

**This is an electronic reprint of the original article.
This reprint *may differ* from the original in pagination and typographic detail.**

Author(s): Varga, Sandra; Kytöviita, Minna-Maarit

Title: Sexes in gynodioecious *Geranium sylvaticum* do not differ in their isotopic signature or photosynthetic capacity

Year: 2017

Version:

Please cite the original version:

Varga, S., & Kytöviita, M.-M. (2017). Sexes in gynodioecious *Geranium sylvaticum* do not differ in their isotopic signature or photosynthetic capacity. *Plant Biology*, 19(6), 896-903. <https://doi.org/10.1111/plb.12606>

All material supplied via JYX is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of the repository collections is not permitted, except that material may be duplicated by you for your research use or educational purposes in electronic or print form. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone who is not an authorised user.

DR. SANDRA VARGA (Orcid ID : 0000-0001-9799-714X)

Article type : Research Paper

Handling Editor: Prof. W. Adams

Sexes in gynodioecious *Geranium sylvaticum* do not differ in their isotopic signature or photosynthetic capacity

Sandra Varga^{1*} & Minna-Maarit Kytöviita²

¹School of Life Sciences, Joseph Banks Laboratories, Green Lane, University of Lincoln, LN6 7TS, Lincoln, UK.

²Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35, FIN-40014 Jyväskylä, Finland

*Corresponding author: svarga@lincoln.ac.uk or sandravarga30@hotmail.com

Abstract

- In gynodioecious plants, females are expected to produce more or better seeds than hermaphrodites in order to be maintained within the same population. Even though rarely measured, higher seed production can be achieved through differences in physiology.
- In this work, we measured sexual dimorphism in several physiological traits in the gynodioecious plant *Geranium sylvaticum*. Photosynthetic rate, stomatal conductivity,

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/plb.12606

This article is protected by copyright. All rights reserved.

transpiration rate, water use efficiency and isotopic signatures were measured in plants growing in two habitats differing in light availability.

- Females have been reported to produce more seeds than hermaphrodites. However, we did not observe any significant difference in seed output between the sexes in these experimental populations. Similarly, the sexes did not differ in any physiological trait measured. Seed production was strongly limited by light availability. Likewise, differences between plants growing in full light vs. low light were detected in most physiological parameters measured.
- Our results show that the sexes in *G. sylvaticum* do not show any evidence of sexual dimorphism in physiology which concurred with the lack of sexual differences in seed output.

Key words: *Geranium sylvaticum*, gynodioecy, isotopic signatures, photosynthesis, sexual dimorphism, shade, $\delta^{15}\text{N}$, $\delta^{13}\text{C}$

Introduction

Sexually dimorphic plant species (i.e. where more than one sexual morph can be recognised) often display sexual differences in relation to reproductive allocation and physiology due to the different reproductive costs associated with each sexual function (Geber *et al.* 1999; Obeso 2002; Case & Ashman 2005). Seeds are considered more costly to produce but have a higher chance of producing offspring than pollen (Charnov 1982). In gynodioecious species, female plants must compensate for not fathering offspring as hermaphrodites do in order to be maintained within the same population (Lewis 1941; Charlesworth & Charlesworth 1978). This compensation is usually observed as an increased overall seed production in females or the production of better quality seeds than hermaphrodites (Shykoff *et al.* 2003). Due to the

trade-offs between plant functions, the increased reproductive output in females can be explained by differences in resource allocation patterns between the sexes (e.g. Eckhart 1992), the avoidance of inbreeding depression (Dufay & Billard 2012), or by differences between the sexes in mutualistic and antagonistic interactions (Clarke & Brody 2015; Van Etten & Chang 2014).

Differences in reproductive output between the sexes are sometimes accompanied by sex-specific differences in physiology (Case & Ashman 2005). Even though the available evidence for gynodioecious systems is limited, 12 studies have evaluated physiological traits in gynodioecious plants and the results are contradictory (Table 1). For example, the higher reproductive output seen in females could be explained by increased photosynthetic rates. However, when differences in photosynthetic rates between the sexes have been observed, females showed higher carbon assimilation rates than hermaphrodites only in 2 out of 10 studies. Looking at this limited evidence, it seems that the sexes in gynodioecious species show little sexual dimorphism in physiological traits, including carbon and nitrogen discrimination (Table 1). Nevertheless, physiological differences, together with the mechanisms to compensate for increased female reproductive output, determine the performance of each sex in different habitats and ultimately determine population structure.

Stable isotope ratios of carbon ($^{13}\text{C}:^{12}\text{C}$) and nitrogen ($^{15}\text{N}:^{14}\text{N}$) are increasingly being used in ecological studies as they provide a relatively fast and economic method to link physiological differences to nutrient use (Bhat & Bhat 2010; Silvertown *et al.* 2015). Isotope ratios are expressed as delta values (δ) and are measures of a parts-per-thousand ratio between the isotope ratio of a sample and that of an international standard. Because heavier isotope (^{13}C or ^{15}N) reaction affinity is different than that of lighter isotopes (^{12}C or ^{14}N), reaction processes result in substrates with relatively more heavy isotopes (more positive or enriched substrates), or substrates with relatively fewer of the heavy isotopes (and thus more

negative or depleted). For example, the carbon isotope ratio in leaves can be used as a proxy of stomatal conductance and integrated water-use efficiency (WUE) (Mole *et al.* 1994; Dawson *et al.* 2002) through the positive relationship between $\delta^{13}\text{C}$ and photosynthetic WUE (Farquhar *et al.* 1982). Plants growing without water limitation will have their stomata open and stomatal resistance will be minimal, thus the intercellular CO_2 concentration will be high and they will increasingly discriminate against $^{13}\text{CO}_2$ during photosynthesis, thus resulting in low (i.e., less positive) $\delta^{13}\text{C}$ values. In contrast, $\delta^{13}\text{C}$ values will be higher (i.e. more positive) under conditions of water stress (Farquhar *et al.* 1989). Similarly, the plant $\delta^{15}\text{N}$ value provides information about the source, absorption and assimilation of nitrogen in plants (Evans 2001).

Sexes in sexually dimorphic plant species have been shown to differ in C discrimination but whether the sexes differ in N discrimination as well is not known. Studies are limited especially in gynodioecious species (Table 1). Because sexual dimorphism can be modified by resource availability (Hesse & Pannell 2011), including light (Dykstra *et al.* 2009), we investigated whether females and hermaphrodites in the gynodioecious plant *Geranium sylvaticum* differ in seed production and physiological traits in two habitats differing in light availability. Light is likely to be a particularly important ecological factor for this species as *Geranium sylvaticum* grows in both high light (meadows and road verges that receive full sky light conditions) and low light (under forest canopy) conditions. Light availability may affect plant allocation patterns and thus plant reproductive output (Jacquemyn *et al.* 2010). In *G. sylvaticum*, light levels have been shown to limit seed production similarly in both sexes (Varga *et al.* 2015; Varga & Kytöviita 2016). Therefore, our specific research questions were: 1) Do the sexes differ in their reproductive output? 2) Do the sexes show sexual dimorphism in physiology and/or isotopic signatures? 3) How does light availability determine these responses?

Material and methods

Study species

Geranium sylvaticum L. is a rhizomatous, perennial plant with Eurasian distribution. Its habitats include damp woodlands, meadows, herb-rich forests and verges, so plants can be found in open habitats with full sunshine but also in shadowed habitats like forest understories. Populations are usually gynodioecious, with female frequency varying between 0.4 and 27.2% in Finland (Vaarama & Jääskeläinen 1967; Horovitz & Galil 1972; Asikainen & Mutikainen 2003). Female frequency seems to be related to light availability and females appear more common in shadow habitats compared to full light habitats (Kytöviita, unpublished results) and light availability has been shown to influence sex expression in this species (Varga & Kytöviita 2016). Bumblebees, bees, syrphid flies and other Hymenoptera pollinate the plants. Regarding pollen production, plants can be either classified as male-steriles (i.e. female plants) or males. Female plants have rudimentary, non-functional stamens and male plants possess flowers with one to ten functional stamens, so plants can be further classified as full hermaphrodites (i.e. producing only perfect flowers with 10 functional stamens), and intermediates (i.e. producing perfect flowers with one to nine functional stamens or with a mixture of pistillate and staminate flowers). The intermediate plants are probably the result of a partial male sterility restoration. In this work, we refer to the sexual expression of the plant at the individual level, so hermaphrodites may contain a variable number of fully hermaphroditic, fully female or intermediate flowers. Regardless of their sexual expression, all flowers have penta-locular ovaries and contain 2 ovules per locule, even though usually up to five seeds develop within each flower.

Experimental setup

Soil and leaf samples were collected in the beginning of July 2013 in three experimental populations established to evaluate long term reproductive output in response to light availability (see Varga & Kytöviita 2016 for details). Briefly, in 2010 we selected three meadows dominated by *G. sylvaticum* plants near Jyväskylä (Finland) with similar natural history (designated Site 1, Site 2 and Site 3). In each site, two habitats were chosen differing in the amount of light 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). Permanent plots were established and permanently marked in 2010 and all aboveground vegetation was removed. Altogether, 374 plants were used (see Varga & Kytöviita 2016).

Reproductive measurements

During the flowering period (end of May until the beginning of July), the number of open flowers and the number of functional stamens in each flower was recorded every fourth day. Floral shoots were collected at the end of the fruiting season and the number of flowers and fruits were counted in each plant to estimate total flower production. To estimate total seed production per plant, the number of seeds produced in each fruit was scored by counting the number of seed scars on the base of each fruit (*G. sylvaticum* fruits produce up to 5 seeds per fruit). Total stamen production per plant was estimated at the end of the flowering period by multiplying the average number of stamens recorded per plant by the total number of flowers produced.

¹⁵N and ¹³C determination

Soil samples were taken with a soil core (3 cm diameter) from the top 10 cm near the shoots of each plant. For the leaves, a whole fully expanded rosette leaf with no signs of damage was randomly selected per plant at about 25-30 cm height. Leaves and soil samples were dried at 36°C until constant weight and finely ground either manually in a mortar (soil) or using a FastPrep® FP120 Cell Disrupter (leaf). Samples were passed through a 0.125 mm sieve and 1.2 mg and 6.0 mg of leaf and soil sample, respectively, was weighed and wrapped into pre-weighed tin cups (Elemental Microanalysis, UK). Foliar and soil $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, N% and C% were determined using a Flash EA1112 element analyser (Carlo Erba) connected to a Finnigan Deltaplus Advantage (Thermo Electron Corp., Waltham, USA) continuous flow isotope ratio mass spectrometer.

Photosynthetic measurements

Photosynthetic measurements were conducted on plants growing in the experimental sites, but not included in the transplant experiment in June 2010 using Li-Cor 6400 Portable Photosynthesis System (Li-Cor, Lincoln, USA) equipped with the leaf chamber fluorometer. Measurements were made on clear days during 10:00 to 16:00. In addition, light response curves (LRC) for each sex were measured on a fully extended rosette leaf at about 25-30 cm height on each site and habitat. Five female and five hermaphrodite plants per site and habitat were chosen (N = 58 plants). For all LRCs, CO₂ flow in the reference chamber was set to 400 $\mu\text{mol s}^{-1}$, the leaf temperature was set to 25°C and the stomatal ratio was set to 0 (since stomata are present only in one side of the leaf). We measured the response of photosynthesis to five differing light levels (0, 100, 500, 1000 and 2000 $\mu\text{mol s}^{-1} \text{m}^{-2}$) using the Li-Cor 6400's internal red + blue light source. Leaves were allowed to acclimate for at least two

minutes before steady-state gas exchange properties were observed, logged and changed to the next light level using the Li-Cor 6400 light curve program.

Statistical analyses

Analyses were carried out with R 3.1.2 (R Core Team 2014). To test for significant differences in the number of flowers and seeds produced, we used ANOVA after using Generalised Linear Mixed Effects models (GLMER) with a negative binomial distribution to correct for the overdispersion observed in the data. The models included plant sex (Female/Hermaphrodite), light treatment (Low/High), and their interaction as fixed factors and experimental site was included as a random factor. Whether light availability affected stamen production in hermaphrodites was analysed with a GLMER including light treatment (Low/High) as fixed factor and experimental site as a random effect.

Data on leaf and soil ^{13}C , ^{15}N and C and N concentration in leaves were analysed with Linear Mixed Effects (LMER) models. Correlations between these soil and leaf parameters were performed with Spearman's correlations.

LMER were also fitted to the data on physiological traits (maximum photosynthetic rate, stomatal conductivity, transpiration rate and WUE) including plant sex and light habitat as fixed factors and experimental site as a random component. Finally, to examine the relationship between photosynthetic rate and PAR, LMER with plant sex (Female/Hermaphrodite), light availability (High/Low), PAR (0, 100, 500, 1200, 2000), and their interactions were included as fixed factors and experimental site as random factors. Differences due to the significant interaction between light and PAR level were investigated with Tukey's planned comparisons using the 'lsmeans' package (Lenth 2015).

Results

Reproductive output

In 2013, a similar proportion of females (69.4%) and hermaphrodites (71.6%) flowered ($\chi^2_1 = 2.14$, $P = 0.14$) regardless of the Light treatment ($\chi^2_1 = 3.37$, $P = 0.07$ and $\chi^2_1 = 2.03$, $P = 0.15$, for the main effect of light and its interaction with plant sex respectively). Plants from the Low light habitat produced 31% less flowers than plants from the High light habitats ($\chi^2_1 = 20.31$, $P < 0.01$; Fig. 1a) and there was no statistically significant difference between the sexes ($\chi^2_1 = 1.67$, $P = 0.20$) and no significant interaction between plant sex and light treatment was detected ($\chi^2_1 = 0.12$, $P = 0.73$).

Total seed production was also significantly lower in Low light plants ($\chi^2_1 = 43.43$, $P < 0.01$; Fig. 1b) and the sexes produced similar amount of seeds ($\chi^2_1 = 0.27$, $P = 0.60$; and $\chi^2_1 = 3.03$, $P = 0.08$ for the effect of sex and the interaction with light, respectively).

In hermaphrodites, total stamen production per plant was significantly reduced by light availability ($\chi^2_1 = 37.00$, $P < 0.01$). Hermaphrodites from High light produced 638.37 ± 71.7 stamens per plant compared to 189.7 ± 31.0 stamens per plant in Low light.

Foliar isotopic signatures and N and C concentration

The sexes had similar foliar N and C isotopic signatures and concentrations (Table 2).

However, significant differences in all foliar parameters analysed were detected between High and Low light habitat plants (Table 2). Plants growing in the Low light habitats had lower $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ than plants growing in the High light (Supplementary Table S1; Fig. 2). Moreover, leaves in Low light habitats had 2% less C and 0.4% less N than leaves in the High light habitats (Table S1).

Soil isotopic signatures and N and C concentration

Whether the soil was collected below a female or a hermaphrodite plant had no effect on the soil isotopic signatures or N and C concentrations (Table 3, Supplementary Table S2).

Moreover, whilst the light habitat did not influence C isotopic signature nor concentration, soil in Low light habitats had lower $\delta^{15}\text{N}$ and N concentration than soil in High light habitats (Table 3, Supplementary Table S2).

Relationship between foliar and soil N and C isotopic signatures

Leaf ^{15}N covaried positively with soil ^{15}N ($t_{130} = 7.11$, $P < 0.01$) and leaf N% ($t_{130} = 6.04$, $P < 0.01$). Opposite to this, there was no significant relationship between ^{13}C in the leaf and in the soil ($t_{130} = 0.070$, $P = 0.94$) even though leaf ^{13}C was positively correlated with C concentration in the leaf ($t_{130} = 7.51$, $P < 0.01$; Fig. 2).

Photosynthetic measurements

Light response curves were very similar between the sexes (Fig. 3). We observed that plants in High light had lower light compensation points (i.e. the light intensity where the rate of photosynthesis matches exactly the rate of respiration) than Low light plants (Fig. 3).

Photosynthetic rates increased with PAR ($F_{4,221} = 336.27$, $P < 0.01$) and were overall higher in High light plants ($F_{1,53} = 52.35$, $P < 0.01$), but there was a significant interaction between habitat and PAR ($F_{4,221} = 8.64$, $P < 0.001$). While in High light plants maximum photosynthetic rate was already achieved at 1200 PAR (Fig. 3A), in Low light plants photosynthetic rate was maximum at 2000 PAR even though it did not reach a plateau (Fig. 3B). No significant differences between the sexes were detected in photosynthetic rate at any PAR ($F_{1,53} = 0.95$, $P = 0.34$; and $F_{4,221} = 1.28$, $P = 0.28$ for the effect of sex and the

Accepted Article
interaction between sex and PAR, respectively). All other interactions were not statistically significant (all $P > 0.28$).

Under High light conditions, plants also had higher transpiration (E), conductance (gs), and WUE than plants growing under Low light conditions, and there was no significant sexual dimorphism in any of these traits (Table 4).

Discussion

Physiological differences between the sexes in gynodioecious species are predicted due to the different costs of reproduction associated with each sex (Geber *et al.* 1999; Reekie & Bazzaz 2005) and should be more apparent when the costs of reproduction and the pattern of resource allocation are very different between sexes. We detected few differences between the sexes in the physiological parameters measured, which agrees with the largely similar reproductive output observed in these populations.

Even though the reproductive effort of *G. sylvaticum* has been found to differ between the sexes in several studies (see Table 1 in Elzinga & Varga 2017), we did not observe any difference in the proportion of flowering plants between the sexes nor in flower or seed production during the study period. Estimating the costs of reproduction is challenging (Ashman 1994; Obeso 2002) and even more so in gynodioecious perennial plants, where both sexual functions are present within the hermaphrodite plants. Moreover, unless long-term observations are made, it is virtually impossible to make accurate estimates of the demographic costs imposed by the costs of reproduction in any long-lived perennial. The lifespan of *G. sylvaticum* plants has been estimated to be more than 20 years (Klimesová & de Bello 2009). Nevertheless, following the same plants for five consecutive years, we did not detect any significant difference in the total number of seeds produced by the two morphs

in the present study populations (Varga & Kytöviita 2016) and therefore we conclude that total seed production is similar between the genders in our experimental populations. Moreover, even though we did not investigate seed germination in these experimental populations, previous studies have showed that pre-dispersal seed predation is similar in both genders (Asikainen & Mutikainen 2005b; Varga 2014) and seed germination is also not related to the gender producing the seeds (Asikainen & Mutikainen 2003; Varga 2015) and therefore, there is no reason to expect differences in our study populations.

Besides seed production, total reproductive costs entail also the costs associated with floral structures and pollen production. For any given flowering event, given the larger floral size together with the higher production of nectar and pollen in hermaphrodite *G. sylvaticum* (Varga *et al.* 2013), we could hypothesise that the total costs of reproduction might have been larger in hermaphrodite plants. Therefore, we expected the hermaphrodites to increase their photosynthetic rates to fulfil the energetic demands of the larger reproductive costs. Giving some support to this idea, we observed that the photosynthetic light compensation point was lower in hermaphrodites when compared to females in shaded habitats. Overall, and corroborating previous findings (Varga *et al.* 2015; Varga & Kytöviita 2016) light was an important factor limiting seed production but similarly in both genders. Females and hermaphrodites both achieved higher photosynthetic rates, transpiration, stomatal conductance and water use efficiency when grown under full light. The two genders had similar light response curves and isotopic signatures in the leaves, suggesting little sexual dimorphism in physiological traits. Similar results have been reported for other gynodioecious plants including the close species *G. maculatum* (Table 1).

It should be pointed out, that besides the differences in light and nutrient availability measured in the two habitats (Varga & Kytöviita 2016), it is sensible to assume that, although not measured, there might have been differences also in water availability and temperature.

All these parameters may influence seed production even though they do not seem to affect the plants in a sex-specific manner in our study. Combined, the available evidence suggests that the sometimes reported increased reproductive output in females may not be due to physiological mechanisms increasing photosynthetic capacity, but by other mechanisms such as habitat selection or inbreeding depression avoidance. While the latter remains to be measured in natural populations of *G. sylvaticum*, certain habitat selection by the two genders has been noted, as female frequency is higher in shaded environments (Kytöviita, unpublished results). Even though the sex ratio seems to be related to some extent to light availability, the flowering frequency of the two sexes was similar when studying the same populations for five years (Varga & Kytöviita 2016).

Concurring with the physiological differences between high and low light habitat plants, foliar isotopic signatures were also statistically significantly different. The less negative values of $\delta^{13}\text{C}$ in high light plants may reflect the drier conditions in high light habitats or the higher WUE of plants growing under full light (Farquhar *et al.* 1989). Moreover, there was a negative relationship between foliar N% and $\delta^{13}\text{C}$ hermaphrodites, but not in females suggesting higher nitrogen use efficiency of photosynthesis in hermaphrodites under high light. Plant $\delta^{15}\text{N}$ usually reflects the soil $\delta^{15}\text{N}$ (Kahmen *et al.* 2008), which was the case here as well.

To conclude, this study demonstrates that the sexes in *G. sylvaticum* do not differ markedly in physiology or isotopic signatures, which concurs with a similar reproductive output and reproductive costs. Hermaphrodites appeared to have better photosynthetic nitrogen use in high light. This physiological difference does not provide any explanations for the evolutionary maintenance of females. We have previously shown that the sexes do not differ in their tolerance to light limitation during seed maturation, a period when plants may receive less light due to the natural closing of the canopy or as a result of self-shading (Varga

et al. 2015). In the present case, both genders decreased seed production similarly in response to shade, suggesting that light limitation is not an important factor determining female maintenance in this gynodioecious species. Having ruled out differences in physiology, the importance in inbreeding avoidance in explaining female maintenance in this species remains to be tested.

Acknowledgments

We thank Pasi Reunanen for help establishing the experimental sites. Metsähallitus for permission to use the field sites, and Tuula Sinisalo for the isotopic analyses. Funding was provided by the Academy of Finland (project number 250911) to SV.

References

- Ashman T.-L. (1994) Reproductive allocation in hermaphrodite and female plants of *Sidalcea oregana* ssp. *spicata* (Malvaceae) using four currencies. *American Journal of Botany*, **81**, 433–438.
- Asikainen E., Mutikainen P. (2003) Female frequency and relative fitness of females and hermaphrodites in gynodioecious *Geranium sylvaticum* (Geraniaceae). *American Journal of Botany*, **90**, 226–234.
- Asikainen E., Mutikainen P. (2005a) Pollen and resource limitation in a gynodioecious species. *American Journal of Botany*, **92**, 487–494.
- Asikainen E., Mutikainen P. (2005b) Preferences of pollinators and herbivores in gynodioecious *Geranium sylvaticum*. *Annals of Botany*, **95**, 879–886.
- Bhat M.I., Bhat A. (2010) Applications of stable and radioactive isotopes in soil science. *Current Science*, **98**, 1458–1471.
- Caruso C.M., Yakobowski S.J. (2008) Selection on floral and carbon uptake traits of *Lobelia siphilitica* is similar in females and hermaphrodites. *Journal of Evolutionary Biology*, **21**, 1514–1523.
- Caruso C.M., Maherali H., Jackson R.B. (2003) Gender-specific floral and physiological traits: implications for the maintenance of females in gynodioecious *Lobelia siphilitica*. *Oecologia*, **135**, 524–531.
- Case, A.L., Ashman T.-L. (2005) Sex-specific physiology and its implications for the cost of reproduction. In: Reekie E.G., Bazzaz, F.A. (Eds) *Reproductive Allocation in Plants*.

Elsevier Academic Press, San Diego, CA: 126-154.

- Case A.L., Barrett S.C.H. (2001) Ecological differentiation of combined and separate sexes of *Wurmbea dioica* (Colchicaceae) in sympatry. *Ecology*, **82**, 2601–2616.
- Chang S.M. (2006) Female compensation through the quantity and quality of progeny in a gynodioecious plant, *Geranium maculatum* (Geraniaceae). *American Journal of Botany*, **93**, 263–270.
- Charlesworth B., Charlesworth D. (1978) A model for the evolution of dioecy and gynodioecy. *American Naturalist*, **112**, 975–997.
- Charnov E.L. (1982) *The Theory of Sex Allocation*. Princeton University Press: 355 pp.
- Clarke G.L., Brody A.K. (2015) Gender inequality in predispersal seed predation contributes to female seed set advantage in a gynodioecious species. *Ecology*, **96**, 1309–1317.
- Culley T.M., Dunbar-Wallis A.K., Sakai A.K., Weller S.G., Mishio M., Campbell D.R., Herzenach M. (2006) Genetic variation of ecophysiological traits in two gynodioecious species of *Schiedea* (Caryophyllaceae). *New Phytologist*, **169**, 589–601.
- Dawson T.E., Mambelli S., Plamboeck A.H., Templer P.H., Tu K.P. (2002) Stable isotopes in plant ecology. *Annual Review of Ecology and Systematics*, **33**, 507–559.
- Delph L.F., Carroll, S.B. (2001) Factors affecting relative seed fitness and female frequency in a gynodioecious species, *Silene acaulis*. *Evolutionary Ecology Research*, **3**, 487–505.
- Dufayé M., Billard E. (2012) How much better are females? The occurrence of female advantage, its proximal causes and its variation within and among gynodioecious species. *Annals of Botany*, **109**, 505–519.
- Dykstra A.B., Brock M.T., Delph L.F., Weinig C. (2009) Sex-specific trade-offs and responses to foliar shade in the gynodioecious species *Silene vulgaris* (Caryophyllaceae). *International Journal of Plant Sciences*, **170**, 575–583.
- Eckhart V.M. (1992) Resource compensation and the evolution of gynodioecy in *Phacelia linearis* (Hydrophyllaceae). *Evolution*, **46**, 1313–1328.
- Elzinga J.A., Varga S. (2017) Prolonged stigma and flower lifespan in females of the gynodioecious plant *Geranium sylvaticum*. *Flora*, **226**, 72–81.
- Evans R.D. (2001) Physiological mechanisms influencing plant nitrogen isotope composition. *Trends in Plant Science*, **6**, 121–126.
- Farquhar G.D., Ehleringer J.R., Hubick K.T. (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Biology*, **40**, 503–537.
- Farquhar G.D., O'leary M.H., Berry J.A. (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Functional Plant Biology*, **9**, 121–137.
- Geber M.A., Dawson T.E., Delph L.F. (1999) *Gender and Sexual Dimorphism in Flowering*

Plants. Springer-Verlag, Berlin: 305 pp.

- Hesse E., Pannell J.R. (2011) Sexual dimorphism in a dioecious population of the wind-pollinated herb *Mercurialis annua*: the interactive effects of resource availability and competition. *Annals of Botany*, **107**, 1039–1045.
- Horovitz A., Galil J. (1972) Gynodioecism in east Mediterranean *Hirschfeldia incana*. Cruciferae. *Botanical Gazette*, **133**, 127–131.
- Jacquemyn H., Brys R., Jongejans E. (2010) Size-dependent flowering and costs of reproduction affect population dynamics in a tuberous perennial woodland orchid. *Journal of Ecology*, **98**, 1204–1215.
- Kahmen A., Wanek W., Buchmann N. (2008) Foliar $\delta^{15}\text{N}$ values characterize soil N cycling and reflect nitrate or ammonium preference of plants along a temperate grassland gradient. *Oecologia*, **156**, 861–870.
- Klimešová J., de Bello F. (2009) CLO-PLA: the database of clonal and bud bank traits of Central European flora. *Journal of Vegetation Science*, **20**, 511–516.
- Leigh A., Cosgrove M.J., Nicotra A.B. (2006) Reproductive allocation in a gender dimorphic shrub: anomalous female investment in *Gynatrix pulchella*? *Journal of Ecology*, **94**, 1261–1271.
- Lenth R. (2015). lsmeans: Least-squares means. R Package v.2.20-23. <http://CRAN.R-project.org/package=lsmeans>.
- Lewis D. (1941) Male sterility in natural populations of hermaphrodite plants the equilibrium between females and hermaphrodites to be expected with different types of inheritance. *New Phytologist*, **40**, 56–63.
- Miller J.S., Stanton-Geddes J.L. (2007) Gynodioecy in *Lobelia siphilitica* and *L. spicata* (Lobeliaceae) from Western Massachusetts. *Journal of the Torrey Botanical Society*, **134**, 349–361.
- Mole S., Joern A., Oleary M.H., Madhavan S. (1994) Spatial and temporal variation in carbon-isotope discrimination in prairie graminoids. *Oecologia*, **97**, 316–321.
- Obeso J.-R. (2002) The costs of reproduction in plants. *New Phytologist*, **155**, 321–348.
- Poot P. (1997) Reproductive allocation and resource compensation in male-sterile and hermaphroditic plants of *Plantago lanceolata* (Plantaginaceae). *American Journal of Botany*, **84**, 1256–1256.
- Poot P., Broek T., van Damme J.M.M., Lambers H. (1997) A comparison of the vegetative growth of male-sterile and hermaphroditic lines of *Plantago lanceolata* in relation to N supply. *New Phytologist*, **135**, 429–437.
- Poot P., Pilon J., Pons T.L. (1996) Photosynthetic characteristics of leaves of male-sterile and hermaphrodite sex types of *Plantago lanceolata* grown under conditions of contrasting nitrogen and light availabilities. *Physiologia Plantarum*, **98**, 780–790.

- R Development Core Team. (2014) R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.
- Ramula S., Mutikainen P. (2003) Sex allocation of females and hermaphrodites in the gynodioecious *Geranium sylvaticum*. *Annals of Botany*, **92**, 207–213.
- Reekie E.G., Bazzaz F.A. (2005) *Reproductive Allocation in Plants*. USA, Elsevier, Academic Press: 247 pp.
- Sakai A.K., Weller S.G., Chen M.-L., Chou S.-Y., Tasanont C. (1997) Evolution of gynodioecy and maintenance of females: the role of inbreeding depression, outcrossing rates, and resource allocation in *Schiedea adamantis* (Caryophyllaceae). *Evolution*, **51**, 724–736.
- Schultz S.T. (2003) Sexual dimorphism in gynodioecious *Sidalcea hirtipes* (Malvaceae). I. Seed, fruit, and ecophysiology. *International Journal of Plant Sciences*, **164**, 165–173.
- Schultz S.T. (2009) Leaf gas exchange, water status, spatial dispersion, and gender in gynodioecious *Bidens sandvicensis* (Asteraceae). *International Journal of Plant Sciences*, **170**, 200–209.
- Schultz S.T., Ganders F.R. (1996) Evolution of unisexuality in the Hawaiian flora: A test of microevolutionary theory. *Evolution*, **50**, 842–855.
- Shibata A., Kudo G. (2016) Size-dependent sex allocation and reproductive investment in a gynodioecious shrub. *AoB Plants*:plw089.
- Shykoff J.A., Kolokotronis S.-O., Collin C.L., López-Villavicencio M. (2003) Effects of male sterility on reproductive traits in gynodioecious plants: a meta-analysis. *Oecologia*, **135**, 1–9.
- Silvertown J., Araya Y., Gowing D. (2015) Hydrological niches in terrestrial plant communities: a review. *Journal of Ecology*, **103**, 93–108.
- Vaarama A., Jääskeläinen O. 1967. Studies on gynodioecism in the Finnish populations of *Geranium sylvaticum* L. *Annales Academiæ Scientiarum Fennica Series A IV Biologica*, **108**, 1–39.
- Van Etten M.L., Chang S.-M. (2014) Frequency-dependent pollinator discrimination acts against female plants in the gynodioecious *Geranium maculatum*. *Annals of Botany*, **114**, 1769–1778.
- Van Etten M.L., Prevost L.B., Deen A.C., Ortiz B.V., Donovan L.A., Chang S.M. (2008) Gender differences in reproductive and physiological traits in a gynodioecious species, *Geranium maculatum* (Geraniaceae). *International Journal of Plant Sciences*, **169**, 271–279.
- Varga S. (2014) Pre-dispersal seed predation in gynodioecious *Geranium sylvaticum* is not affected by plant gender or flowering phenology. *Arthropod-Plant Interactions*, **8**, 253–260.
- Varga S. (2015) Effects of arbuscular mycorrhizal fungi and maternal plant sex on seed

germination and early plant establishment. *American Journal of Botany*, **102**, 358–366.

Varga S., Kytöviita M.-M. (2016) Light availability affects sex lability in a gynodioecious plant. *American Journal of Botany*, **103**, 1928–1936.

Varga S., Kytöviita M.-M., Siikamäki P. (2009) Sexual differences in response to simulated herbivory in the gynodioecious herb *Geranium sylvaticum*. *Plant Ecology*, **202**, 325–336.

Varga S., Laaksonen E., Siikamäki P., Kytöviita M.-M. (2015) Absence of sex differential plasticity to light availability during seed maturation in *Geranium sylvaticum*. *PLoS One*, **10**:e0118981.

Varga S., Nuortila C., Kytöviita M.-M. (2013) Nectar sugar production across floral phases in the gynodioecious protandrous plant *Geranium sylvaticum*. *PLoS One*, **8**:e62575.

Table 1. Available studies reporting physiological traits and seed output in gynodioecious plants. Differences in seed production between sexes are expressed as total seed production per plant unless stated otherwise.

Species	Seeds	A	Gs	WUE	C	N	References
<i>Bidens sandvicensis</i>	H < F ¹	H = F	H = F	H = F	H = F		Schultz & Ganders 1996, Schultz 2009
<i>Daphne jezoensis</i>	H < F ²	H = F					Shibata & Kudo 2016
<i>Geranium maculatum</i>	H ≤ F	H = F				H = F	Chang 2006, Van Etten <i>et al.</i> 2008
<i>Geranium sylvaticum</i>	H ≤ F						Ramula & Mutikainen 2003, Asikainen & Mutikainen 2005a, Varga <i>et al.</i> 2009, Varga 2014
<i>Gynatrix pulchella</i>	H < F	H = F	H = F	H = F	H = F	H = F	This study.
<i>Lobelia siphilitica</i>	H ≤ F	H < F	H < F	H = F			Leigh <i>et al.</i> 2006
<i>Plantago lanceolata</i>	H < F	H ≤ F	H = F	H < F			Caruso <i>et al.</i> 2003, Miller & Stanton-Geddes 2007, Caruso & Yakobowski 2008
<i>Schiedea adamantis</i>	H < F	H = F	H = F	H = F			Poot <i>et al.</i> 1996, Poot 1997, Poot <i>et al.</i> 1997
<i>Schiedea salicaria</i>	H < F	H = F	H = F	H = F			Sakai <i>et al.</i> 1997, Culley <i>et al.</i> 2006
<i>Sidalcea hirtipes</i>	H < F ¹	H = F	H = F	H = F			Culley <i>et al.</i> 2006
<i>Silene acaulis</i>	H = F ¹					H = F	Schultz 2003
<i>Wurmbea dioica</i>	H < F ¹			H = F			Delph and Carroll 2001
							Case & Barrett 2001

Notes: ¹Seed set; ²Fruit set. A: photosynthetic rate; Gs: stomatal conductance; WUE: water use efficiency; C: carbon discrimination; N: nitrogen discrimination.

Table 2. ANOVA results from the linear mixed effects models for the N and C contents and isotope ratio measurements in *Geranium sylvaticum* leaves. Significant results ($P < 0.05$) are shown in bold.

	df	Foliar ^{13}C		Foliar C%		Foliar ^{15}N		Foliar N%	
		F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>
Light	1,126	380.37	<0.01	73.00	<0.01	5.52	0.02	40.79	<0.01
Sex	1,126	0.29	0.59	2.01	0.16	3.68	0.06*	3.63	0.06*
Light \times Sex	1,126	0.62	0.43	1.83	0.18	0.12	0.73	0.17	0.68

*Cohen's *d* for the effect of sex on ^{15}N leaf and ^{15}N soil were 0.31 and 0.28, respectively, and therefore considered small.

Table 3. ANOVA results from the linear mixed effects models for the C and N contents and isotope ratios in the soil samples near *Geranium sylvaticum* plants. Significant results ($P < 0.05$) are shown in bold.

	df	^{13}C soil		C% soil		^{15}N soil		N% soil	
		F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>
Light	1,126	0.07	0.79	0.40	0.53	5.95	0.02	7.84	0.01
Sex	1,126	1.19	0.28	0.08	0.77	0.25	0.62	0.46	0.50
Light \times Sex	1,126	0.12	0.73	0.58	0.45	0.13	0.72	0.29	0.59

Table 4. Mean maximum net photosynthetic rate (A), transpiration (E), stomatal conductance (g_s) and water use efficiency (WUE) in female and hermaphrodite *Geranium sylvaticum* plants in High and Low light habitats. Values are means \pm SE (N = 15 except for Hermaphrodite plants in Low light habitat where N = 14). Letters within a column indicate significant differences. F and P values are given for the effect of Sex, Light and the interaction between Sex and Light. Maximum measurements were measured within PAR 500 – 2000 depending on the plant.

Sex	A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	E ($\text{mmol m}^{-2} \text{s}^{-1}$)	g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	WUE ($\mu\text{mol CO}_2 \text{mmol}^{-1} \text{H}_2\text{O}$)
High light:				
Female	12.1 ± 1.4^a	4.3 ± 0.3^a	0.33 ± 0.02^a	3.20 ± 0.49^a
Hermaphrodite	12.1 ± 1.4^a	3.7 ± 0.4^a	0.30 ± 0.03^a	3.64 ± 0.58^a
Low light:				
Female	5.5 ± 0.8^b	2.9 ± 0.3^b	0.18 ± 0.02^b	2.73 ± 0.61^b
Hermaphrodite	6.7 ± 1.4^b	3.0 ± 0.4^b	0.19 ± 0.02^b	2.85 ± 0.55^b
F_{sex}	0.57	0.73	0.15	0.28
F_{light}	42.94***	11.15**	35.74***	4.67*
$F_{\text{sex} \times \text{light}}$	0.30	0.19	0.29	0.29

Figure captions

Fig. 1. A) Number of flowers and B) number of seeds produced in female (white bars) and hermaphrodite (dark bars) *Geranium sylvaticum* plants in Low and High light habitats. Mean \pm SE are indicated, N = 356. Significant differences ($P < 0.05$) between light treatments are indicated with different letters above the groups.

Fig. 2. Relationship between carbon isotope discrimination ($\delta^{13}\text{C}$) and leaf N concentration (%DW) in female (open symbols) and hermaphrodite (filled symbols) *Geranium sylvaticum* individuals growing in A) Low and B) High light habitats. N = 66.

Fig. 3. Light response curves of leaves from female (thin line) and hermaphrodite (thick line) *Geranium sylvaticum* plants from A) High light habitats and B) Low light habitats. Mean photosynthetic rates \pm SE are indicated (N=59).

Figure 1.

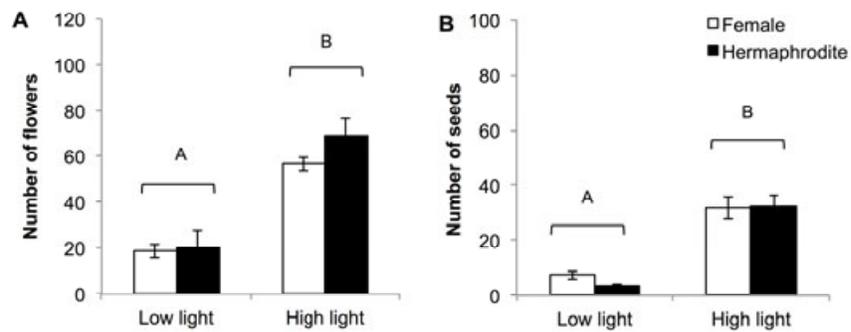


Figure 3.

