Gynodioecy in *Lobelia siphilitica* and *L. spicata* (Lobeliaceae) from western Massachusetts[1]

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MILLER, J. S. (Department of Biology, McGuire Life Sciences Building, Amherst College, Amherst, MA 01002) AND J. L. STANTON-GEDDES (Department of Ecology, Evolution, and Behavior, 1987 Upper Buford Circle, 100 Ecology Building, University of Minnesota, St. Paul, MN 55108). Gynodioecy in *Lobelia siphilitica* and *L. spicata* (Lobeliaceae) from western Massachusetts. J. Torrey Bot. Soc. 134: 349–361. 2007.—Gynodioecy is a dimorphic breeding system in which male-sterile individuals (i.e., females) coexist with hermaphroditic individuals in populations. Previous studies of two species of *Lobelia* in North America have documented gynodioecy in parts of their ranges and here, we document gynodioecy for a single population of *L. siphilitica* and in two populations of *L. spicata* from western Massachusetts. Our objectives were to (1) determine sex ratios in natural populations of these species, (2) use controlled pollinations to investigate the capacity and extent of self fertilization in hermaphrodites and (3) compare the relative fertility and magnitude of sexual dimorphism in floral morphology between females and hermaphrodites. The frequency of females was only 3% in *L. siphilitica*, whereas both populations of *L. spicata* contained 12% females. Hermaphrodites in both species were self-compatible and estimates of mating system parameters confirmed mixed mating in *L. spicata*. Females of *L. spicata* had higher fruit and seed production compared to hermaphrodites and there was significant floral size dimorphism. In contrast, there was no sexual dimorphism and no differences in fruit and seed set between females and hermaphrodites in *L. siphilitica*. We also used herbarium records to document that flowering of these two congeners is well separated temporally in the Northeast and that northeastern collections of *L. spicata*, but not *L. siphilitica*, flower significantly later in the season compared to herbarium specimens collected from outside this region. Further, herbarium collections were used to test previous hypotheses concerning the validity of infrageneric classifications in *Lobelia spicata*; specifically, we confirmed that *L. spicata* var. *campanulata* is a male-sterile form of this species.

Key words: gynodioecy, herbarium specimens, *Lobelia siphilitica*, *Lobelia spicata*, mating system, self fertilization, sex ratios, sexual dimorphism.

Gynodioecy is a dimorphic breeding system in which hermaphroditic individuals and male-sterile (hereafter, female) individuals co-exist in populations. Although gender dimorphism is a relatively rare condition, occurring in fewer than 10% of angiosperm species (Geber et al. 1999), its repeated evolution among many unrelated taxa makes it significant evolutionarily. There has been considerable research related to the theories concerning the maintenance and spread of females in populations (Lloyd 1975, Charlesworth and Charlesworth 1978), expectations for sex-ratio variation (Delannay et al. 1981, Frank 1989, Gouyon et al. 1991, Bailey et al. 2003), and empirical studies of gynodioecious species (reviewed in Webb 1999). Generally, there is strong support implicating self-fertilization and consequent inbreeding depression in the evolution of gynodioecy (Thomson and Barrett 1981, Webb 1999). Further, the relative seed fertility of female and hermaphroditic plants is thought to be important to both the maintenance of females and in the dynamics of sex-ratio evolution in gynodioecious populations (Delph and Carroll 2001).

In many species, the genetic basis of gynodioecy involves both cytoplasmic and nuclear control (Delph et al. 2007); male-sterility is the result of cytoplasmic mutations,

whereas restoration of male function is achieved via nuclear genes (Lewis 1941, Kaul 1988, Frank 1989, Charlesworth and Laporte 1998, Dudle et al. 2001). One feature of species with nucleo-cytoplasmic control of gynodioecy is widespread and complex variation in the sex-ratio (i.e., the proportions of female and hermaphroditic plants) in populations (Olson et al. 2005, Nilsson and Ågren 2006, Caruso and Case 2007). Both ecological and genetic factors have been suggested as important in regulating the frequency of females in populations. For example, Nilsson and Ågren (2006) demonstrated that for *Plantago maritima* (Plantaginaceae), in which male-sterility shows nucleo-cytoplasmic inheritance, both stochastic processes acting in small populations and the relative fertility of the mating types affected sex ratios.

In populations that are dimorphic for gender, there is often a tendency toward sexual dimorphism in secondary reproductive characteristics. Such floral specialization has provided opportunities to investigate the morphological divergence of females and hermaphrodites in gynodioecious populations or females and males in dioecious populations (e.g., Darwin 1877, Baker 1948, Lloyd and Webb 1977, Delph et al. 1996, Ashman 1999, 2000, Schultz 2003). In particular, Eckhart (1999) and Delph and colleagues (Delph 1996, Delph et al. 1996) have observed that corollas on females are almost always smaller than those on hermaphroditic plants in gynodioecious populations spanning a wide variety of plant families. Several hypotheses have been suggested to explain such size differences, including selection for increased male function in hermaphrodites (Bell 1985) or resource savings in females (Eckhart 1992, Ashman 1994), and developmental associations between pollen fertility and flower size (Plack 1957, 1958, Wíden and Wíden 1999). Whatever the ultimate cause, certainly the pattern among gynodioecious populations is generally one of larger flowers in hermaphrodites compared to females (reviewed in Baker 1948, Delph 1996, Eckhart 1999).

In North America, there are relatively few representatives of the large genus *Lobelia* (Lobeliaceae; McVaugh 1936, Molano-Flores 2002) and even fewer (7 spp.) that extend into northeastern North America (Magee and Ahles 1999). However, two of these, *Lobelia siphilitica* L. and *L. spicata* Lam., have been

reported as gynodioecious (Yetter 1989, Molano-Flores 2002), and Dudle et al. (2001) showed that inheritance of male-sterility in *L. siphilitica* is nucleo-cytoplasmic. *Lobelia siphilitica* is well studied in the Midwest, both in terms of its floral morphology (Dudle 1999, Caruso et al. 2003a,b, Caruso 2004), mating systems (Johnston 1992, Dudle 1999), and recently with regard to sex-ratio variation (Caruso and Case 2007). The only published study of *L. siphilitica* from the Northeast is from northwestern Connecticut (Pigliucci and Schlichting 1995), but it is not clear from this study if populations contained females. In contrast, there are few published studies involving *L. spicata*, and only in Illinois and Ohio. This species has been described as gynodioecious (Cruden 1962, Molano-Flores 2002), and the frequency of females in populations varies widely (2–85%; Byers et al. 2005). In McVaugh's (1936) systematic treatment of *Lobelia* from eastern North America, he named five varieties of *L. spicata* (*L. spicata* var. *campanulata*, *L. spicata* var. *hirtella*, *L. spicata* var. *leptostachys*, *L. spicata* var. *originalis*, and *L. spicata* var. *scaposa*); note that *L. spicata* var. *originalis* is presently included within *L. spicata* var. *spicata*. However, in later work Bowden (1959) recognized only four varieties (*L. spicata* var. *hirtella*, *L. spicata* var. *leptostachys*, *L. spicata* var. *scaposa*, and *L. spicata* var. *spicata*); in particular, he excluded *L. spicata* var. *campanulata*, which Bowden (1959) interpreted as a male-sterile form of *L. spicata* var. *spicata*.

In the present study, we investigate the sexual system and reproductive ecology of *Lobelia siphilitica* and *L. spicata* in western Massachusetts. Previous studies (Dudle 1999, Caruso and Case 2007) suggest decreasing female frequency with increasing latitude in *L. siphilitica* but to date there are no reports of gynodioecy for either species in northeastern populations. Here, we document gynodioecy in Massachusetts for both *Lobelia siphilitica* and *L. spicata* and report the frequency of female plants in natural populations. We also present results from controlled pollinations and allozyme estimates of mating-system parameters to investigate the capacity and extent of self-fertilization in hermaphrodites. Further, we explore the magnitude of sexual dimorphism in floral morphology, flower production, and relative seed fertility between females and hermaphrodites. We use herbar-

ium surveys to detect the extent of temporal separation in flowering among these congeners in the Northeast, as well as, to contrast flowering times for northeastern and non-northeastern collections of each species. Finally, we test Bowden's (1959) hypothesis that *L. spicata* var. *campanulata* is male-sterile by examining herbarium specimens and recording the incidence of pollen sterility for different varieties of this species.

Materials and Methods. FIELD POPULATIONS. *Study Species and Sites. Lobelia siphilitica* (great blue lobelia) and *L. spicata* (palespike lobelia) are herbaceous, short-lived perennials producing basal rosettes and leaves along terminal racemes. In Massachusetts, *L. siphilitica* is known natively only from Berkshire Co. in the southwestern part of the state (P. Somers, Massachusetts Division of Fisheries and Wildlife), whereas *L. spicata* is present throughout the state. *Lobelia siphilitica* is more robust than *L. spicata* in terms of overall size and stature (50–150 cm versus 30–100 cm tall, respectively) and flower production [3–100 for *L. siphilitica* (Mutikainen and Delph 1998) versus 2–70 for *L. spicata* (Molano-Flores 2002)]. Further, flowers on *L. siphilitica* are bright blue and larger compared to the pale blue to white flowers of *L. spicata* (Fig. 1A).

We studied *Lobelia spicata* in two populations in Hampshire Co., Massachusetts. The first population was in the Mount Holyoke Range (42°17′28.02″N, 72°34′28.38″W), and plants were located in clearings along power lines on the south side of the range. The second population was partially located in the Upper Fort River Conservation area (42°21′26.70″N, 72°30′1.08″W) in Amherst; plants were present in open fields and were accessible along ca. 2 km of the Upper Fort River trail system. We studied *Lobelia siphilitica* in Berkshire Co., Massachusetts, at Bartholomew's Cobble (42°3′26.85″N, 73°21′2.73″W), a natural preserve managed by the Trustees of Reservations, Sheffield, MA. Plants were accessible in the reserve from the Borland and Tulip Tree trails in open, moist fields.

Sex Ratios and Flowering Phenology. Populations were sampled several times over the season to determine population sex ratios and flowering phenology. Female plants are easily distinguished from hermaphrodites by their papery white anther tubes (compare Figs. 1B and 1C) that do not contain pollen. Populations of *Lobelia spicata* were surveyed either five (Holyoke Range population) or nine (Upper Fort River population) times from 7 July to 8 August 2003. *Lobelia siphilitica* populations were surveyed six times from 21 August until 30 September 2003. As populations were small (< 600 plants), all flowering plants in populations could be sexed. A total of 421 flowering individuals in two populations of *L. spicata* (153 in Holyoke Range and 268 in Upper Fort River) and 552 flowering individuals of *L. siphilitica* were included in this study.

Relative Fertility of Females and Hermaphrodites and Controlled Pollinations. We monitored female and hermaphroditic plants to assess natural levels of reproduction and compare flower production, fruit set, and seed production for the two sexual morphs. Twenty-six hermaphrodites and 18 females of *L. siphilitica* and 34 hermaphrodites and 33 females of *L. spicata* in the Upper Fort River population were permanently tagged, but flowers were left open to receive natural pollination (control flowers). Marked plants were monitored twice a week throughout flowering and total flower and fruit production was recorded for individuals. Up to five fruits per individual were collected, dried and weighed individually to the nearest mg. Seed number was counted under a Nikon SMZ800 dissecting scope for 25 and 47 fruits of *L. siphilitica* and *L. spicata*, respectively. For each species, we regressed seed number on fruit weight; seed number was significantly correlated with fruit weight (*L. siphilitica*: $R^2 = 0.68$, $F_{1,23} = 50$, $P < 0.0001$; *L. spicata*: $R^2 = 0.90$, $F_{1,45} = 393$, $P < 0.0001$). We then used fruit weight to estimate seed number in the remaining sample of fruit (*L. siphilitica*, $n = 287$ fruits; *L. spicata*, $n = 336$ fruits).

To determine if hermaphroditic plants of *Lobelia siphilitica* and *L. spicata* were self-compatible, we compared fruit and seed production of flowers pollinated with either self or outcross pollen in natural populations of these species. Flowering inflorescences with a minimum of 10 unopened buds were covered with mesh bags (0.75 by 0.25 mm mesh size) to prevent insect visitation. Open flowers on inflorescences (typically < 5 flowers) were
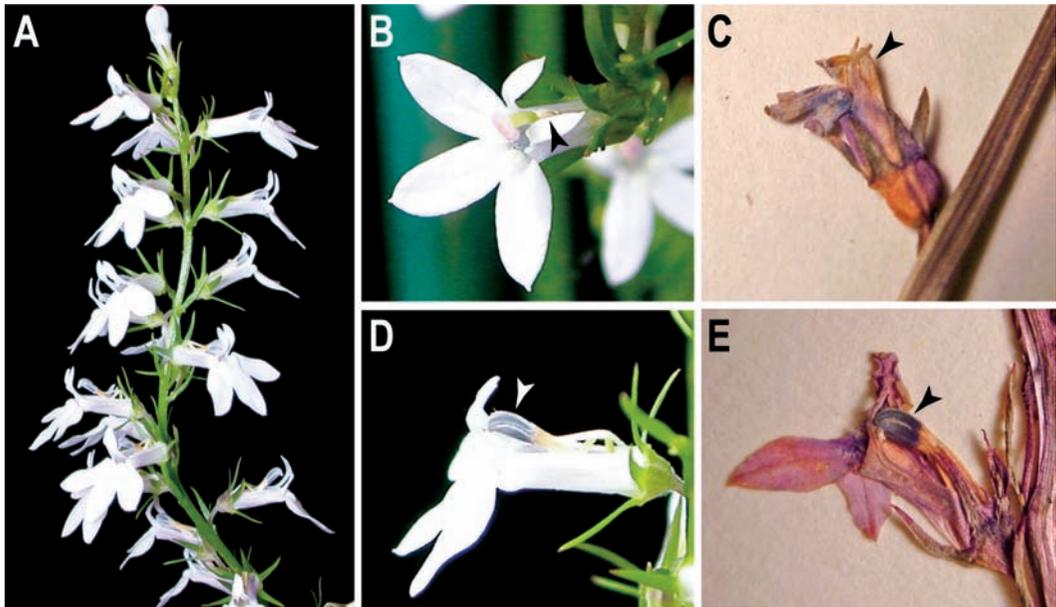
Fig. 1.　A flowering inflorescence of *Lobelia spicata* (A). Flowers on female plants (B and C) have reduced, white anther tubes (indicated with arrows) that lack pollen, whereas flowers on hermaphroditic plants (D and E) have well-developed, blue anther tubes (indicated with arrows) that contain pollen. Panels C and E are herbarium specimens showing flowers from female (C) *Rogers McVaugh 5111* (MIN) and hermaphroditic (E) *C. E. Garton 1249* (MIN) plants.

removed. Over the next several mornings, plants were revisited and newly opened (within 24 h) flowers were emasculated by removing the anther tube with fine forceps. Although autogamous pollination in *Lobelia* is not likely given their pollen-presentation mechanism and strong protandry within flowers (Johnston 1991, Molano-Flores 2002), emasculation was done to prevent autogamy, force hermaphroditic flowers into the pistillate phase (Devlin and Stephenson 1984, Dudle 1999), and to collect pollen for crosses. Flowers on plants were pollinated using pollen collected from a minimum of two flowers either from the same plant (self treatment) or pooled from several different plants (cross treatment) collected from at least 10 m away. A total of 83 flowers were pollinated with self pollen and 86 flowers with outcross pollen on 33 plants of *L. siphilitica*. In *L. spicata*, we pollinated 74 flowers with self pollen and 76 flowers with outcross pollen on a total of 26 plants. Pollination treatments were usually paired on plants and every effort was made to replicate treatments within plants. We used Mann-Whitney U tests with Dunn-Sidak corrections (Sokal and Rohlf 1995) to determine if fruit and seed production were significantly differ-

ent following outcross versus self pollination in hermaphrodites, as well as for open control flowers on female versus hermaphrodite plants.

Allozyme Estimates of Mating System. We used cellulose-acetate gel electrophoresis to estimate the proportion of selfed and outcrossed seeds in hermaphrodites. Leaf material from 20 (*Lobelia siphilitica*) and 25 (*L. spicata*, Upper Fort River population) hermaphrodites was collected and frozen immediately. We also collected fruits from these plants, germinated seeds, and harvested seedlings 3–4 weeks following germination. Samples were processed in 30 or 100 μl of homogenization buffer (Werth 1985) and centrifuged (6,000 rpm) at 4°C. Cellulose-acetate plates were soaked in the running buffer (tris-glycine pH 8.0, Hebert and Beaton 1989) prior to electrophoresis. On each plate, at least one replicate aliquot from a previous run was used as a control for contamination and to facilitate banding pattern interpretation across gels. Samples were run at 150 volts for 45 min on ice. Following initial screens of several enzymes, only phosphoglucose isomerase (*Pgi*), phosphoglucomutase (*Pgm*), and malate de-

hydrogenase (*Mdh*) showed adequate enzyme activity and, of these, only *Pgi* and *Pgm* showed interpretable variation (recipes modified from Richardson et al. 1986, Hebert and Beaton 1989). A total of 112 progeny from seven maternal parents of *L. spicata* and 200 progeny from 10 maternal parents of *L. siphilitica* were scored. We verified Mendelian inheritance of *Pgm* and *Pgi* by comparing progeny arrays within maternal parents (Wendel and Weeden 1989, Kephart 1990). No variability in banding patterns was observed among parents or progeny arrays in *L. siphilitica*. By contrast, in *L. spicata*, two loci (each with multiple alleles) were present for *Pgi* and *Pgm*, and these were included in mating-system estimates.

Parameters of the mating system of *Lobelia spicata* were estimated using the multi-locus mating-system program MLTR (Ritland 2002), based on the models in Ritland and Jain (1981). Several parameters are estimated in this program, including the multi-locus outcrossing rate and the inbreeding coefficient of maternal parents. Maximum-likelihood parameter estimation used the default settings and the Newton-Raphson numerical method as implemented in MLTR. Standard errors of the estimates were calculated using 1000 bootstrap replicates, with families as the unit of observation.

Sexual Dimorphism. Recently opened (< 1 day old) flowers from females and hermaphrodites of *Lobelia spicata* (Upper Fort River population) were collected and preserved in FAA. Including 26 flowers from 15 female plants and 88 flowers from 41 hermaphroditic plants, we measured corolla tube length, corolla tube width, corolla lobe length, and corolla lobe width (following Caruso et al. 2003a). All measurements were made to 0.1 mm using a Nikon SMZ800 dissecting scope with an ocular micrometer. For *L. siphilitica*, flowers from 43 hermaphroditic plants were collected, and corolla measurements (to 0.1 mm) were made using digital calipers. These flowers are protandrous; hence pistillate ($n = 21$) and staminate ($n = 22$) phase hermaphroditic flowers were compared for the four corolla traits. Only seven flowers from females were collected; thus, comparisons across genders in this species were not possible. We used Mann-Whitney U-tests with Dunn-Sidak corrections (Sokal and Rohlf 1995) to test for sexual dimorphism in corolla traits for *L. spicata* and to determine if pistillate- and staminate-phase hermaphroditic flowers in *L. siphilitica* differed in size.

HERBARIUM SURVEY. *Phenology.* Flowering phenology was determined using collections housed at the University of Massachusetts (MASS), University of Connecticut (CONN), L. H. Bailey Herbarium (BH) at Cornell University, Philadelphia Academy of Natural Sciences (PH), Missouri Botanical Garden (MO), University of Minnesota (MIN), the New England Botanical Club (NEBC), as well as recent records obtained from the Massachusetts Natural Heritage Endangered Species program (Massachusetts Division of Fisheries and Wildlife). For each specimen, we recorded the locality (state, county, and town), date of collection, and whether the specimen had flowers. All dates were assigned a number corresponding to the week of the year that each specimen was collected, and specimens were further characterized if they were collected from the Northeast (as defined by Magee and Ahles 1999). Duplicate specimens were counted a single time and only those specimens with complete date information (e.g., month, day and year) were included. We recorded data for 169 and 267 herbarium collections of *L. siphilitica* and *L. spicata*, respectively. We used a general linear model to analyze the flowering phenology of herbarium specimens. Specifically, flowering times for the two species, as well as for Northeast and non-northeastern collections were compared. Effects included in the model were species (*L. siphilitica* and *L. spicata*), locale (Northeast or non-northeastern) and their interaction.

Sexual Expression of Lobelia spicata var. campanulata. To test the hypothesis that *Lobelia spicata* var. *campanulata* is a male-sterile form of this species, we examined 452 herbarium sheets from BH, MASS, MO, MIN, and PH. When possible, we determined the sex (compare Figs. 1C and 1E) of all plants on herbarium collections and recorded the variety if indicated. Of the 452 herbarium sheets surveyed, we determined the sex of 747 plants on 423 herbarium sheets (29 specimens could not be sexed reliably). Twenty-six herbarium accessions labeled *L. spicata* var. *campanulata* were included, as well as 397 additional accessions of which 28% ($n = 110$)

Table 1. Fruit and seed production among pollination treatments (cross and self) for hermaphrodite plants of *Lobelia siphilitica* and *L. spicata*. Fruit, seed, and flower production are also presented for naturally pollinated control flowers for both female (F) and hermaphroditic (H) plants. Comparisons of female and hermaphroditic plants of *L. spicata* were significant for all three variables measured (in bold). Values are means ± standard errors.

Treatment	*L. siphilitica*			*L. spicata*		
	Fruit set	No. seeds	Flower no.	Fruit set	No. seeds	Flower no.
Cross pollination	0.88 ± 0.04	498.7 ± 30.2	—	0.82 ± 0.05	46.1 ± 7.5	—
Self pollination	0.92 ± 0.04	487.1 ± 31.4	—	0.89 ± 0.04	42.9 ± 5.6	—
Open control (H)	0.82 ± 0.03	467.7 ± 43.6	22.9 ± 3.0	**0.76 ± 0.04**	**51.2 ± 5.6**	**31.1 ± 2.5**
Open control (F)	0.83 ± 0.03	471.7 ± 31.7	28.2 ± 6.7	**0.92 ± 0.02**	**181.2 ± 10.1**	**16.7 ± 1.6**

were labeled to varieties other than var. *campanulata* [*L. spicata* var. *hirtella*, *L. spicata* var. *leptostachys*, *L. spicata* var. *scaposa*, and *L. spicata* var. *spicata* (including those specimens labeled *L. spicata* var. *originalis* and *L. spicata* var. *parviflora*)]. We used a 2×2 contingency test to determine if plants on herbarium sheets labeled *L. spicata* var. *campanulata* were more often female than plants not labeled this variety. Chi-square values with the Yates correction (Yates 1934) were used to assess significance. Herbarium specimens often contained several plants per sheet; analyses were conducted for individual plants.

Results. FIELD POPULATIONS. *Sex Ratios in Massachusetts.* The two species of *Lobelia* studied in western Massachusetts were gynodioecious, and populations consisted of plants that were either hermaphroditic or male-sterile. The frequency of female plants was similar in the two populations of *L. spicata* (Holyoke Range: 19 females/153 individuals, 12.4%; Upper Fort River: 33 females/268 individuals, 12.3%), whereas females were uncommon in *L. siphilitica* (Bartholomew's Cobble: 18 females/552 individuals, 3.3%).

Relative Fertility of Females and Hermaphrodites and Controlled Pollinations. There were no differences in fruit set ($U = 144$, $P = 0.984$, $n_F = 12$, $n_H = 24$), seed production ($U = 131$, $P = 0.834$, $n_F = 11$, $n_H = 25$), or flower number ($U = 186$, $P = 0.576$, $n_F = 16$, $n_H = 26$) between open control flowers on females and hermaphrodites of *Lobelia siphilitica*. In contrast, in *L. spicata* control flowers on females had higher fruit ($U = 420$, $P = 0.0003$, $n_F = 33$, $n_H = 33$) and seed production ($U = 420$, $P < 0.0001$, $n_F = 21$, $n_H = 20$). Females of *L. spicata* also had fewer flowers compared to hermaphrodites ($U = 894$, $P <$

0.0001, $n_F = 33$, $n_H = 34$; Table 1). Hermaphroditic plants of both species were self-compatible; there were no differences in fruit set or seed production following selfing and outcrossing for either *L. siphilitica* (Table 1; fruit set: $U = 561.5$, $P = 0.67$, $n_{OUT} = 33$, $n_{SEL} = 32$; seed number: $U = 481$, $P = 0.84$, $n_{OUT} = 32$, $n_{SEL} = 31$) or *L. spicata* (fruit set: $U = 353$, $P = 0.61$, $n_{OUT} = 26$, $n_{SEL} = 25$; seed number: $U = 324$, $P = 0.98$, $n_{OUT} = 26$, $n_{SEL} = 25$).

Allozyme Estimates of Mating System. Four enzyme loci for *Lobelia spicata* were used for the mating-system estimates reported here. Two alleles for each locus were present, and their frequencies were as follows: *Pgi-1* (0.76 and 0.24), *Pgi-2* (0.943 and 0.057), *Pgm-1* (0.963 and 0.037), and *Pgm-2* (0.873 and 0.127). The multilocus outcrossing rate was 0.489 (SE = 0.197) and the inbreeding coefficient of maternal parents was 0.682 (SE = 0.290). No variability in banding patterns was observed among parents or progeny arrays in *L. siphilitica*.

Sexual Dimorphism. There were no significant differences for any of the corolla traits between pistillate- and staminate-phase flowers in *Lobelia siphilitica*, so we pooled these to calculate averages for hermaphrodites (Table 2). Though there was a trend for hermaphroditic flowers to have longer and wider corolla tubes compared to flowers on females in *L. siphilitica*, significance could not be reliably assessed given the small number of females in our sample. However, sexual dimorphism in corolla traits was present in *L. spicata*. Flowers on hermaphrodites had longer ($U = 534$, $P < 0.0001$, $n_F = 15$, $n_H = 41$) and wider ($U = 481$, $P = 0.0014$, $n_F = 15$, $n_H = 41$) corolla lobes, and longer corolla tubes ($U = 496$, $P = 0.0005$, $n_F = 15$, $n_H = 41$) compared to flowers on female plants (Table 2).

Table 2.   Floral measurements (in mm) for flowers from females (F) and hermaphrodites (H) in *Lobelia siphilitica* and *Lobelia spicata*. The number of plants (*n*) included for each species and sexual type is indicated. Comparisons in bold were significant for Mann-Whitney U tests after the Dunn-Sidak correction for multiple tests. Values are means ± standard errors.

Floral trait	*Lobelia siphilitica*		*Lobelia spicata*	
	F (*n* = 7)	H (*n* = 43)	F (*n* = 15)	H (*n* = 41)
Corolla lobe width	4.2 ± 0.29	4.1 ± 0.10	**1.6 ± 0.06**	**2.0 ± 0.04**
Corolla lobe length	8.6 ± 0.25	8.8 ± 0.15	**4.2 ± 0.16**	**4.8 ± 0.07**
Corolla tube width	3.2 ± 0.21	3.6 ± 0.09	1.5 ± 0.06	1.4 ± 0.03
Corolla tube length	18.0 ± 0.61	19.4 ± 0.21	**5.3 ± 0.13**	**5.9 ± 0.10**

HERBARIUM SURVEY. *Phenology. Lobelia spicata* flowers significantly earlier than *L. siphilitica* in both northeastern and non-northeastern collections (main effect of species; $F_{1,432} = 610$, $P < 0.0001$). The average week of flowering for northeastern collections was 28.1 for *L. spicata* and 35.8 for *L. siphilitica* (Fig. 2A).

Though the main effect of locale was not significant ($F_{1,432} = 2$, $P = 0.10$), specimens of *Lobelia spicata* collected from the Northeast flowered, on average, about two weeks later than did conspecific specimens collected outside the Northeast (mean week of flowering, 28.1 versus 26.1; Fig. 2B). However, the same was not found for *L. siphilitica*; the mean week of flowering was 35.8 ($n = 39$ specimens) for northeastern collections and 36.6 ($n = 130$ specimens) for non-northeastern collections (Fig. 2C). This difference was responsible for the significant species by locality interaction term in our analyses ($F_{1,432} = 13$, $P = 0.0003$).

Sex Expression of Lobelia spicata var. campanulata. Twenty-six sheets labeled *L. spicata* var. *campanulata* contained 41 plants, and 38 of these plants were female and three were hermaphroditic (93% female frequency, Table 3). By contrast, of 706 plants included on 397 sheets not identified as *L. spicata* var. *campanulata*, 74 and 632 were female and hermaphroditic, respectively (10% female frequency). A 2 × 2 contingency test revealed that herbarium collections labeled *L. spicata* var. *campanulata* are more often female than expected given the frequency of females in *L. spicata* collections overall ($\chi^2 = 199$, $P < 0.0001$).

Discussion. FIELD POPULATIONS. *Sex Ratios in Massachusetts.* Populations of *Lobelia spicata* and *L. siphilitica* in Massachusetts are gynodioecious and contain male-sterile plants. Several previous reports of sex ratios in populations of *L. siphilitica* suggest that there is wide variation in female frequency (and mating system) across the species range. For example, populations in southern Indiana and Ohio have female frequencies ranging from near 50% to over 90% (Yetter 1989, Mutikainen and Delph 1998, Dudle 1999). By contrast, female plants are reported only rarely from Michigan populations of *L. siphilitica* (Johnston 1992), and Caruso et al. (2003a) also report low female frequencies (1–9%) in populations from Jasper County, Iowa. Interestingly, among published studies, populations with low (< 10%) female frequencies are from more northern locations, whereas higher female frequencies are associated with populations in southern Indiana and Ohio. In a large-scale survey of *L. siphilitica*, Caruso and Case (2007) sampled female frequency across 53 populations throughout the Midwest (IA, WI, IL, IN, OH, MI, and WV). The authors found that female frequency was negatively correlated with latitude; that is, populations at more northern latitudes contained fewer females. However, they also documented a significant effect of population size on female frequency; specifically, female frequencies were higher in small (< 100 individuals) versus large (> 100 individuals) populations. Caruso and Case (2007) note that their data are consistent with previous studies of gynodioecious taxa with nucleo-cytoplasmic inheritance that document the importance of stochastic forces (i.e., genetic drift) in regulating female frequency (Manicacci et al. 1996, Byers et al. 2005, Nilsson and Ågren 2006). Here, we document low female frequency (3%) in a large (> 550 individuals) population of *L. siphilitica* from southwestern Massachusetts. Thus, our data are consistent with Caruso and Case's (2007) analysis of sex-ratio variation in *L. siphilitica* and its relationship to geography and population size. There
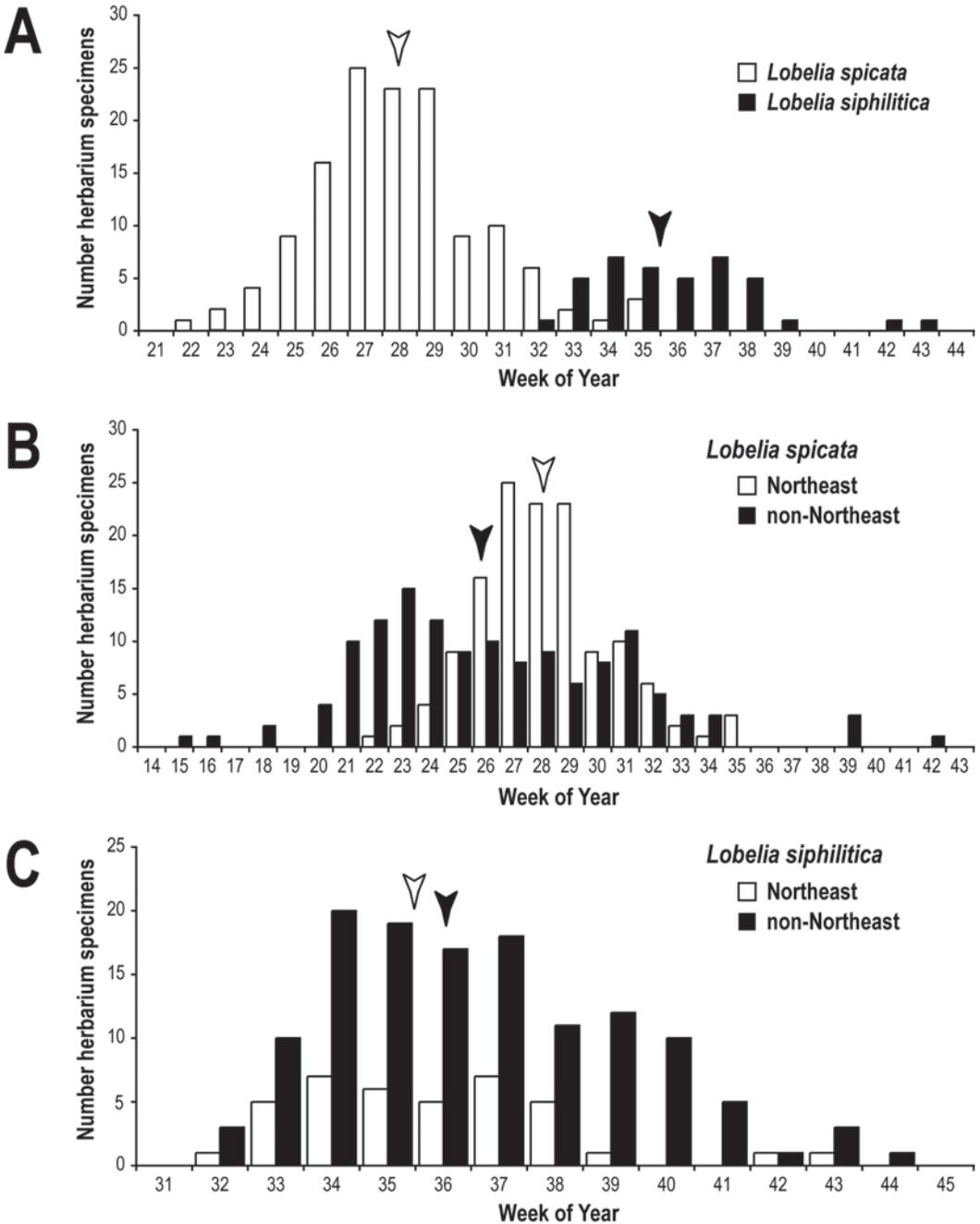
FIG. 2.   Flowering phenology of (A) *Lobelia spicata* and *L. siphilitica* in the Northeast, and (B) *Lobelia spicata* and (C) *Lobelia siphilitica* collected from throughout their ranges. Phenology was determined from herbarium specimens located at BH, CONN, MASS, MIN, MO, NEBC, PH and the Massachusetts Natural Heritage Endangered Species Program. Arrows indicate the mean flowering week.

is only one report of sex-ratio data for *Lobelia spicata* (Byers et al. 2005) and the frequency of females varies widely [ranging from near zero (2%) to 85%] among prairie populations in Illinois. We report relatively low female frequency (12%) for two populations in western Massachusetts. Two of the experimental populations in Byers et al. (2005) were

Table 3. The number of herbarium sheets of *Lobelia spicata* examined to assess the relationship of varietal classification and sexual phenotype. The total number of plants and the number of female (F) and hermaphroditic (H) plants is indicated, as is the proportion of females for accepted varieties of *L. spicata*. Note that *L. spicata* var. *spicata* includes those specimens labeled *L. spicata* var. *originalis* (6 specimens, 14 plants) and *L. spicata* var. *parviflora* (2 specimens, 2 plants). Herbarium specimens were from BH, MASS, MIN, MO, and PH.

Variety	No. sheets examined	Total no. plants	No. F plants	No. H plants	Proportion females
Lobelia spicata var. *campanulata*	26	41	38	3	0.93
Total NON *L. spicata* var. *campanulata*	397	706	74	632	0.10
L. spicata (no variety indicated)	287	506	47	459	0.09
L. spicata var. *spicata*	81	158	15	143	0.09
L. spicata var. *leptostachys*	22	28	7	21	0.25
L. spicata var. *hirtella*	5	11	5	6	0.45
L. spicata var. *scaposa*	2	3	0	3	0.00

of similar sizes to the populations in the present study (ca. 300 plants), but comparisons are limited given that their populations were chosen because of divergent female frequencies. Generalities about patterns of sex-ratio variation in *L. spicata* await future large-scale studies as in Caruso and Case (2007).

Relative Fertility of Females and Hermaphrodites and Controlled Pollinations. In the present study for both species, fruit and seed set for the pollination treatments either exceeded or were equivalent to those in unmanipulated hermaphroditic controls (Table 1); thus, there were no effects of floral manipulation in our study. Further, there were no differences in either fruit or seed set following pollination with self and outcross pollen. These data are in accordance with observations of self-compatibility for *L. spicata* in Illinois (Molano-Flores 2002) and *L. siphilitica* (Yetter 1989, Johnston 1992, Dudle 1999). In addition, our controlled crosses are consistent with allozyme estimates of outcrossing rates in *L. spicata*, which indicate that this species has a mixed mating system. Despite strong intrafloral protandry (Molano-Flores 2002), a significant amount of geitonogamous pollen transfer likely occurs.

The two species differed in the relative fertility of females and hermaphrodites. Whereas fruit set, seed production, and flower number all differed significantly between females and hermaphrodites in *Lobelia spicata*, none of these differed for *L. siphilitica*. In *L. spicata*, females had both higher fruit and seed set compared to hermaphrodites, but they produced fewer total flowers (Table 1). Our data contrast with Molano-Flores (2002), who

reported uniformly high fruit set among open pollinated females and hermaphrodites (80% versus 83%, respectively). Though we found comparable levels of fruit set in hermaphroditic plants from Massachusetts (76%, see Table 1), females had significantly higher fruit set (92%) and seed production compared to hermaphrodites. In *L. siphilitica*, Yetter (1989) reported significantly higher fruit set in females for one population of *L. siphilitica*, but no difference for two other populations, and Dudle (1999) reported higher fruit set in females. In addition, Yetter (1989) documented no differences in seed number between females and hermaphrodites following controlled pollinations in the greenhouse. Thus, our data are not outside the range of previous work on this species. Instead, it may be the case that the relative fruit and seed production of females and hermaphrodites varies among populations of these two species. Asikainen and Mutikainen (2003) documented higher fruit and seed production in females compared to hermaphrodites for *Geranium sylvaticum* (Geraniaceae), and argued that such female advantages may at least in part explain the maintenance of females within gynodioecious *Geranium* populations. In addition, these authors noted a negative correlation of female frequency with the relative seed fitness of hermaphrodites. Indeed, several authors have argued that the relative seed fertility of hermaphrodites can affect population sex ratios in gynodioecious populations (Delph and Carroll 2001, Asikainen and Mutikainen 2003, Nilsson and Ågren 2006). In particular, female frequency is expected to decline with increasing seed fertility in hermaphrodites; that is, when hermaphrodites have high female

function, females are more rare in populations. Recent comprehensive studies (e.g., Nilsson and Ågren 2006, Caruso and Case 2007) investigating the influence of both stochastic (e.g., population size) and ecological forces (e.g., relative seed fertility in females and hermaphrodites, herbivory, inbreeding) point the way toward an understanding of the complicated dynamics of sex-ratio variation among gynodioecious populations.

Sexual Dimorphism. Not only do hermaphrodites produce more flowers than females in *Lobelia spicata* (Table 1), these flowers are also significantly larger than those on female plants for three of four corolla traits (Table 2). These data are consistent with Byers et al. (2005), who report that corollas on hermaphrodites are wider than those on females. Interestingly, in his description of *L. spicata* var. *campanulata*, McVaugh (1936) reports that both flower size and number are reduced in this variety. Thus, if Bowden (1959) is correct in that *L. spicata* var. *campanulata* is, in fact, a male-sterile form of *L. spicata* (see data below), then our data for females are also consistent with McVaugh's (1936) observations.

Although females of *Lobelia spicata* produced fewer flowers than hermaphrodites, they had significantly greater fruit and seed production; thus, it is possible that the relative increase in the seed fitness of females (compared to hermaphrodites, Table 1) is the result of resource compensation in females. However, the pattern of increased floral displays in hermaphrodites (more and larger flowers compared to females, Tables 1, 2) is also consistent with the hypothesis of selection for greater male function in hermaphrodites. These two mechanisms (reduction in female flower size with subsequent compensation and increased hermaphroditic flower size to attract pollinators) are not mutually exclusive, and both may affect flower size in gynodioecious populations. Molano-Flores (2002) reported that primarily small bees visited *L. spicata* in Illinois; however, the effectiveness of these visitors is unknown. Given that flowers on female plants do not produce nectar (Molano-Flores 2002), and that females produce fewer, smaller flowers than those on conspecific hermaphrodites, further studies of how these sex-specific differences affect pollinator service are warranted.

In contrast to *Lobelia spicata*, no differences in either flower number or corolla size measures were detected for *L. siphilitica* (Tables 1, 2). There are, however, several reports of flower number and floral morphology for this species in the literature (Yetter 1989, Dudle 1999, Caruso et al. 2003a,b, Caruso 2004). Not surprisingly, floral morphological traits are generally smaller in natural populations (this study; Caruso et al. 2003a) as compared to greenhouse-raised plants (Caruso et al. 2003b, Caruso 2004). Likewise, flower number was almost twice as great for plants raised in the greenhouse (Caruso et al. 2003b, Caruso 2004) compared to natural populations (Caruso et al. 2003a). Our study included flowers collected from a single population in Massachusetts, and these data are generally within the range reported for natural populations studied by Yetter (1989) and Caruso et al. (2003a). However, whereas we did not find differences between flowers from female and hermaphroditic plants, Caruso et al. (2003b) reported significant sexual size dimorphism for corolla lobe length, and corolla tube length and width. Similarly, Yetter (1989) found that flowers on hermaphroditic plants were significantly longer, but not wider, than those on females. Despite a trend for longer and wider corolla tubes in the present data, our sample size for females is too low to detect significant differences.

HERBARIUM SURVEY. *Phenology. Lobelia spicata* flowers significantly earlier than *L. siphilitica* in both northeastern and non-northeastern collections, and there is very little overlap in flowering between the two species in the Northeast. Specifically, the average week of flowering for northeastern specimens was 28.1 for *L. spicata* versus 35.8 for *L. siphilitica*; thus, the two species are well separated temporally, and our fieldwork overlapped with historical records of peak flowering for both species. Molano-Flores (2002) also observed that *L. spicata* was temporally separated from other species of North American *Lobelia*, including *L. siphilitica*.

Lobelia spicata collected in the Northeast flowered significantly later than conspecifics collected from outside the Northeast. However, the same was not found for *L. siphilitica*; there was no difference in the onset or mean time of flowering for northeastern versus non-

northeastern populations of *L. siphilitica*. The difference in flowering phenology of northeastern versus non-northeastern populations of *L. spicata* is consistent with the shorter flowering season in the Northeast. In contrast, *L. siphilitica* flowers later than *L. spicata* both in the Northeast and throughout its range. Further, as *L. siphilitica* has a late summer phenology throughout its range, delayed flowering may not be an option given early frost in the Northeast.

Sex Expression of Lobelia spicata var. campanulata. As first suggested by Bowden (1959), it appears that the taxonomy of *Lobelia spicata* is confounded with sexual phenotype. In his revision of eastern North American *Lobelia*, McVaugh (1936) distinguished five varieties of *L. spicata*. Two of these, *L. spicata* var. *originalis* (currently synonymized with *L. spicata* var. *spicata*) and *L. spicata* var. *campanulata*, co-occur and are distinguished primarily by floral morphology. In his description, McVaugh (1936) distinguishes between these varieties using the color of the anther tube (blue in *L. spicata* var. *originalis* versus white in *L. spicata* var. *campanulata*) and flower size (large in *L. spicata* var. *originalis* versus small in *L. spicata* var. *campanulata*). Our analysis of herbarium specimens indicates that specimens identified as *L. spicata* var. *campanulata* are overwhelmingly female, whereas the frequency of females was significantly less for specimens of other varieties or for those not identified to variety (Table 3). Thus, our data are consistent with previous suggestions that *L. spicata* var. *campanulata* is not a true taxonomic variety, but instead is the female morph of this species (Bowden 1959, McGregor 1985).

Conclusions. In summary, both *Lobelia siphilitica* and *L. spicata* are gynodioecious in Massachusetts. Our research corroborates previous studies of these species in other parts of their ranges (Yetter 1989, Molano-Flores 2002) and is the first report of gynodioecy in the Northeast for either species. Examination of herbarium collections revealed that *L. spicata* var. *campanulata* (McVaugh 1936) is predominantly male-sterile and not a valid variety (see also Bowden 1959). Our herbarium observations also suggest that gynodioecy may also be present in other species of *Lobelia* (e.g., *L. puberula*, Bowden 1959, A. L. Case,

Kent State University, pers. comm.). Across populations, there is extensive variation in the frequency of females for *L. siphilitica* (Caruso and Case 2007) and probably also for *L. spicata*, though comprehensive surveys of the latter species are lacking at present. Future studies to dissect the contributions of drift and selection as they relate to the frequency of females are necessary. In addition, comparative phylogenetic studies of species relationships and breeding systems would be instructive in understanding the evolution of cosexual and dimorphic breeding systems in *Lobelia*. Finally, the inheritance of gynodioecy in *Lobelia* is controlled both by cytoplasmic male-sterility factors and nuclear alleles that restore male function (Dudle et al. 2001), yet not all members of *Lobelia* are gynodioecious; thus, more generally, this genus provides a model for the study of the dynamics of nuclear and cytoplasmic interactions.

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