Light availability affects sex lability in a gynodioecious plant[1]

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Running head: Sex lability in *Geranium sylvaticum*.

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Abstract

Premise of the study: Sex lability (i.e. gender diphasy) in plants is classically linked to the larger resource needs associated with the female sexual function (i.e. seed production) compared to the male function (i.e. pollen production). Sex lability in response to the environment is extensively documented in dioecious species, but has been largely overlooked in gynodioecious plants.

Methods: Here, we tested whether environmental conditions induce sex lability in the gynodioecious *Geranium sylvaticum*. We conducted a transplantation experiment in the field where plants with different sex expression were reciprocally transplanted between high light and low light habitats. We measured plants' reproductive output and sex expression over 4 years.

Key results: Our results show that sex expression was labile over the study period. The light level at the destination habitat had a significant effect on sexual expression and reproductive output, as plants decreased their reproductive output when transplanted to the low light habitat. Transplantation origin did not affect any parameter measured.

Conclusions: This study shows that sex expression in *Geranium sylvaticum* is labile and related to light availability. Sexually labile plants did not produce more seeds or pollen, and thus, there was no apparent fitness gain in sexually labile individuals. Sex lability in gynodioecious plants may be more common than previously believed as detection of sex lability necessitates data on the same individuals over time which is rare in sexually dimorphic herbaceous plants.

Key-words: functional gender, Geraniaceae, gynodioecy; light availability; reproductive output; sex lability; sexual dimorphism.

INTRODUCTION

Gynodioecy is a relatively common breeding system found in about 7% of angiosperms (Silvertown and Charlesworth, 2001). In gynodioecious populations, male sterile (i.e. females) plants coexist with male fertile (i.e. hermaphrodite) individuals. The evolution and maintenance of such sexual polymorphism has been extensively investigated by evolutionary biologists for decades and is one of the most important transitions in plant breeding system evolution (Barrett, 2008). Understanding the causes and consequences behind female maintenance is challenging, because several opposing selective forces may be involved (e.g. Caruso and Case, 2012; Dornier and Dufaÿ, 2013).

Sex determination in gynodioecious species falls into two categories. Male sterility is caused either solely by nuclear genes (nuclear gynodioecy, NG, such as in *Fragaria virginiana*, Ashman, 1999) or, most commonly, male sterility is determined both by nuclear and cytoplasmic genes (nuclear-cytoplasmic gynodioecy, NCG, such as in *Silene vulgaris,* McCauley et al., 2000). How male sterility is determined is important to understand the fitness advantage needed for females to coexist with hermaphrodites (Bailey et al., 2003). In NG, females require at least a two-fold fitness advantage to invade and coexist within hermaphrodite populations, whereas in NCG, a small fitness advantage is enough, because cytoplasmic genes are only transmitted through ovules (see e.g. Caruso and Case, 2012).

Regardless of the nature of the determination of male sterility, plant sexual expression can be modified by the environment (Golenberg and West, 2013). Sexual plasticity in response to the environment has been extensively documented in dioecious species (reviewed in Korpelainen, 1998). Sexual lability (gender diphasy) is the potential to reproduce as one sex in one year and as the other in another year. In dioecious plants, changes in sexual expression have been linked to the resource needs associated with each sexual function (e.g. Freeman et al., 1980), as the female function (i.e. seed production) usually requires larger energy investment than the male function (i.e. pollen production). In this regard, two main hypotheses have been developed to explain why plants change their sexual expression (recently reviewed in Vega-Frutis et al., 2014). First, the size-advantage model predicts a change towards achieving more fitness through the female function as plants grow larger and accumulate resources above a certain threshold that allows reproduction (e.g. Yamashita, 2002). Second, the patchy environment hypothesis predicts that changes in sex expression are the result of the abiotic conditions experienced in spatially heterogeneous environments (Freeman et al., 1980). Male and female functions have different costs (reviewed in Delph, 1999). Seeds are considered more costly to produce, but they have a higher probability of contributing offspring to the next generation than pollen (Charnov 1982). In dioecious plants, sex change towards the less energy demanding sex (i.e. the pollen producer) should be selected in resource-limited habitats whereas in more nutrient-rich habitats, the opposite should be true. In gynodioecious plants, estimates of reproductive costs in each gender are not as straightforward as hermaphrodites have both sexual functions. In this system, even though females usually produce more seeds than hermaphrodites, hermaphrodites usually have similar or even larger overall reproductive costs. Hermaphrodites may produce similar or smaller number of flowers than females. However, in addition to pollen, the hermaphrodite flowers are larger and produce more nectar than those in females (Shykoff et al., 2003; Dufaÿ and Billiard, 2012). Overall, this results in similar total reproductive costs in both sexes (e.g. Ashman, 1994; van Etten et al., 2008, but see e.g. Delph, 1990; Gibson and Diggle, 1997).

As shown above, environmental resource limitation should theoretically translate into male-biased populations in dioecious systems. This has been shown also empirically: unfavorable conditions caused by drought, low nutrient levels, low light availability or low temperature have been linked to changes towards maleness in dioecious plants (Korpelainen, 1998). In theory, resource limitation could also affect sex expression in gynodioecious populations. Evidence for environmental factors inducing sex change in gynodioecious species is relatively limited (Table 1). As far as we know, less than 20 studies have reported sexual plasticity and only two of these studies have investigated and linked sexual plasticity to an environmental factor (low temperature, see Koelewijn and van Damme, 1996; Horovitz and Galil, 1972). The available evidence suggests that females tend to increase in frequency as resource availability declines (reviewed in Ashman, 2006). This somehow unexpected pattern has been ascribed to the effect of additional factors affecting sex ratios in gynodioecious populations through mating and pollination (see Case and Ashman, 2009).

Light availability can be a limiting resource and thus could in principle affect differently female and hermaphrodite plants even though this ecological factor has been largely overlooked. Light levels are very low under dense forest canopies and under layers of herbaceous vegetation. Assuming higher seed production in females (theoretically needed in order to be maintained within the same population with hermaphrodites), but larger or similar overall reproductive costs to hermaphrodites, high light availability should promote changes towards maleness in gynodioecious plants. We tested this hypothesis using the perennial gynodioecious herb *Geranium sylvaticum* as a model species. Light is likely to be a particularly important ecological factor for this species as the plants grow in both high light (meadows and road verges that receive full sky light conditions) and low light (under forest canopy) conditions.

We performed a reciprocal transplantation experiment in the field where we dug out individuals differing in their sex expression from low light and high light habitats and transplanted them into low light or high light habitats. We monitored the plants for five years and measured the reproductive output and their sex expression. We expected *G. sylvaticum* plants to change their sex expression when transplanted into different light habitats.

MATERIAL AND METHODS

Study species—*Geranium sylvaticum* (Geraniaceae) is a self-compatible, protandrous perennial plant with Eurasian distribution (Hultén and Fries, 1986) found in herb-rich forests, meadows and along roads. Most populations are gynodioecious (Asikainen and Mutikainen, 2003), containing both female and hermaphrodite individuals. In most populations, plants with an intermediate sexual expression can be found. The intermediate plants include individuals with a mixture of hermaphrodite and female flowers (e.g. gynomonoecious plants) and individuals displaying variability in the number of functional anthers among flowers (one to nine). The existence of intermediate phenotypes has been proposed to be the result of a polygenic restoration of male fertility in hermaphrodites (Koelewijn and van Damme, 1996; Ehlers et al., 2005; Dufaÿ et al., 2008). Hermaphrodites have been reported to produce more numerous and larger flowers that are visited more frequently by pollinators, but females usually have greater seed production than hermaphrodites with some differences between years and populations (Asikainen and Mutikainen, 2003).

Experimental setup—We selected three sites (Site 1: 61º 46' 38" N, 25º 49' 48" E; Site 2: 62º 21' 10" N, 24º 54' 23" E; Site 3: 62º 1' 12" N, 26º 17' 17" E) with abundant *G. sylvaticum* near Jyväskylä (Finland) and similar land-use history (Appendix S1, see Supplemental Data with the online version of this article). In each site, we selected two habitats differing in the amount of light that plants received (referred as High and Low light habitats hereafter). Light intensity in the Low habitats was below 30 KLux and between 140 – 150 KLux in the High habitats (measured with a HD 9221 Lux meter, Delta OHD, Padova, Italy). Distance between habitats within a site was at least 100 meters.

To evaluate whether plants were able to change their sex expression and the overall reproductive performance of the plants according to the light environment, four types of reciprocal transplantations were performed. The experimental plants were randomly chosen during the peak of flowering in June 2010 and classified as either male-sterile (referred as females here after) or male-fertile (referred as hermaphrodites here after) depending on whether they produced pollen (see below *Plant sexual expression*). Females and hermaphrodites from the low light habitat were transplanted to the high light habitat (Low to High transplantation) and transplanted back to low light habitat (Low to Low transplantation), and female and hermaphrodite plants from the high light habitat were transplanted back to high light (High to High transplantation) and low light habitat (High to Low transplantation) within each site. We aimed to use 20 females and 20 hermaphrodites of similar size in each site and habitat. However, it was not always possible to find that many plant pairs especially in the low light habitats, where the number of females was low. Altogether, 374 plants were transplanted and permanently marked. The number of plants used in each site is shown in Table 2. Unfortunately, due to human activities we lost the low light habitat from Site 3 in spring 2014. Therefore, data for 2014 were only collected from the two remaining experimental sites.

Plant sexual expression— In 2010, plants were classified as either female (i.e. with 0 functional stamens) or hermaphrodite (i.e. with ≥ 1 functional stamen) based on the number of functional stamens when the experiment was established. During the following years, flowering phenology of each individual was monitored throughout the flowering period until 2014. During the flowering period, the number of open flowers and the number of functional stamens in each open flower was recorded every second to fourth day. The sex expression (SEXP) in each plant was calculated every year at the end of each flowering period as the average number of functional stamens present in all flowers per plant. For each flower, the number of functional stamens (with fully-developed filaments and swollen pollen sacs containing purplish pigmented pollen grains) was recorded and values from 0 to 10 given according to the number of functional stamens present in each flower (e.g. 0 for a female flower with zero functional stamens; 10 for hermaphroditic flower with 10 functional stamens; 4 for an intermediate flower with four functional stamens). Therefore, SEXP of a plant ranges from 0 (all flowers produced zero functional stamens) to 10 (all flowers produced ten functional stamens), with intermediate plants with intermediate values.

Plants were classified at the end of the four-year study as sexually labile or sexually constant according to the constancy of their SEXP across all the study years. A plant was considered labile when the difference between the maximum and the minimum SEXP calculated through the study period was equal or larger than 1. Sexually constant plants were further divided into females and hermaphrodites. Female plants showed always a SEXP < 1 across the flowering seasons, whereas plants were classified as hermaphrodites when their SEXP was equal or larger than 1 within a flowering season. Intermediate plants were included in the hermaphrodite category as they produce pollen.

***Plant functional gender*—**Plant functional gender (G) was calculated for each plant following Lloyd's seminal paper (1980). Functional gender takes into account whether plants obtained their fitness through the production of seeds, pollen, or both. Ideally, functional gender should be obtained by considering the paternity of the seeds produced, which was not possible in our study. Therefore, we calculated functional gender as 1 minus the total number of seeds divided by the sum of seeds and stamens produced. Plants with a G = 0 reproduced exclusively as females, plants with G = 1 reproduced exclusively as males, whereas intermediate G values indicate reproduction via seed and pollen production.

***Reproductive measurements*—**Floral shoots were collected at the end of each fruiting season and the number of flowers and fruits were counted in each plant to estimate total flower and fruit production. To estimate the total seed production per plant, the number of seeds produced in each fruit was scored by counting the number of seed scars each fruit (*G. sylvaticum* fruits produce up to 5 seeds per fruit). Total stamen production per plant was calculated as the average number of functional stamens observed per open flower multiplied by the total number of flowers produced and it is used as a proxy for fitness through the male function.

***Soil nutrient analysis*—**Soil samples were collected in summer 2013 using a 6 cm in diameter soil core. The top 15-20 cm soil horizon was sampled in five randomly selected positions for each site and light habitat. The samples were air dried for one week at room temperature, weighed, and sieved through a 2 mm sieve. Soil organic matter (OM), pH, total nitrogen, potassium and phosphorus were analyzed by Suomen Ympäristöpalvelu Laboratorio—a laboratory certified by FINAS (Finnish Accreditation Service). Methods employed were SFS-EN 13037 (solid to liquid ratio of 1:5 v/v) for soil pH, SFS-EN 13039 (loss upon ignition at 550°C for 4 h, for organic matter content), SFS-EN 13654-1 (Kjeldahl method) for total nitrogen, and EPA3051 (microwave-assisted HNO_3 extraction) for total potassium and phosphorus.

***Statistical analyses*—**All statistical analyses were conducted in R (R Core Team, 2014). Differences in soil nutrient parameters were explored with ANOVA after fitting linear mixed-effect models (lmer) to the data using light habitat origin as the fixed factor and experimental site as the random component.

To infer between year variation in the proportion of flowering plant, generalized linear mixed-effects models were fitted to the data. In the model, we included year, light habitat origin (High, Low), light habitat destination (High, Low) and their interactions and plant gender (Female, Hermaphrodite, Labile) as fixed factors. Experimental site (1, 2, 3) and individual plants were included as random factors.

Generalized linear mixed-effects models (glmer) with binomial error structure were used to explore differences in whether or not plants showed sex lability between the light treatments. In the model, we included light habitat origin (High, Low), light habitat destination (High, Low) and their interaction as fixed factors and experimental site (1, 2, 3) as a random factor. A generalized linear mixed-effects model (glmer) with binomial error structure was used to explore differences in whether plants flowered after transplantation. In this model, we included plant sexual expression (continuous, 0 to 10), gender (Female, Hermaphrodite, Labile), light habitat origin (High/Low), light habitat destination (High/Low) and their interactions as fixed factors and experimental site (1, 2, 3) as a random factor. The models were fitted by Laplace approximation. Similarly, a generalized linear mixed-effects model with a negative binomial error structure was used to explore the differences in the accumulated number of seeds produced during the experimental period, using the same fixed and random components as above. For the differences in the accumulated number of functional stamens produced, a linear mixed-effect model was fitted after log-transformation of the data.

Finally, we analyzed whether light affected the functional gender of the plants with linear mixed-effects models (lmer) including light habitat origin (High/Low), light habitat destination (High/Low) and their interactions as fixed factors and experimental site (1, 2, 3) as a random factor. After fitting an adequate model, model residuals were always visually inspected. Means ± 1SE are indicated throughout the text.

RESULTS

***Soil nutrient analysis*—**Soil OM content and total N contents were higher in low light habitats ($F_{1,26} = 13.94$, $P < 0.01$; and $F_{1,26} = 4.12$, $P = 0.05$; for OM and N% respectively, Table 2) whilst soil pH and total P contents were slightly higher in high light habitats ($F_{1,26} = 5.60$, $P = 0.03$; and $F_{1,28} = 5.24$, $P = 0.03$; for pH and P% respectively, Table 2). Both habitats had a similar K content in the soil ($F_{1,26} < 0.01$, $P = 0.95$; Table 2).

***Plant sex lability*—**Out of the 326 plants that flowered more than once, 161 changed their sex expression during the experiment based on our definition of sex lability. Only the light level at the transplantation destination significantly affected plant lability ($\chi^2_1 = 3.72$, $P = 0.05$; $\chi^2_1 = 0.25$, $P = 0.62$; and $\chi^2_1 = 0.02$, $P = 0.89$ for the effect of transplantation destination, origin, and the interaction between these two factors respectively). Overall, 55.5% of plants showed lability when transplanted to High light habitats against 42.5% of plants transplanted to Low light habitats.

***Plant sex expression and functional gender across years*—** There was some variation between years in flowering, but we did not detect differences related to sex lability (Fig. 1; Appendix S2, see Supplemental Data with the online version of this article). On average, experimental plants flowered during 3.3 ± 0.1 seasons out of the 5 years the experiment lasted. The experimental plants showed variation in their sex expression also between years (Appendix S2; Fig. 2A, B). While 27.3% and 23.3% of the plants were constant females and hermaphrodites throughout the study period, 49.4% of the plants were labile. In addition, plants also showed variation in functional gender between years (Appendix S2; Fig. 3A, 3B). For all years, there was a binomial distribution of functional gender: plants' reproductive contribution was either through the female or the male function, with few individuals with similar female/male contributions (Fig. 3A). None of the light factors included in the model significantly affected functional gender ($\chi^2_1 = 0.10$, $P = 0.75$; and $\chi^2_1 = 0.88$, $P = 0.35$ for the effect of light at origin and destination respectively, all $P > 0.33$ for the interactions; Appendix S2).

***Total reproductive output*—**There was variation among years in all reproductive parameters investigated as well as several significant interactions between year and light at the transplantation origin and destination (Appendix S2). Overall, the accumulated total flower production during the experimental period was not related to sex lability, plant sex expression or light at the transplantation origin (Table 3). However, transplantation destination greatly influenced flower production (Table 3), with plants transplanted to the low light habitats producing 44% less flowers than plants transplanted to high light habitats (184.7 ± 11.8 vs. 81.8 ± 4.6 for High and Low light respectively; Fig. 4). Total seed production was also unrelated to sex lability, sex expression and the transplantation origin (Table 3) but again transplantation destination significantly affected the number of flower produced, which was 46% lower in plants transplanted to low light habitats (Fig. 5). Finally, the light levels at the transplantation origin or destination did not influence the total number of stamens produced during the study (Table 3). Constant females produced on average 3.4 ± 1.9 stamens while constant hermaphrodites and labiles produced 1428.8 ± 161.5 and 1038.2 ± 90.4 stamens respectively. The difference in the total number of stamens produced by hermaphrodites and labiles was not statistically significant (Tukey's *P* = 0.56).

DISCUSSION

In this long-term transplantation experiment, 49% of the plants changed their sex expression during the course of the study according to our classification of lability. Light availability at the destination habitat strongly influenced most reproductive parameters investigated, including the sex expression of the plants. Opposite to that, the habitat of origin of the plants did not have a significant effect on most parameters investigated.

Effects of plant gender and light availability on sex lability—According to our literature review sex lability has been examined in 21 gynodioecious species and about half of them appear able to change their sexual expression (Table 1). In the present study, almost half of the plants that reflowered at some point during the study period changed their sex expression as thus were classified as labile. Previous studies have reported either female plants (Widén and Widén 1999), or hermaphrodites (Koelewijn and van Damme 1996) to be more prone to show lability. However, these previous studies relied on classifying plants as either females or hermaphrodites by their sex expression in a given year, which in labile species is problematic. Male sterility in these systems as well as in *G. sylvaticum* is thought to be under cytonuclear control (Asikainen 2004). Therefore, male fertility factors are required to restore the male function. Consequently, the only explanation why some female plants are able to produce pollen at some point must be that these plants were in fact partial male steriles (PMS) that did not produce pollen when sexed. PMS have been reported in *G. sylvaticum* and other gynodioecious species (Koelewijn and van Damme, 1996). PMS individuals may possess a mixture of hermaphrodite and female flowers or display variability in the number of functional anthers among flowers (Varga and Kytöviita, 2014).

Light and other abiotic and biotic factors affect sex expression in dioecious species (Korpelainen, 1998). Among these factors, temperature has been observed to alter the expression of recessive male sterility in *Brassica oleracea* (Nieuwhof 1968) and *Hirschfeldia incana* (Horovitz and Galil 1972). Until now, temperature is the only factor linked to sex lability in gynodioecious plants: Koelewijn and Van Damme (1996) found that temperature influenced the sex expression of PMS *Plantago coronopus*, but pure females and hermaphrodites were stable in their sex expression. In the present experiment, light and nutrient availability were markedly different in the two habitats and it is sensible to assume there might have also been differences in water availability and temperature, although not measured. We observed that sex lability was related to habitat: plants transplanted in and to high light habitats were more labile in sexual expression than plants transplanted in and to low light habitats. This finding suggests that sex lability is related to resource acquisition.

What could be the reasons for the observed instability in sex expression in this species? Firstly, the mechanism resulting in male sterility may inherently not be stable through time and be a consequence of the restorer genetics behind male sterility, as previously discussed in the case of *P. coronopus* (Koelewijn and van Damme, 1996). Until the genetical mechanisms behind male sterility in *G. sylvaticum* are fully elucidated, this point cannot be solved. Secondly, higher plasticity may be a result of evolutionary selection. This assumes that there is a fitness benefit in lability and that lability can be inherited. In the present work, labile plants did not produce more seeds or more stamens than non-labile plants, so the fitness gain was not through increased female or male function. Lastly, lability could be related to plant aging and accumulating more resources (i.e. size-advantage model, Yamashita, 2002). However, from our data no clear pattern emerges as plants showed some variation between years in their sex expression and even functional gender. Even though this was a relatively long-term experiment, *G. sylvaticum* is a long-lived perennial. Important fitness consequences could take place later on during its life cycle. Taken all evidence together, the reasons and mechanisms behind sex lability in this species remain elusive and warrant further experiments.

***Effect of plant sex expression and light availability on reproductive output*—**Our long term results indicate that a similar proportion of plants re-flowered each year regardless of their sex expression. Environment induced plasticity in reproductive output in response to light has been previously investigated in the greenhouse using the close species *G. maculatum* (Van Etten et al., 2008) and in the field during seed maturation in *G. sylvaticum* (Varga et al., 2015). In both studies, seed production was limited by light availability and both sexes showed a similar reduction. This result can be explained by the lack of sexual dimorphism in physiological traits

(Van Etten et al., 2008). The current study corroborates these previous findings as flower and seed production were constrained by light availability and plants transplanted to the high light habitats produced 54% and 56% more flowers and seeds than plants transplanted to the low light habitats, regardless of their sexual expression.

In most gynodioecious species, females are usually reported to produce more flowers and more seeds than hermaphrodites (Shykoff et al., 2003), including *G. sylvaticum* (e.g. Asikainen and Mutikainen, 2003; Ramula and Mutikainen, 2003). Larger seed production has been proposed to be the main reason explaining female maintenance in this gynodioecious system (see e.g. Asikainen and Mutikainen, 2003). In contrast to our original hypothesis and the previous reports, the accumulated number of seeds produced by the two sexes did not differ significantly in the present three populations over five years. Taken all available evidence together, female maintenance cannot be simply explained by larger reproductive output in females (i.e. number of seeds). Moreover, females do not seem to produce larger seeds of better quality (germination) than hermaphrodites (Asikainen and Mutikainen, 2003; Varga, 2014) and previous research has shown that pre-dispersal seed predation exert a similar selective pressure on both genders (Varga, 2014). Having ruled out most of the explanations given to explain female maintenance in gynodioecious plants in general, the possibility of inbreeding depression avoidance in females appears as a likely candidate to explain the existence of females in *G. sylvaticum*. However, limited evidence suggests that inbreeding depression is minor in this species, at least in the seedling stage (Varga et al., 2013). Because the magnitude of inbreeding depression can differ during plant life cycle (Husband and Schemske, 1996), and inbreeding depression can be affected by different environmental stresses (see e.g. Heschel et al., 2005), we cannot completely rule out this possibility without targeted long-term experiments.

CONCLUSIONS

Sexes in *G. sylvaticum* showed lability in their sex expression related to the amount of light they received in their transplantation destination. Sex lability in gynodioecious plants may be more common than previously believed as detection of sex lability necessitates data on the same individuals over time which is rare in sexually dimorphic herbaceous plants. Commonly reported in dioecious species, we could also link sex lability to environmental factors in the present system, namely light and nutrient levels even though other unmeasured factors could have also contributed. The binomial distribution of the functional gender supports the view of gynodioecy as a pathway to dioecy in this species.

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TABLES

Table 1. Studies in which sex lability in gynodioecious species has been observed or mentioned. The number of plants observed and the proportion of individuals showing sex lability are given in brackets. NA: Data not available.

Species	Family	Lability	Contrast	Type of experiment, duration	Reference
Hirschfeldia incama	Brassicaceae	Yes (NA, NA)	NA	Greenhouse	Horovitz and Galil, 1972
Pachycerous pringlei	Cactaceae	No (168, 0%)	-	Field, 2 years	Molina Freaner et al., 2003
Gypsophila repens	Caryophyllaceae	No (800, 0%)	-	Field, 2 years	López-Villavicencio et al., 2003
Moehringia laterifolia	Caryophyllaceae	No (NA, 0%)	-	Greenhouse	Sugawara 1993
Schiedea adamantis	Caryophyllaceae	No (267, 2%)	-	Field, 7 years	Sakai et al., 1997
Silene acaulis	Caryophyllaceae	Yes (296, 8%)	NA	Field/greenhouse, 2 years	Klaas and Olson, 2006
		No (15, 0%)	-	Field, 2 non consecutive years	Philipp et al., 2009
Geranium sylvaticum	Geraniaceae	Yes (326, 49%)	F > H	Field, 4 years	This study
Glechoma hederacea	Lamiaceae	Yes (23, 9%)	F > H	Greenhouse, 2 years	Widen and Widén, 1999
Glechoma longituba	Lamiaceae	No (429, 0%)	-	Field, 3 years	Zhang et al., 2008
Thymus vulgaris	Lamiaceae	No (NA, 0%)	-	Field and greenhouse	Dommée and Assouad, 1978
Sidalcea malviflora	Malvaceae	No (NA, 0%)	-	Pers. obs.	Graff, 1999
Fuchsia microphylla	Onagraceae	Yes (18, 6%)	ns	Greenhouse	Arroyo and Raven, 1975
Fuchsia thymifolia	Onagraceae	Yes (55, 4%)	ns	Greenhouse	Arroyo and Raven, 1975
Plantago coronopus	Plantaginaceae	Yes (172, 6%)	ns	Field, 2 years	Koelewijn and van Damme, 1996
		Yes (148, 13%)	H > F	Growth chamber	Koelewijn and van Damme, 1996
Ochradenus baccatus	Resedaceae	No (150, 0%)	-	Field, 2 years	Wolfe and Shmida, 1995
Prunus mahaleb	Rosaceae	No (52, 0%)	-	Field, 4 years	Jordano, 1993
Rhodiola rosea	Rosaceae	No (NA, 0%)	-	Field, 2 years	Molau 1993
Lycium californicum	Solanaceae	No (52, 0%)	-	Field, 3 years	Miller and Venable, 2002
Lycium exsertum	Solanaceae	No (94, 0%)	-	Field, 5 years	Miller and Venable, 2002
Lycium fremontii	Solanaceae	No (130, 0%)	-	Field, 5 years	Miller and Venable, 2002
Daphne laureola	Thymelaeaceae	No (1737, 0%)	-	Field, 5 years	Alonso and Herrera, 2001
Thymelaea hirsuta	Thymelaeaceae	Yes (671, 16%)	-	Field, 6 years	Ramadan et al., 1994

Table 2. Number of *Geranium sylvaticum* plants transplanted and soil fertility parameters analyzed in 2013 for the Low and High light availability experimental habitats. Statistically significant differences between habitats are indicated by different subscripts within each column.

Light availability	Transplanted plants	pH	OM %	Total N %	Total P %	Total K %
Low	129	5.11 ± 0.09^a	11.6 ± 0.84^a	0.36 ± 0.03^a	0.06 ± 0.01^a	0.10 ± 0.02
High	235	5.36 ± 0.07^b	9.3 ± 0.47^b	0.32 ± 0.02^b	0.07 ± 0.01^b	0.10 ± 0.02

OM = organic matter, N = nitrogen, P = phosphorus, K = potassium.

Table 3. Statistical results for the effects of sex expression, plant gender, light availability at the transplantation origin and destination and their interactions on several reproductive traits in *Geranium sylvaticum.* Significant results are shown in bold.

		Reflowering		Total flower production		Total seed production		Total stamen production	
	df	χ	*P*	χ	*P*	χ	*P*	χ	*P*
Sex expression	1	2.35	0.13	3.15	0.08	3.58	0.06	146.39	**<0.01**
Plant gender (Gen)	2	0.56	0.76	1.75	0.42	2.22	0.33	152.96	**<0.01**
Transplantation origin (Ori)	1	1.40	0.24	0.93	0.34	0.17	0.69	0.24	0.62
Transplantation destination (Des)	1	0.84	0.36	13.11	**<0.01**	5.61	**0.02**	0.08	0.78
Gen × Ori	2	3.90	0.14	1.98	0.37	4.29	0.12	1.23	0.54
Gen × Des	2	1.20	0.55	1.62	0.45	3.01	0.22	3.29	0.19
Ori × Des	1	0.02	0.90	0.86	0.35	0.71	0.40	0.01	0.93
Gen × Ori × Des	2	0.88	0.64	1.09	0.58	2.84	0.24	0.02	0.99

FIGURE CAPTIONS

Fig. 1. Proportion of *Geranium sylvaticum* individuals flowering each year according to their sex expression.

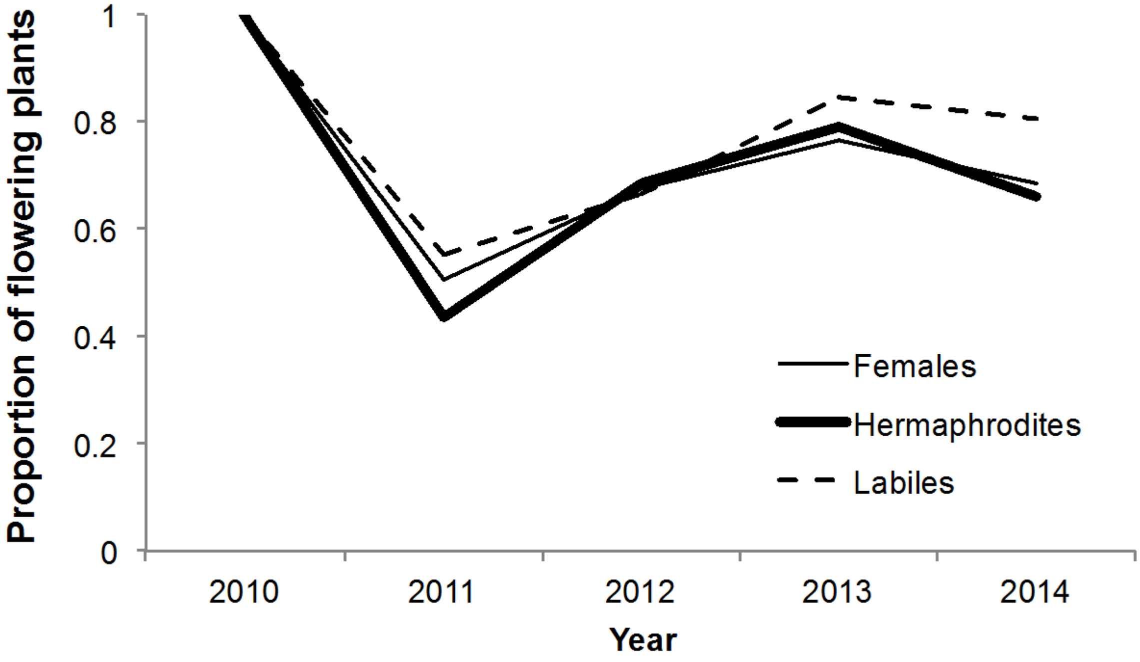


Fig. 2. A) Frequency of *Geranium sylvaticum* plants according to their sexual expression in each study year and B) Sex expression in the individual plants over the study years. Sex expression refers to the average number of functional stamens per plant. In B) constant females are shown by the horizontal line at 0 whereas constant hermaphrodites are shown by the horizontal line at 10. Each individual trajectory represents sex lability over time by one plant. See Material and methods for details.

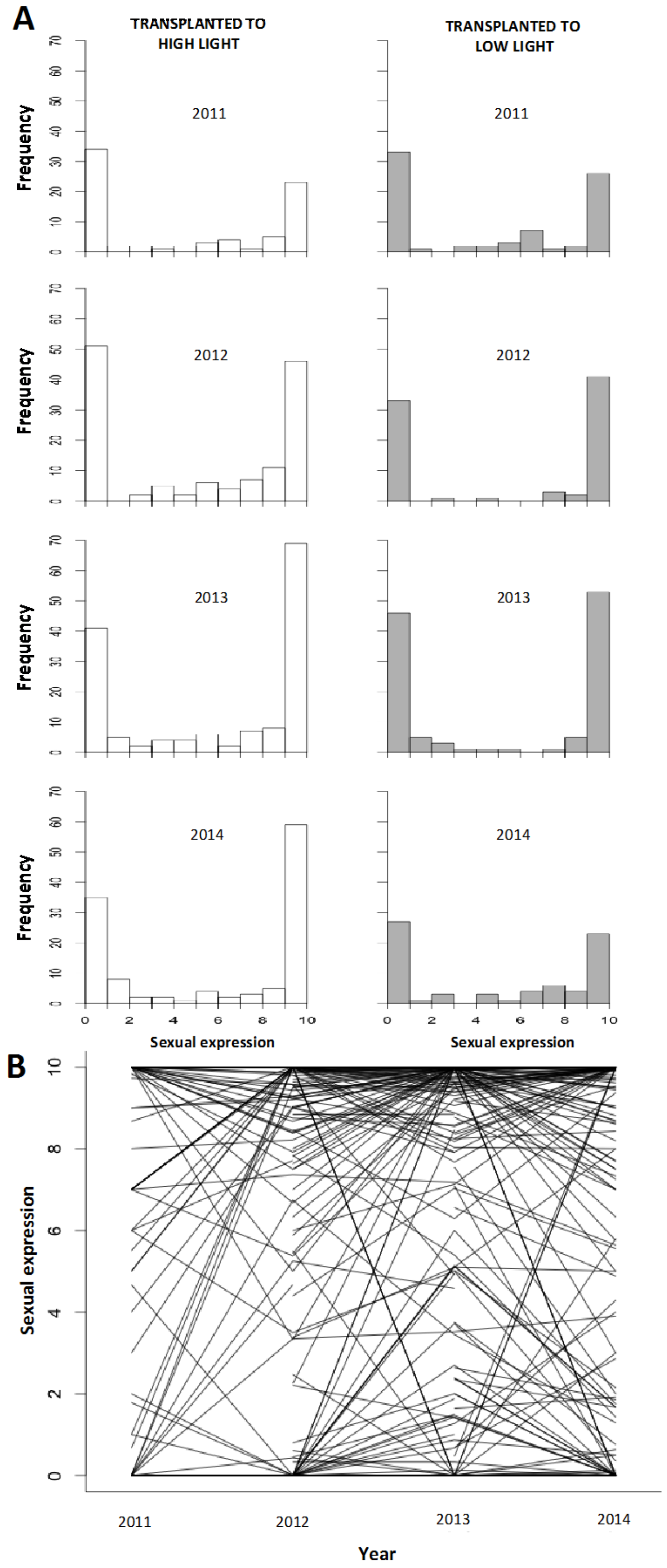

A
TRANSPLANTED TO HIGH LIGHT
TRANSPLANTED TO LOW LIGHT
Frequency
0
10
20
30
40
50
60
70
2011
2012
2013
2014
0
2
4
6
8
10
Sexual expression
B
Sexual expression
2011
2012
2013
2014
Year

Fig. 3. A) Frequency of *Geranium sylvaticum* plants according to their functional gender in each year and B) Functional gender variation in the individual plants over the study years. For any given year, 0 indicates plants that reproduced only by seeds (i.e. females); 1 indicates plants that reproduced only by pollen (i.e. males); whereas intermediate values indicate plants that reproduced both via seeds and pollen. In B) constant females are shown by the horizontal line at 0. See Material and methods for details.

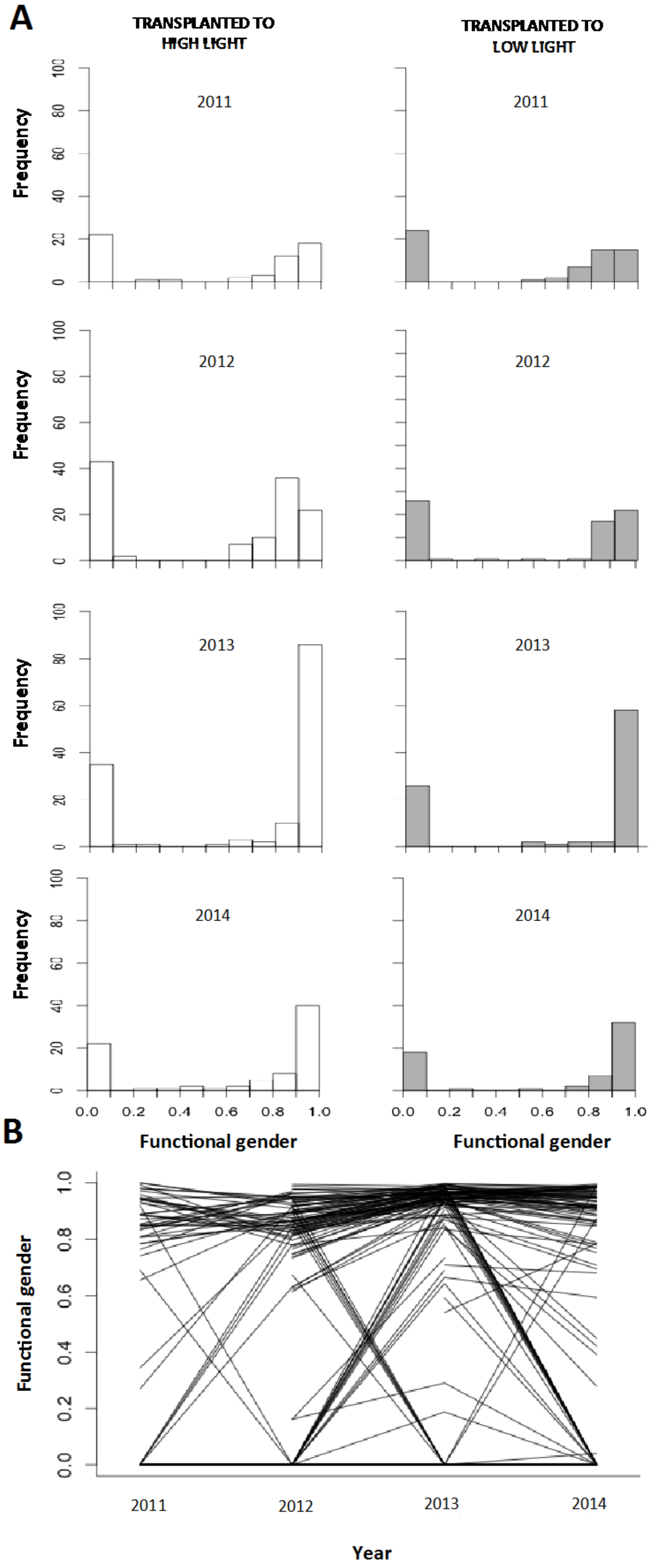

A
TRANSPLANTED TO HIGH LIGHT
TRANSPLANTED TO LOW LIGHT
2011
2012
2013
2014
Frequency
0
20
40
60
80
100
0.0
0.2
0.4
0.6
0.8
1.0
Functional gender
B
Functional gender
Year
2011
2012
2013
2014

Fig. 4. Total number of flowers produced during the experimental period (2010 – 2014) in female (white bars), hermaphrodite (black bars) and labile (grey bars) *Geranium sylvaticum* experimental plants transplanted to High or Low light habitats. Letters above the bars denotes significant differences between transplantation destinations at *P* < 0.05.

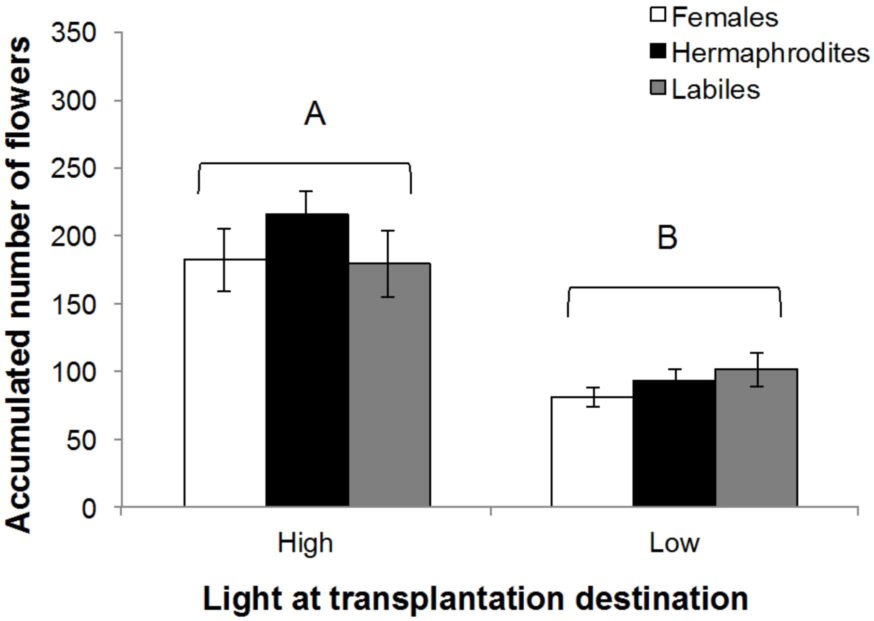
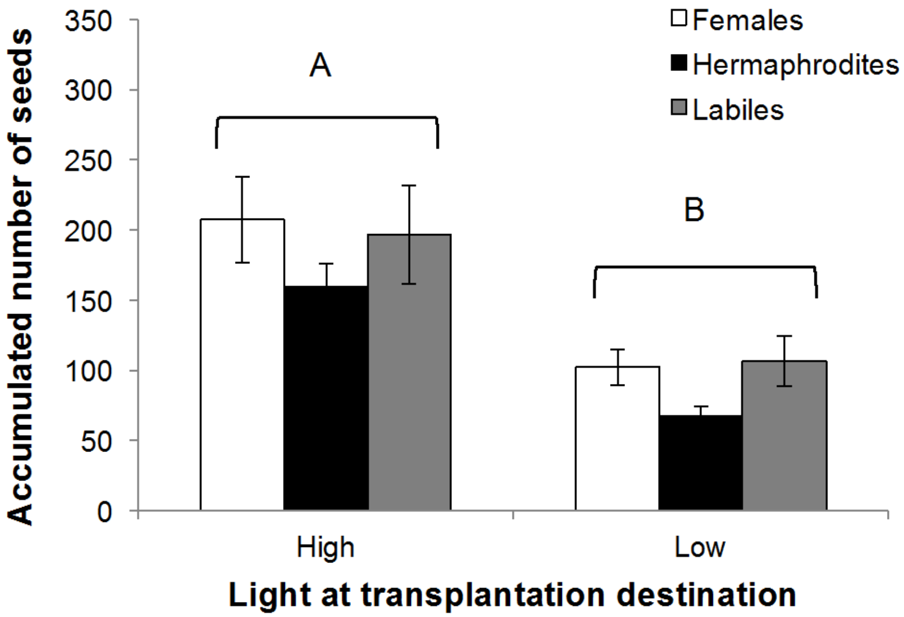


Fig. 5. Total number of seeds produced during the experimental period (2010 – 2014) in female (white bars), hermaphrodite (black bars) and labile (grey bars) *Geranium sylvaticum* individuals transplanted to High or Low light habitats. Letters above the bars denotes significant differences between transplantation destinations at *P* < 0.05.


Accumulated number of seeds
350
300
250
200
150
100
50
0
□ Females
■ Hermaphrodites
▨ Labiles
A
B
High
Low
Light at transplantation destination