraniaceae) as a potential contributing factor to the female advantage observed in this and other closely related *Geranium* species (Table 1). *Geranium sylvaticum* is assumed to have a mixed nuclear-cytoplasmic sex determination system (Asikainen, 2004). The female frequency varies considerably between populations and ranges from 0 to 27% with an average of about 15% (Vaarama and Jääskeläinen, 1967; Asikainen and Mutikainen, 2003; Volkova et al., 2007). Female flowers are markedly smaller and have lower nectar sugar content than hermaphrodite flowers (Table 1). Also, insect visitation rates have been found to be lower for females (Table 1), although visitation rate by bumblebees may not differ between the sexes (Varga and Kytöviita, 2010). According to Asikainen and Mutikainen (2005a) pollen limitation is generally quite low and there is no difference in pollen limitation between sexes in this species. Hermaphrodite flowers are protandrous with the stigmatic lobes closely joined during initial pollen presentation (Varga et al., 2013), believed to strongly reduce autogamy. Female flowers start unfolding their stigma early after flower opening (Varga et al., 2013). Individual flower lifespan has not been measured, but for the closely related *G. richardsonii* it was suggested, but not measured, that female flowers have 2–3 days longer lifespan than hermaphrodites (Williams et al., 2000). Some *G. sylvaticum* plants produce female and hermaphrodite flowers, or flowers with a reduced number of anthers (flowers of intermediate sexual expression) (Putrament, 1962). The reported frequency of intermediates in populations ranges from 0 to 43% (Putrament, 1962; Volkova et al., 2007).

In this study, we tested whether the maximum flower and stigma lifespan in the absence of pollination is longer in females than in hermaphrodites of *G. sylvaticum*. We monitored individual flowers and recorded how long the flower and stigma remained open and receptive. A variable number of functional anthers in intermediate plants allowed us to test whether the level of male gender expression is correlated with flower and stigma lifespan. We discuss the potential mechanisms through which increased stigma lifespan can lead to a female advantage in this and other gynodioecious species.

2. Materials and methods

2.1. Plant species

Wood cranesbill, *Geranium sylvaticum* L. is a very common perennial herb that grows from heavily shadowed to non-shadowed habitats like deciduous forests, moist meadows and roadsides in large parts of Eurasia. In Central Finland flowering starts at the end of May with a peak in June and usually ends in July. According to Asikainen and Mutikainen (2005a), female plants start flowering three days earlier than hermaphrodites. The protandrous hermaphrodite flowers produce 10 stamens (Fig. 1A) with the anthers presenting pollen shortly after flower opening (Varga et al., 2013). For a variable period of time during this male phase, the five lobes of the stigma remain closely joined, after which the female phase starts when the lobes curl outwards thus exposing the pollen receptive surface (Fig. 2). In naturally pollinated, unmanipulated plants, the stigma unfolding occurs frequently after all the anthers have dropped (Müller, 1881). In females (Fig. 1B) the stigma generally opens during or shortly after flower opening. Nectar is produced at the base of the flower (Varga et al., 2015). Flower visitors and potential pollinators include bumblebees and other hymenopterans, syrphid flies and other diptera, and a pre-dispersal seed predator specialist weevil *Zacladus geranii* (Varga and Kytöviita, 2010; Varga, 2014). When the flower senesces, the petals drop, usually within a short period of time or all at once due to a disturbance (e.g. wind or a bumblebee visit, pers. obs.). Whereas Müller (1881) anecdotally reports a few observations of nectar consuming insect visitors on petal free flowers, Willson et al. (1979) did not observe any visitors after all petals were abscised in *Geranium maculatum*. Flowers have 10 ovules but usually a maximum of five seeds ripen per flower.

Most populations are gynodioecious with female plants producing male sterile flowers that have degenerated stamens and do not produce functional pollen (Fig. 1B). Stamen degeneration ranges from flowers having fully developed stamens but with the anther only producing sterile deformed pollen, through degenerated anthers to missing anthers and rudimentary filaments. Also, in many populations plants with intermediate flowers can be found