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Gender-specific floral and physiological traits: implications for the maintenance of females in gynodioecious *Lobelia siphilitica*

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Abstract A common gender dimorphism in angiosperms is gynodioecy, in which hermaphrodites and females co-occur. Females are at an inherent disadvantage because they can transmit their genes only through ovule production. One mechanism by which females can compensate for the loss of male function is by producing more seeds than hermaphrodites. As such, females should: (1) increase resource uptake to support higher seed production; and (2) allocate resources saved by the loss of male function to seed production. To test this hypothesis, we measured physiological and floral traits of gynodioecious *Lobelia siphilitica*, controlling for both environmental and genetic variation through a comparison of greenhouse-grown siblings. Pre-reproductive females had 14% higher area-based ($Z=2.14$; $P=0.04$) and 32% higher mass-based ($Z=1.96$; $P=0.05$) photosynthetic rate than hermaphrodites, suggesting that they have increased carbon acquisition by altering photosynthetic physiology. Female *L. siphilitica* produced flowers with 4–8% smaller corollas than hermaphrodites (all $P<0.05$), suggesting that females allocate resources away from floral structures used for pollinator attraction. The genetic correlation between genders for four floral and four physiological traits was significantly less than one but greater than zero, indicating that the evolution of gender dimorphism in

response to sex-differential selection will be constrained. The allocation of resources saved by the loss of male function has been viewed as the most important mechanism allowing females of gynodioecious species to support higher seed production. Our data suggest that increased resource acquisition by females at pre-reproductive stages can also contribute to the maintenance of gender dimorphism in gynodioecious species.

Keywords Flower size · Photosynthesis · Plant mating systems · Plant gender · Resource allocation

Introduction

Although the majority of angiosperms bear hermaphroditic flowers, gender dimorphism has evolved independently in many flowering plant families (e.g. Renner and Ricklefs 1995). A common dimorphism is gynodioecy, in which female and hermaphrodite individuals co-occur. Because females can transmit genes only through ovules, they are at an inherent disadvantage relative to hermaphrodites. For gender dimorphism to be maintained, females must compensate for the loss of male function (e.g. Lewis 1941; Lloyd 1975).

This compensation can occur if females produce more seeds or higher-quality seeds than hermaphrodites (Lewis 1941). The relative seed production of females compared to hermaphrodites can be high [e.g. female *Silene acaulis* produce 2.9 times more seeds (Shykoff 1988)], although in some gynodioecious species fecundity does not differ between the genders [e.g. *Sidalcea oregana spicata* (Ashman 1994)]. Because a seed is costlier to produce than a pollen grain (e.g. Ashman 1994), it has been predicted that females: (1) increase resource uptake to support higher seed production (Dawson and Geber 1999); and (2) allocate resources saved by the loss of male function to seed production (Darwin 1877). Increased carbon uptake may occur through the production of more photosynthetic tissue and/or higher photosynthetic rate. Photosynthetic gas-exchange physiology has

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been measured in few gynodioecious species (Poot et al. 1996). However, females do frequently have greater vegetative biomass than hermaphrodites (e.g. Kesseli and Jain 1984; Eckhart 1992a; Poot 1997), suggesting that they have increased carbon acquisition by producing more photosynthetic tissue. In females, resources saved by the loss of male function may be allocated to seed production. Pollinators commonly prefer flowers with large corollas (e.g. Galen and Newport 1987), and increased visitation is thought to have a stronger effect on pollen export (i.e. male function) than female function (e.g. Bell 1985). Because corollas are typically non-photosynthetic and lose water, they are physiologically costly to maintain (e.g. Galen et al. 1999). The production of flowers with smaller corollas by females of almost all gynodioecious species [reviewed in Baker (1948); Delph (1996); Eckhart (1999)] may therefore represent the allocation of resources away from floral structures that disproportionately influence male function.

Gender dimorphism in physiological and floral traits that allow females to produce more seeds could result from selection or the pleiotropic effects of gender-determining genes. If the genetic correlation between a trait expressed in females and the same trait expressed in hermaphrodites is significantly less than one, then the trait can evolve independently in response to sex-differential selection (Slatkin 1984; Lande 1980). However, if the cross-gender correlation is also significantly greater than zero, then selection in one gender may cause a correlated response in the other, slowing the adaptive evolution of sexual dimorphism (Lande 1980). For example, Eckhart (1993) found that a correlation across the genders for corolla diameter of *Phacelia linearis* might promote an increase in corolla diameter of females in response to positive directional selection on this trait in hermaphrodites. Genetic correlations between genders are rarely estimated [see Eckhart (1993); Ashman (1999)], but they may play an important role in the evolution of dimorphism in species where the origin of gynodioecy is recent and females are rare (Eckhart 1999). When females are rare, gender dimorphism in physiological and floral traits may also be an immediate pleiotropic effect of gender-determining genes that influence plant hormone production [reviewed in Kaul (1988); Sawhney and Shukla (1994)].

Because most plants cannot be sexed before flowering, studies of resource acquisition and allocation in gynodioecious species have generally focused on the reproductive portion of the plant's life cycle. Although physiological traits can in principle be measured prior to reproduction, theory suggests that gender dimorphism in physiology should not arise until resources are invested in male function [i.e. bud formation; Seger and Eckhart (1996); Eckhart and Seger (1999)]. Pre-reproductive physiological traits have been measured for few gynodioecious species [*Plantago lanceolata* (Poot et al. 1996, 1997; Poot 1997) and *Phacelia linearis* (Eckhart and Chapin 1997)], and no gender-specific differences were detected.

As part of a larger quantitative genetic analysis, we examined gender-specific differences in floral traits and

pre-reproductive physiological traits, as well as genetic correlations across the genders, for gynodioecious *Lobelia siphilitica*. We addressed the following questions: (1) Do females (A) increase carbon acquisition prior to reproduction and (B) produce flowers with smaller corollas, as predicted if changes in resource acquisition and allocation contribute to the maintenance of gender dimorphism in *L. siphilitica*?, and (2) Can physiological and floral traits of *L. siphilitica* hermaphrodites and females evolve independently in response to sex-differential selection? Female *L. siphilitica* from southern Indiana produce more fruits than co-occurring hermaphrodites, suggesting that increased seed production may contribute to the maintenance of females in this species (Dudle 1999). However, the importance of increased resource acquisition by pre-reproductive plants and the allocation of resources saved by producing flowers with smaller corollas in supporting higher fruit set of female *L. siphilitica* is not known.

Materials and methods

Study species

Lobelia siphilitica (Lobeliaceae) is a short-lived, herbaceous perennial wildflower that grows in wet meadows and woods throughout eastern North America [Johnston (1991a) and references therein]. Its 3 cm long blue flowers (Fig. 1) are primarily pollinated by *Bombus* spp. (Beaudoin Yetter 1989). In natural populations in Iowa, plants flower from early August until early October and fruits dehisce from mid-September through November (C.M. Caruso, personal observation). Hermaphroditic flowers are protandrous and pollen is shed from a tube formed by the fused anthers and filaments (Johnston 1991a). Although *L. siphilitica* is self-compatible (Johnston 1992), the complete separation between staminate and pistillate phases of flower development ensures that self-fertilization of hermaphrodites is the result of geitonogamy rather than autogamy (Johnston 1991b). Females from southern Indiana produce ~8% more fruits/flower than co-occurring hermaphrodites [calculated from data in Dudle (1999)]. *Lobelia siphilitica* can also reproduce clonally via rosettes that overwinter and produce a flowering stalk the following summer (Beaudoin Yetter 1989).

Gender in *L. siphilitica* is determined by the interaction of cytoplasmic male sterility factors and nuclear genes that restore male function (Dudle et al. 2001). As in other gynodioecious species where gender is under nuclear-cytoplasmic control (e.g.

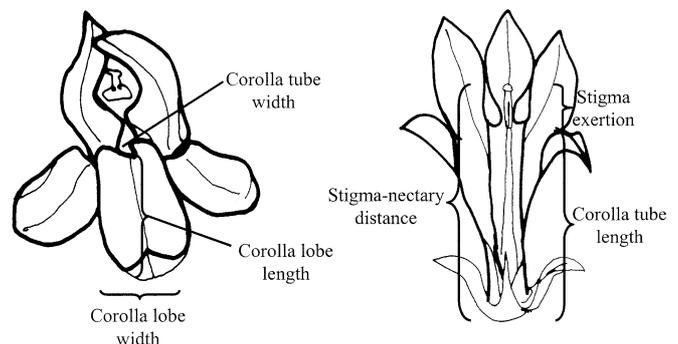


Fig. 1 Front and top views of *Lobelia siphilitica* flowers, showing the six aspects on floral morphology examined in this study. Drawings are not to scale.

Thymus vulgaris; Dominee et al. 1978), female frequencies vary widely among *L. siphilitica* populations. Populations in Iowa (C.M. Caruso, unpublished data), Wisconsin (Dudle 1999), and Michigan (Johnston 1991a; Dudle 1999) contain 0–9% females. In contrast, *L. siphilitica* populations in southern Indiana (Mutikainen and Delph 1998; Dudle 1999) and Ohio (Beaudoin Yetter 1989) contain 45–90% females. Spatial segregation of the genders has not been documented in *L. siphilitica*.

Experimental design

Our data were collected in the course of a quantitative genetic analysis of naturally occurring variation in floral and physiological traits of *L. siphilitica*. To create half-sib families, we collected open-pollinated fruits from two *L. siphilitica* populations [CERA (N 41° 41.0', W 92° 52.2') and Krumm (N 41° 42.3', W 92° 47.2')] in Jasper Co., Iowa, USA. The percentage of females in CERA and Krumm was estimated in 1999 and 2000 and varied from 1% to 9%. Fruits were collected from 116 plants in CERA and 67 in Krumm. Because *L. siphilitica* can reproduce clonally, we attempted to sample fruits from only one ramet per genet. Seeds from each maternal family were placed on moist filter paper in a petri dish, wrapped in parafilm, and stratified at 4°C for 8 weeks (Johnston 1992). Approximately 20 seeds per pot were sown onto moist MetroMix 380 (Scotts Company, Marysville, Ohio USA) and placed in standing water in the greenhouse at Grinnell College, Grinnell, Iowa USA. Two cells were planted for each maternal family. After 8 weeks, we transplanted 1–9 seedlings/family (57 from CERA and 55 from Krumm) into 9×9 cm plastic pots. Plants were watered as necessary, fertilized with Osmocote 14–14–14 (Scotts Company), and exposed to supplemental light (16-h days).

At flowering, we crossed one randomly chosen plant with male-phase flowers to two randomly chosen plants with female-phase flowers to create a series of full-sib families nested within half-sib families (50 half-sib families from CERA and 75 from Krumm). All crosses were to other members of the same population and individuals used in crosses were sampled without replacement. We pollinated 3 flowers/female plant. Although female flowers used in the crosses were not bagged, few flowers that were not hand-pollinated set fruit (C.M. Caruso, personal observation), indicating that accidental pollination was rare. Fruits were collected from successful crosses (31 half-sib families from CERA and 51 from Krumm) and seeds were stratified as described above.

Approximately 20 seeds/pot were sown onto moist MetroMix 360 (Scotts Company) and placed in standing water in the greenhouse at Duke University, Durham, NC, USA. Twenty-four cells were planted for each half-sib family, equally divided between the two nested full-sib families. After 6 weeks, we transplanted 18 seedlings/half-sib family, again equally divided between the two nested full-sib families, into 9×9 cm plastic pots. Plants were watered as necessary and fertilized weekly. By growing females and hermaphrodites in a common greenhouse environment, we were able to control for the effects of any cryptic spatial segregation of the genders in field habitats.

Phenotypic measurements

All physiological traits were measured 12–16 weeks after planting, when plants had formed rosettes but not yet flowered. We therefore made physiological measurements while blind to plant gender. The magnitude of gender-specific differences in physiology can vary across the life cycle (e.g. Laporte and Delph 1996), suggesting that our measurements of pre-reproductive plants cannot be extrapolated to reproductive or post-reproductive *L. siphilitica*. We randomly assigned one-third of the offspring in each full-sib family to be measured in each of three 10-day blocks. We measured chlorophyll concentration, quantum efficiency, rosette size, leaf mass, leaf size, and specific leaf area (SLA) on all plants. Chlorophyll concentration was measured on 3 leaves/plant using a portable chlorophyll meter (SPAD 502, Minolta, Ramsey, NJ, USA). We measured the

maximum quantum efficiency of PSII (F_v/F_m) with a field portable pulse modulated chlorophyll fluorimeter (Model FMS2, Hansatech Instruments Ltd., King's Lynn, Norfolk, UK). Measurements were made on one leaf per plant, and leaves were dark-adapted with leaf clips for 20 min prior to fluorescence measurements. To minimize temporal variation in F_v/F_m , all measurements were made between 0900 and 1100 hours EST. Rosette size was estimated as the average of two measurements of rosette diameter, taken at 90° angles to each other. One randomly chosen, fully expanded leaf from each plant was excised and its area measured with a leaf area meter (LI-3000, Li-Cor Inc., Lincoln, Neb., USA). These leaves were dried to constant mass in a forced convection oven at 65°C for 24 h and weighed. Specific leaf area was calculated by dividing leaf size by leaf mass.

We measured photosynthetic rate (A), stomatal conductance (g_s), the ratio of intercellular to ambient CO₂ (C_i/C_a), and water-use efficiency (WUE) for 32 randomly selected half-sib families from Krumm. Within each of these families, we randomly selected eight plants for measurement, evenly divided between nested full-sib families. Steady state leaf gas-exchange was measured at saturating irradiance (1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) with an open gas-exchange system (LI-6400, Li-Cor) between 0900 and 1200 hours EST in June 2001. During measurements, incident irradiance was provided by red-blue light-emitting diodes and ambient CO₂ concentration was maintained at 380 $\mu\text{mol mol}^{-1}$. A Peltier cooling module maintained leaf temperature at approximately ambient conditions (20–25°C) during each measurement period. We maintained the leaf-to-air vapor pressure deficit (D) at 0.9–1.1 kPa, which permitted the measurement of maximum g_s . To calculate g_s and intercellular CO₂ concentration (C_i) we used a boundary layer conductance of 1.42 $\text{mol m}^{-2} \text{s}^{-1}$, which was calculated on the basis of leaf area and fan speed using the energy balance algorithms of the LI-6400. Following enclosure in the leaf cuvette, leaves reached steady-state values (e.g. when the coefficients of variation of CO₂ and H₂O within the chamber were <0.25%) within 5 min. To express these area-based measurements on a per unit mass basis, we multiplied by SLA. Instantaneous WUE was calculated as A/g_s . To ensure that samples experienced similar soil moisture during data collection, all plants were watered to field capacity the evening prior to measurement.

At flowering, we measured six floral traits per plant: corolla lobe length, corolla lobe width, corolla tube length, corolla tube width, stigma-nectary distance, and total flower number (Fig. 1). Floral morphology was measured for three flowers/plant using hand-held digital calipers. An additional floral morphology trait (stigma exertion) was estimated by subtracting corolla length from stigma-nectary distance (Fig. 1). Because flowers within a plant are not independent, we used the mean of three flowers per individual in all analyses of floral morphology. Our estimates of stigma-nectary distance are approximately one-third greater than those reported by Johnston (1991a) for Michigan populations of *L. siphilitica*, perhaps because of differences in measurement techniques. We estimated total flower number as the sum of the senescent flowers, open flowers, and unopened buds on each plant. Floral traits were measured for approximately 1,000 haphazardly chosen plants. Plants were also categorized as females or hermaphrodites. 27% and 24% of the full-sib families from Krumm and CERA, respectively, contained at least one female. Because pollinators are necessary for *L. siphilitica* to reproduce, we were unable to measure seed or fruit set of our greenhouse-grown plants.

Statistical analysis

We restricted our analyses to full- or (for gas-exchange traits) half-sib families that contained both females and hermaphrodites. By making family the unit of replication for all analyses, we controlled for differences in floral and physiological traits caused by random genetic variation, resulting in a more powerful test of gender differences. However, analyzing only mixed-gender families could have biased our sampling of hermaphrodites. The hermaphrodites in mixed-gender families must carry both a cytoplasmic male

Table 1 Mean (\pm SE) floral and physiological traits of female and hermaphrodite *Lobelia siphilitica* from CERA, Krumm, or combined across populations. *P*-values are from Wilcoxon paired-sample tests and indicate whether a trait differed significantly between the genders

| Trait | Mean \pm 1 SE | | | | | |
|---|----------------------------------|----------------------------------|------------------------------------|-----------------------------------|---|---|
| | CERA | | Krumm | | Combined | |
| | Female | Hermaphrodite | Female | Hermaphrodite | Female | Hermaphrodite |
| Corolla lobe length (mm) | — | — | — | — | 12.44 \pm 0.22 | 13.45*** \pm 0.20† |
| Corolla lobe width (mm) | — | — | — | — | 5.65 \pm 0.11 | 5.87* \pm 0.09 |
| Corolla tube length (mm) | — | — | — | — | 16.32 \pm 0.23 | 17.07*** \pm 0.15† |
| Corolla tube width (mm) | — | — | — | — | 5.97 \pm 0.14 | 6.46*** \pm 0.14† |
| Stigma-nectary distance (mm) | — | — | — | — | 23.08 \pm 0.28 | 23.60 \pm 0.22 |
| Stigma exertion (mm) | — | — | — | — | 6.85* \pm 0.17 | 6.58 \pm 0.28 |
| Flower number | — | — | — | — | 96.52 \pm 4.85 | 89.55 \pm 3.69 |
| Rosette diameter (cm) | — | — | — | — | 23.01 \pm 0.56 | 23.59 \pm 0.32 |
| Leaf size (m ²) | — | — | — | — | 3.38 $\times 10^{-3}$ \pm 1.23 $\times 10^{-4}$ | 3.70 $\times 10^{-3}$ * \pm 1.05 $\times 10^{-4}$ |
| Leaf mass (g) | — | — | — | — | 0.11 \pm 4.52 $\times 10^{-3}$ | 0.12* \pm 4.39 $\times 10^{-3}$ |
| Specific leaf area (m ² g ⁻¹) | — | — | — | — | 0.031 \pm 7.21 $\times 10^{-4}$ | 0.032 \pm 6.90 $\times 10^{-4}$ |
| F_v/F_m | 0.84 \pm 2.72 $\times 10^{-3}$ | 0.84 \pm 1.57 $\times 10^{-3}$ | — | — | — | — |
| Chlorophyll concentration | 30.59 \pm 0.50 | 32.03 \pm 0.78 | — | — | — | — |
| A (μ mol CO ₂ m ⁻² s ⁻¹) | — | — | 0.84* \pm 1.92 $\times 10^{-3}$ | 0.83 \pm 1.79 $\times 10^{-3}$ | — | — |
| g_s (mol m ⁻² s ⁻¹) | — | — | 31.06* \pm 0.68 | 29.94 \pm 0.68 | — | — |
| A (μ mol CO ₂ g ⁻¹ s ⁻¹) | — | — | 12.95* \pm 0.32 | 11.29 \pm 0.54 | — | — |
| g_s (mol m ⁻² s ⁻¹) | — | — | 0.46* \pm 0.03 | 0.37 \pm 0.03 | — | — |
| g_s (mol m ⁻² s ⁻¹) | — | — | 0.44* \pm 0.02 | 0.33 \pm 0.03 | — | — |
| C _i /C _a | — | — | 0.016* \pm 1.22 $\times 10^{-3}$ | 0.011 \pm 1.44 $\times 10^{-3}$ | — | — |
| WUE | — | — | 0.83 \pm 6.71 $\times 10^{-3}$ | 0.81 \pm 9.91 $\times 10^{-3}$ | — | — |
| N | 15 | — | 29.34 \pm 1.53 | 33.91 \pm 2.13 | — | — |
| | | | 11–22 | | 33–37 | |

* *P*<0.05 ** *P*<0.01 *** *P*<0.001 † Remained significant after Dunn-Sidak correction

sterility factor and a nuclear gene that restores male function. In contrast, hermaphrodites in single-gender families could either carry a male-fertile cytoplasm or a sterile cytoplasm and its nuclear restorer. *Lobelia siphilitica* hermaphrodites that carry more nuclear restorers have lower pollen viability (Bailey 2002), suggesting that hermaphrodites from single- and mixed-gender families could also differ in physiological and floral traits.

Wilcoxon paired-sample tests (Zar 1999) were used to compare physiological and floral traits of females vs. hermaphrodites. To determine if our data could be pooled across populations for the Wilcoxon test, we ran a series of one-way ANOVAs with population as the independent variable and (mean of females – mean of hermaphrodites) as the dependent variable. The difference between females and hermaphrodites was consistent across populations ($P>0.05$) for all traits except quantum efficiency and chlorophyll concentration, so we pooled data for most traits. To determine if floral and physiological traits were genetically correlated across the genders, we calculated Pearson correlation coefficients (and associated P -values) between full- or half-sib family means of hermaphrodites and females. To determine if these correlations were significantly different from both 0 and 1, we calculated 95% confidence intervals as in Sokal and Rohlf (1995). We present P -values for the Wilcoxon tests and correlations both before and after applying the Dunn-Sidak correction (Sokal and Rohlf 1995) for multiple tests within each group (CERA, Krumm, or pooled across populations).

Results

Hermaphrodite *L. siphilitica* produced 8% larger ($Z=2.29$; $n=37$; $P=0.022$) and 7% heavier leaves ($Z=2.06$; $n=37$; $P=0.039$) than females. In contrast to these differences in individual leaf size, pre-reproductive rosette size did not differ between the genders ($Z=1.36$; $n=37$; $P=0.175$). Because increases in leaf size and mass of hermaphrodites were proportional, SLA did not differ between the genders ($Z=0.73$; $n=37$; $P=0.464$). Females had a 14% higher area-based photosynthetic rate ($Z=2.14$; $n=11$;

$P=0.041$) and a 32% higher mass-based photosynthetic rate ($Z=1.96$; $n=11$; $P=0.050$) than hermaphrodites. Area-based stomatal conductance was 24% higher ($Z=2.14$; $n=11$; $P=0.033$) and mass-based conductance 31% higher ($Z=2.13$; $n=11$; $P=0.033$) in females than in hermaphrodites. In contrast to these differences in photosynthesis and conductance, neither C_i/C_a ($Z=1.25$; $n=11$; $P=0.213$) nor instantaneous WUE ($Z=1.51$; $n=11$; $P=0.131$) differed between the genders (Table 1).

Gender-specific differences in quantum efficiency and chlorophyll concentration were not consistent across populations. Female *L. siphilitica* from Krumm had a 1% higher F_v/F_m ($Z=2.40$; $n=22$; $P=0.016$) and a 3% higher chlorophyll concentration ($Z=2.27$; $n=22$; $P=0.023$) than hermaphrodites. In contrast, the F_v/F_m of females and hermaphrodites from CERA did not differ ($Z=0.51$; $n=15$; $P=0.609$). There was a marginally significant trend towards higher chlorophyll concentration in hermaphrodite *L. siphilitica* from CERA ($Z=1.82$; $n=15$; $P=0.069$; Table 1).

Floral morphology, but not flower number, differed between female and hermaphrodite *L. siphilitica* plants. Hermaphrodite plants had 8% longer ($Z=3.67$; $n=33$; $P<0.001$) and 4% wider ($Z=2.10$; $n=35$; $P=0.036$) corolla lobes than females. Corolla tubes were 5% longer ($Z=3.37$; $n=37$; $P=0.001$) and 8% wider ($Z=3.46$; $n=33$; $P=0.001$) in hermaphrodites than females. In contrast, stigmas were 4% more exerted in female *L. siphilitica* than in hermaphrodites ($Z=2.29$; $n=37$; $P=0.022$). Stigma-nectary distance ($Z=1.37$; $n=37$; $P=0.172$) and flower number ($Z=1.31$; $n=37$; $P=0.192$) were the only floral traits that did not differ between the genders (Table 1).

Genetic correlations across the genders for four of seven floral traits and four of eleven physiological traits

Table 2 Correlations (based on full- or half-sib family means) across the genders of gynodioecious *L. siphilitica* from CERA, Krumm, or combined across populations. P -values indicate whether the correlation was significantly different from 0. 95% confidence intervals indicate whether the correlation was significantly different from both 0 and 1

| Trait | Cross sex-morph correlation (95% CI) | | |
|--|--------------------------------------|--------------------------|--------------------------|
| | CERA | Krumm | Combined |
| Corolla lobe length | — | — | 0.437* (0.110, 0.679) |
| Corolla lobe width | — | — | 0.467** (0.158, 0.692) |
| Corolla tube length | — | — | 0.392* (0.078, 0.635) |
| Corolla tube width | — | — | 0.625*** (0.359, 0.797)† |
| Stigma-nectary distance | — | — | 0.276 (–0.053, 0.551) |
| Stigma exertion | — | — | 0.193 (–0.140, 0.487) |
| Flower number | — | — | 0.304 (–0.022, 0.572) |
| Rosette diameter | — | — | 0.296 (–0.031, 0.566) |
| Leaf size | — | — | 0.387* (0.072, 0.632) |
| Leaf mass | — | — | 0.576*** (0.310, 0.758)† |
| Specific leaf area | — | — | 0.346* (0.025, 0.602) |
| F_v/F_m | 0.176 (–0.370, 0.631) | 0.218 (–0.224, 0.586) | — |
| Chlorophyll concentration | 0.353 (–0.194, 0.733) | 0.687*** (0.374, 0.860)† | — |
| A ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) | — | –0.013 (–0.608, 0.591) | — |
| g_s ($\text{mol m}^{-2} \text{ s}^{-1}$) | — | 0.463 (–0.190, 0.832) | — |
| A ($\mu\text{mol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$) | — | –0.026 (–0.616, 0.583) | — |
| g_s ($\text{mol m}^{-2} \text{ s}^{-1}$) | — | 0.014 (–0.591, 0.609) | — |
| C_i/C_a | — | 0.138 (–0.504, 0.681) | — |
| WUE | — | 0.066 (–0.556, 0.641) | — |
| N | 15 | 11–22 | 33–37 |

* $P<0.05$ ** $P<0.01$ *** $P<0.001$ † P -value remained significant after Dunn-Sidak correction

were significantly greater than 0. The mean \pm SE cross-gender correlation was higher for floral (0.385 ± 0.054) than physiological (0.247 ± 0.062) traits. However, correlations for all traits were significantly less than 1 (Table 2).

Discussion

Changes in both resource acquisition and resource allocation appear to contribute to the maintenance of gender dimorphism in gynodioecious *L. siphilitica*, especially in the Krumm population. Female *L. siphilitica* from Krumm produced more chlorophyll, used light more efficiently, and had a higher photosynthetic rate (Table 1), suggesting they have the potential to acquire more carbon than hermaphrodites. Females from both populations also produced flowers with smaller corollas (Table 1). We hypothesize that this combination of increased leaf-level carbon acquisition and the allocation of resources away from floral structures that primarily benefit male function will allow female *L. siphilitica* to produce more fruits [as documented by Dudle (1999)] and therefore be maintained in the Krumm population.

These gender-specific differences in physiological and floral traits could be caused by at least three mechanisms. Firstly, there may be direct selection on females to increase seed production. However, if we assume that females have always been as infrequent (1–9%) at CERA and Krumm as they were in 1999 and 2000, there will have been little opportunity for selection to act directly on their physiological and floral traits (Eckhart 1993). Nonetheless, ~25% of our artificially-created full-sib families contained at least 1 female, and when offspring were pooled across families, 15% were females. This suggests that there may have been more females in CERA and Krumm in the past, as would be expected if there is cyclic variation in gender frequency over time (e.g. Gouyon et al. 1991). Secondly, direct selection on the more common hermaphrodite *L. siphilitica* may result in a correlated response in rare females. However, cross-gender correlations were significantly greater than zero for lobe length, lobe width, corolla length, corolla width, leaf size, leaf mass, and chlorophyll concentration (Table 2). Any selection on these traits in hermaphrodites will therefore result in a positive correlated response in females, slowing any adaptive evolution of gender dimorphism (Eckhart 1999). Thirdly, we cannot rule out the possibility that dimorphism in physiological and floral traits of *L. siphilitica* results from the pleiotropic effects of cytoplasmic male sterility genes and/or their nuclear restorers. For example, nuclear restorers of male fertility have a negative pleiotropic effect on pollen viability of *L. siphilitica* from southern Indiana (Bailey 2002).

Gender-specific differences in chlorophyll concentration, F_v/F_m , and A (Table 1) indicate that female *L. siphilitica* from Krumm have an enhanced photosynthetic physiology that allows them to fix more carbon on a leaf-level basis. Leaf chlorophyll concentration is correlated

with nitrogen content (Scheepers et al. 1992; Chapman and Barreto 1997). Therefore, given that all plants had equal access to soil nitrogen, the higher chlorophyll concentration of Krumm females (Table 1) suggests that they preferentially allocate nitrogen to leaves as a means to increase photosynthetic rate (Evans 1989). Hermaphrodites' allocation of nitrogen to leaves may be more constrained than that of females because *L. siphilitica* pollen is nitrogen-rich (Dudle 1999). Gender-specific photosynthetic gas exchange has been measured in pre-reproductive plants of only one other gynodioecious species (*Plantago lanceolata*), and no differences were detected (Poot et al. 1996). Studies in other species are needed to determine if a gender-specific difference in photosynthetic gas exchange is common in gynodioecious breeding systems.

In the absence of differences in total photosynthetic area, the enhancement of leaf-level photosynthesis in female versus hermaphrodite *L. siphilitica* from Krumm may lead to an increase in total carbon acquisition. Although pre-reproductive female *L. siphilitica* produced smaller and lighter leaves than hermaphrodites, overall rosette size did not differ between the genders (Table 1), suggesting that females do not allocate more biomass to photosynthetic tissue. Nonetheless, we did not directly measure total photosynthetic leaf area because the large number of plants included in the original study (>1000) made this task logistically difficult. Furthermore, direct quantification of total leaf area requires a destructive harvest, which would have prevented us from sexing plants and measuring their floral traits later in the life cycle. Given the uncertainties associated with scaling leaf-level photosynthesis to the whole plant, further studies are needed to determine if whole-plant carbon gain is higher in female than hermaphrodite *L. siphilitica*.

Although female and hermaphrodite *L. siphilitica* from Krumm clearly differ physiologically, our data from CERA suggest that the direction of these gender-specific differences can vary between populations. We did not measure gas exchange traits of plants from CERA, and neither chlorophyll concentration nor F_v/F_m differed significantly ($P > 0.05$) between the genders in this population (Table 1). However, in contrast to Krumm, there was a trend in CERA towards higher chlorophyll concentration and F_v/F_m in hermaphrodites than females. Interpopulation variation in the direction of gender-specific differences in physiology has been documented in dioecious *Salix* spp. where it was attributed to differences in the biotic and abiotic environment of the populations [reviewed in Dawson and Geber (1999)]. Although manipulative experiments would be needed to determine the ecological causes of differences in gender-specific physiology between CERA and Krumm, soil water availability does differ strongly between these populations (C.M. Caruso, unpublished data), suggesting one possible cause.

Our finding of smaller corollas on female *L. siphilitica* from both CERA and Krumm (Table 1) is consistent with studies of other gynodioecious species [reviewed in Baker

(1948); Delph (1996); Eckhart (1999)], but inconsistent with previous studies of *L. siphilitica* (Beaudoin Yetter 1989; Dudle 1999). Working in populations in southern Ohio, Beaudoin Yetter (1989) found that female *L. siphilitica* had significantly longer, but not narrower, corollas. Dudle (1999) found that corolla biomass, length, and width did not differ between female and hermaphrodite *L. siphilitica* from southern Indiana. In contrast, we found that all four measured corolla dimensions were significantly smaller in female *L. siphilitica* from central Iowa (Table 1). Any gender-specific differences in corolla size of *L. siphilitica* flowers from southern Ohio and Indiana may have been obscured by uncontrolled variation in the environment [in Beaudoin Yetter's (1989) study] or in the genetic background of the measured plants [both Beaudoin Yetter (1989) and Dudle (1999)]. By growing plants in the greenhouse and matching genders within families, we controlled for both environment and genetics, increasing our power to detect gender-specific differences in floral and physiological traits. Alternatively, geographic variation in gender-specific differences in floral traits of *L. siphilitica* may reflect variation in selection on these traits. The frequency of female *L. siphilitica* is dramatically higher in southern Ohio and Indiana than in Iowa (see *Methods* for details), and variation in female frequency can influence selection on floral traits in gynodioecious species (Ashman and Diefenderfer 2001).

Although female *L. siphilitica* had consistently smaller corollas than hermaphrodites, stigma exertion was greater in females (Table 1). Females may have more exerted stigmas because they spend more time in the pistillate phase than hermaphrodites (Dudle 1999), resulting in more time for the style to elongate. Female *L. siphilitica* from Indiana have marginally longer styles than co-occurring hermaphrodites (Dudle 1999), supporting our hypothesis. Stigma exertion can influence the amount of pollen deposited by pollinators (e.g. Conner et al. 1995) and therefore increase seed set. For example, *L. siphilitica* plants with more exerted stigmas set significantly more fruits (C.M. Caruso and S.B. Peterson, unpublished data). Based on the strength of phenotypic selection on stigma exertion in CERA ($\beta = 0.049$; C.M. Caruso and S.B. Peterson, unpublished data), we estimate that an *L. siphilitica* with the stigma exertion of an average female would set 8% more fruits than one with the average hermaphrodite exertion. As such, gender-specific differences in stigma exertion may also contribute to the maintenance of rare female *L. siphilitica* in our Iowa populations.

In addition to increased seed production, inbreeding depression can influence the evolutionary maintenance of females in gynodioecious species. If inbreeding depression is severe, then females, which are obligate outcrossers, may produce higher-quality seeds than hermaphrodites (e.g. Lloyd 1975). Hermaphrodite *L. siphilitica* can geitonogamously self-pollinate, and inbreeding depression in fertility (flower number) was ~50% in Michigan populations (Johnston 1992). These

data suggest that inbreeding depression can contribute to the maintenance of gender dimorphism in *L. siphilitica*. However, further studies would be needed to determine the relative importance of increased seed production and decreased inbreeding depression in the maintenance of female *L. siphilitica*.

In summary, female *L. siphilitica* produced smaller flowers and had enhanced leaf-level photosynthetic physiology relative to their hermaphrodite siblings. Increased photosynthesis, if it facilitates higher total plant carbon gain, could contribute to the maintenance of gender dimorphism by allowing females to produce more fruits [as documented in Dudle (1999)]. Significant positive genetic correlations across the genders indicate that selection on floral and physiological traits of hermaphrodite *L. siphilitica* could result in the correlated evolution of these traits in rare females, slowing any adaptive evolution of gender dimorphism. More generally, our results conflict with predictions from sex-allocation theory (Seeger and Eckhart 1996; Eckhart and Seeger 1999) that differences in physiological traits between females and hermaphrodites should not arise until the reproductive phase of the life cycle. The enhanced photosynthetic physiology of pre-reproductive *L. siphilitica* females may disproportionately contribute to their maintenance in natural populations because vegetative growth is exponential. As such, even a relatively small difference between the genders in resource acquisition early in the life cycle should translate to a much larger difference in the resources available for later reproduction (Eckhart 1992a, 1992b).

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References

- Ashman T-L (1994) Reproductive allocation in hermaphrodite and female plants of *Sidalcea oregana* ssp. *spicata* (Malvaceae) using four currencies. *Am J Bot* 81:433–438
- Ashman T-L (1999) Quantitative genetics of floral traits in a gynodioecious wild strawberry *Fragaria virginiana*: implications for the independent evolution of female and hermaphrodite floral phenotypes. *Heredity* 83:733–741
- Ashman T-L, Diefenderfer C (2001) Sex ratio represents a unique context for selection on attractive traits: consequences for the evolution of sexual dimorphism. *Am Nat* 157:334–347
- Bailey MF (2002) A cost of restoration of male fertility in a gynodioecious species, *Lobelia siphilitica*. *Evolution* 56:2178–2186.

- Baker HG (1948) Corolla size in gynodioecious and gynomonocious species of flowering plants. *Proc Leeds Philos Lit Soc* 5:136–139
- Beaudoin Yetter R (1989) The expression of male sterility in *Lobelia siphilitica* L. (Campanulaceae): a life history approach. PhD dissertation, Miami University, Miami, Ohio
- Bell G (1985) On the function of flowers. *Proc R Soc Lond B Biol Sci* 224:223–265
- Chapman SC, Barreto HJ (1997) Using a chlorophyll meter to estimate specific leaf nitrogen of tropical maize during vegetative growth. *Agron J* 89:557–562
- Conner JK, Davis R, Rush S (1995) The effect of wild radish floral morphology on pollination efficiency by four taxa of pollinators. *Oecologia* 104:234–245
- Darwin C (1877) The different forms of flowers on plants of the same species. Appleton, New York
- Dawson TE, Geber MA (1999) Sexual dimorphism in physiology and morphology. In: Geber MA, Dawson TE, Delph LF (eds) Gender and sexual dimorphism in flowering plants. Springer, Berlin Heidelberg New York, pp 175–215
- Delph LF (1996) Flower size dimorphism in unisexual plants. In: Lloyd DG, Barrett SCH (eds) Floral biology: studies on floral evolution in animal-pollinated plants. Chapman and Hall, New York, pp 217–237
- Dominee B, Assouad MW, Valdeyron G (1978) Natural selection and gynodioecy in *Thymus vulgaris* L. *Bot J Linn Soc* 77:17–28
- Dudle DA (1999) Maintenance and consequences of females in the gynodioecious plant, *Lobelia siphilitica*. PhD dissertation, Indiana University, Bloomington, Ind
- Dudle DA, Mutikainen P, Delph LF (2001) Genetics of sex determination in the gynodioecious species *Lobelia siphilitica*: evidence from two populations. *Heredity* 86:265–276
- Eckhart VM (1992a) Resource compensation and the evolution of gynodioecy in *Phacelia linearis* (Hydrophyllaceae). *Evolution* 46:1313–1328
- Eckhart VM (1992b) The genetics of gender and the effects of gender on floral characters in gynodioecious *Phacelia linearis* (Hydrophyllaceae). *Am J Bot* 79:792–800
- Eckhart VM (1993) Do hermaphrodites of gynodioecious *Phacelia linearis* (Hydrophyllaceae) trade off seed production to attract pollinators? *Biol J Linn Soc* 50:47–63
- Eckhart VM (1999) Sexual dimorphism in flowers and inflorescences. In: Geber MA, Dawson TE, Delph LF (eds) Gender and sexual dimorphism in flowering plants. Springer, Berlin Heidelberg New York, pp 123–148
- Eckhart VM, Chapin FS (1997) Nutrient sensitivity of the cost of male function in gynodioecious *Phacelia linearis* (Hydrophyllaceae). *Am J Bot* 84:1092–1098
- Eckhart VM, Seger J (1999) Phenological and developmental costs of male sex function in hermaphroditic plants. In: Vuorisalo TO, Mutikainen PK (eds) Life history evolution in plants. Kluwer, Dordrecht, pp 195–213
- Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C₃ plants. *Oecologia* 72:9–19
- Galen C, Newport MEA (1987) Bumble bee behavior and selection on flower size in the sky pilot, *Polemonium viscosum*. *Oecologia* 74:20–23
- Galen C, Sherry RA, Carroll AB (1999) Are flowers physiological sinks or faucets? Costs and correlates of water use by flowers of *Polemonium viscosum*. *Oecologia* 118:461–470
- Gouyon P, Vichot F, van Damme JMM (1991) Nuclear-cytoplasmic male sterility: single-point equilibria versus limit cycles. *Am Nat* 137:498–514
- Johnston MO (1991a) Natural selection on floral traits in two species of *Lobelia* with different pollinators. *Evolution* 45:1468–1479
- Johnston MO (1991b) Pollen limitation of female reproduction in *Lobelia cardinalis* and *L. siphilitica*. *Ecology* 72:1500–1503
- Johnston MO (1992) Effects of cross and self-fertilization on progeny fitness in *Lobelia cardinalis* and *L. siphilitica*. *Evolution* 46:688–702
- Kaul MLH (1988) Male sterility in higher plants. In Frankel R, Grossman M, Linsken HF, Maliga R, Riley R (eds) Monographs on theoretical and applied genetics. Springer, Berlin Heidelberg New York
- Kesseli R, Jain SK (1984) An ecological genetic study of gynodioecy in *Limnanthes douglasii* (Limnanthaceae). *Am J Bot* 71:775–786
- Lande R (1980) Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution* 34:292–305
- Laporte MM, Delph LF (1996) Sex-specific physiology and source-sink relations in the dioecious plant *Silene latifolia*. *Oecologia* 106:63–72.
- Lewis D (1941) Male sterility in natural populations of hermaphroditic plants. *New Phytol* 50:56–63
- Lloyd DG (1975) The maintenance of gynodioecy and androdioecy in angiosperms. *Genetica* 45:325–339
- Mutikainen P, Delph LF (1998) Inbreeding depression in gynodioecious *Lobelia siphilitica*: among-family differences override between-morph differences. *Evolution* 52:1572–1582
- Poot P (1997) Reproductive allocation and resource compensation in male-sterile and hermaphroditic plants of *Plantago lanceolata* (Plantaginaceae). *Am J Bot* 84:1256–1265
- Poot P, Pilon J, Pons TJ (1996) Photosynthetic characteristics of leaves of male-sterile and hermaphroditic sex types of *Plantago lanceolata* grown under conditions of contrasting nitrogen and light availabilities. *Physiol Plant* 98:780–790
- Poot P, van den Broek T, van Damme JMM, Lambers H (1997) A comparison of the vegetative growth of male-sterile and hermaphroditic lines of *Plantago lanceolata* in relation to N supply. *New Phytol* 135:429–437
- Renner SS, Ricklefs RE (1995) Dioecy and its correlates in the flowering plants. *Am J Bot* 82:596–606
- Sawhney VK, Shukla A (1994) Male sterility in flowering plants: are plant growth substances involved? *Am J Bot* 81:1640–1647
- Schepers JS, Francis DD, Vigil M, Below FE (1992) Comparison of corn leaf nitrogen concentration and chlorophyll meter readings. *Commun Soil Sci Plant Anal* 23:2173–2187
- Seger J, Eckhart VM (1996) Evolution of sexual systems and sex allocation in plants when growth and reproduction overlap. *Proc R Soc Lond B* 263:833–841
- Shykoff JA (1988) Maintenance of gynodioecy in *Silene acaulis* (Caryophyllaceae): stage-specific fecundity and viability selection. *Am J Bot* 75:844–850
- Slatkin M (1984) Ecological causes of sexual dimorphism. *Evolution* 38:622–630
- Sokal RR, Rohlf FJ (1995) Biometry. Freeman, New York
- Zar JH (1999) Biostatistical analysis. Prentice Hall, Upper Saddle River