

POPULATION DYNAMICS AND REPRODUCTIVE ECOLOGY OF THE
GYNODIOECIOUS PRAIRIE SPECIES LOBELIA SPICATA LAM. (CAMPANULACEAE)

BY

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THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Plant Biology
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2012

Urbana, Illinois

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ABSTRACT

Lobelia spicata Lam. (Campanulaceae) is a common prairie species found throughout Illinois. Its breeding system, gynodioecy, is relatively rare, and is characterized by having female and hermaphrodite plant morphs coexisting within populations. Although some data on the reproductive ecology of *L. spicata* has been gathered, the population and breeding system dynamics of this species remain unexplored. Consequently, a literature review and a field study were conducted where population and reproductive output and fitness data were collected for 11 *L. spicata* populations across northern and central Illinois during 2008 and 2009.

Chapter 1 provides a summary of the literature review and includes the classification of and phylogenetic relationships within *Lobelia* L. for determination of *L. spicata*'s closest relatives. Information is also provided on breeding system distribution within *Lobelia*, as well as patterns of variation in pollination type, floral compatibility and pollination syndrome for the closest relatives of *L. spicata*. It was found that the classification of *Lobelia* has undergone multiple transformations throughout history, but the most recent system divides the genus into 18 sections based on phenotypic characters, biogeographical patterns and molecular phylogenies. *L. spicata* is grouped with the 21 other eastern North American species into *Lobelia* section *Lobelia*. Members of *Lobelia* are exclusively synoecious with the exception of section *Hypsela*, which contains dioecious species, and section *Lobelia*, which contains two gynodioecious species including *L. spicata*. For *L. spicata*'s closest relatives in *Lobelia* section *Lobelia*, only 6 species out of 22 have had data on pollination type and floral compatibility collected, and among those species the combinations are highly variable. Further, 21 of the 22 species (e.g. *L. spicata*) are insect-pollinated with white, blue or purple corollas. *L. cardinalis* is the only species exhibiting an avian pollination syndrome, most likely due to its large, red flowers. It was concluded that the construction of comprehensive, molecular-based phylogenies for the North American *Lobelia* as well as additional research on individual *Lobelia* species will increase understanding of breeding system evolution within the genus.

Chapter 2 examines the population dynamics of *L. spicata* overall by examining the impact of population size and density on reproductive output and fitness measurements. No relationship was found between population size or density and any of the four reproductive measurements across two sampling years with three exceptions. Population size exhibited a marginally significant negative correlation with fruit set in 2008 and a positive correlation with seed number per fruit in 2009, while population density was positively correlated with seed number per fruit in 2008. Size of *L. spicata* populations may be having an indirect impact on fruit set and seed number per fruit during intermittent years due to its influence on the availability of compatible, out-crossed pollen. Alternatively, high plant density may be linked to increased visitation of flowers by pollinators, causing an increase in the number of seeds produced per fruit. Thus, population dynamics such as size and density could impact future reproductive success and population persistence in *L. spicata*.

Chapter 3 examines the gynodioecious breeding system of *L. spicata* to determine if reproductive differences exist between plant genders, and if so how those differences are impacted by female frequency and/or gender density. Female plants were found to produce greater fruit sets, seed numbers per fruit and percent seed germination than hermaphrodites, though these reproductive gender differences varied among populations and between 2008 and

2009. Female frequency, female density and hermaphrodite density did impact the gender-based reproductive measurements, particularly fruit set, presumably due to their influence on pollen quantity and quality.

Chapter 4 assesses whether two abiotic factors, temperature and precipitation, are driving the relationship between female frequency, gender morph density and reproduction measurements across a latitudinal gradient. Female frequency within *L. spicata* populations was negatively correlated with latitude, such that there were higher percentages of females in southern than in northern populations. Temperature is likely the driving force behind the latitude/female frequency relationship because it negatively correlated with latitude and positively correlated with temperature across sampling years. However, less precipitation in southern populations during intermittent years may also account for some of the latitudinal variation in female frequency. Gender-based reproductive success measurements and female advantage did not correlate with geographic location or abiotic conditions with few exceptions. Both hermaphrodite and female seed biomass and percent seed germination were negatively correlated with latitude and positively correlated with temperature. Thus, stressful conditions such as high temperatures and low precipitation may somehow favor the success of female plants within populations of *L. spicata*, while high temperatures result in the production of larger and better germinating seeds by hermaphrodite and female plants.

In summary, this study has provided detailed information on many aspects of *Lobelia spicata*'s reproductive ecology that was formerly unknown. By starting with the larger issues of breeding system distribution within *Lobelia* (Chapter 1) and species-level reproductive variation with population size and density (Chapter 2), it was possible to critically evaluate trends existing due to *L. spicata*'s gynodioecious breeding system such as gender morph differences and the impact of female frequency and gender morph density on female advantage in reproduction (Chapter 3). Further, formerly unknown patterns of latitudinal variation in female frequency and reproduction were uncovered for the species (Chapter 4). In a broader context, these data could have important implications for the management of species with polymorphic breeding systems in the face of global climate change.

ACKNOWLEDGEMENTS

There are a number of people I must acknowledge for the role they played in bringing this research to fruition. First and foremost, I would like to offer my sincerest gratitude to my advisor, Brenda Molano-Flores, for her unfailing guidance and patience. Through her counsel and example, I have learned what a good researcher (and person) should be. Her constant enthusiasm and encouragement through the trials and tribulations of field and lab work, statistical analysis and manuscript preparation were invaluable to me, without which this study would never have been completed. I would also like to express my appreciation to the members of my Master's Committee, Stephen Downie and Ken Paige, who provided helpful feedback on my project at various stages of its completion.

To my labmates Katherine Chi, Greg Spireas and Jason Zylka and to my friends Amy Dombrowski, Katrina Call, Jenn Onderisin, Rhiannon Peery and Laura Stein, I offer my sincere thanks. Some of these individuals have assisted with field data collection, and in doing so have braved with me the many perils of field work (e.g. inclement weather, allergens, painfully-armed plants and blood-sucking insects). Others have provided advice on data analysis or feedback on sections of my thesis manuscript while sipping coffee in the more festive environment of Café Kopi. In any case, the countless memories of fun (or not so fun) times and laughter which include these individuals will never be forgotten.

I am also exceedingly grateful to my parents Don and Sharon Ruffatto, my younger brothers Don, Matt and Ken Ruffatto and my little dog Willy Cooper for their love and moral support. Their belief in me fueled my determination to reach this stage of my graduate career and without them I would not be who I am today.

Lastly, this study would not have been possible without the financial support of the University of Illinois at Urbana-Champaign. I am grateful for the internal grants that I have received (i.e. University of Illinois First-year Fellowship Grant, University of Illinois Fellowship Grant, Francis M. and Harlie M. Clark Research Support Grant, and Conference Travel Grant), as they have allowed me to travel to scientific meetings and support myself while conducting my research on *Lobelia spicata*.

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PHYLOGENETIC CLASSIFICATION AND BREEDING SYSTEMS OF THE NORTH AMERICAN *Lobelia* L. (CAMPANULACEAE) SPECIES

ABSTRACT

The breeding system of a plant species describes not only gender distribution among plant morphs (gender system), but can also include pollination type, floral compatibility, and pollination syndrome. Since breeding system traits are likely to be genetically inherited, predictions about evolutionary relationships within a family or genus can be made by examining variation in breeding system dynamics. In this literature review, the classification of and phylogenetic relationships within *Lobelia* L. were examined to determine the closest relatives of *Lobelia spicata* Lam. (Campanulaceae), a gynodioecious prairie species. Breeding system distribution was assessed for *Lobelia* overall, and patterns of variation in pollination type, floral compatibility and pollination syndrome were reviewed for the closest relatives of *L. spicata*. The classification of *Lobelia* has undergone multiple transformations throughout history, but the most recent system divides the genus into 18 sections based on phenotypic characters, biogeographical patterns and molecular phylogenies. *L. spicata* is grouped with the 21 other eastern North American species into *Lobelia* section *Lobelia*. Members of *Lobelia* are exclusively synoecious with the exception of section *Hypsela*, which contains dioecious species, and section *Lobelia*, which contains two gynodioecious species including *L. spicata*. For *L. spicata*'s closest relatives in *Lobelia* section *Lobelia*, only 6 species out of 22 have had data on pollination type and floral compatibility collected, and among those species the combinations are highly variable. Further, 21 of the 22 species (e.g. *L. spicata*) are insect-pollinated with white, blue or purple corollas. *L. cardinalis* is the only species exhibiting an avian pollination syndrome, most likely due to its large, red flowers. The construction of comprehensive, molecular-based phylogenies for the North American *Lobelia* as well as additional research on individual *Lobelia* species will increase understanding of breeding system evolution within the genus.

INTRODUCTION

Angiosperms have evolved to employ a wide range of gender distribution strategies within flowers and individual plants. The broadest way to differentiate between strategies is to classify them as either monomorphic or polymorphic (Silverton and Charlesworth 2001). Monomorphic species, though they may possess flowers of varying gender, exhibit the same sexual phenotype in all individuals. Alternatively, polymorphic species will have two or three different plant morphs within a population, where each morph is distinguished by the presence of characteristic flower genders. Plants exhibiting a monomorphic gender system may be classified as synoecious, monoecious, gynomonoeious, andromonoecious or trimonoecious (Table 1.1). Approximately 72% of all angiosperms display a synoecious gender system, in which all plants have perfect flowers (Yampolsky and

Yampolsky 1922). Polymorphic plants can be described as dioecious, gynodioecious, androdioecious or trioecious. The most prevalent polymorphic gender system is gynodioecy followed closely by dioecy, found in 7% and 4% of angiosperms, respectively (Yampolsky and Yampolsky 1922).

The description of a plant's breeding system can be expanded to include pollination type, floral compatibility, and pollination syndrome. Pollination type describes whether pollen is donated to the same flower (autogamy), different flowers on the same plant (geitonogamy), or different flowers on different plants (xenogamy). Thus, autogamous and geitonogamous plants self-pollinate, while xenogamous plants cross-pollinate. The floral compatibility of a flower can be described as either of two character states, namely self-compatible or self-incompatible. A self-compatible flower can produce a zygote using its own pollen while a self-incompatible flower cannot. Pollination syndromes fall into two general categories, namely abiotic or biotic, depending upon the vector used for pollen dispersal. Plants with abiotic pollination syndromes rely mainly on wind and water to serve as vectors. Alternatively, those with biotic mechanisms require pollen to be transferred between individuals within a species by some type of live, motile organism (e.g. insects or birds). The type of pollinator a plant will attract is largely dependent upon flower morphology (i.e. size, shape or color) and the type of reward (i.e. nectar or pollen) that is offered (Eckhart 1991; Johnson et al. 1995; Williams et al. 2000).

Lobelia spicata Lam. is an herbaceous, eastern North American prairie species in the Campanulaceae. Unlike the majority of angiosperms, *L. spicata* exhibits the gynodioecious breeding system (Molano-Flores 2002) where carpellate and hermaphroditic flowers are located on separate plants (Table 1.1). Additionally, the species is xenogamous, self-compatible and insect-pollinated (Molano-Flores 2002). Since breeding system traits are likely to be genetically inherited, it is worthwhile to examine the variation in breeding systems exhibited within *Lobelia*.

Thus, this literature review has three main objectives:

- (1) Determine the classification of and phylogenetic relationships within *Lobelia* to identify the closest relatives of *Lobelia spicata*.
- (2) Examine the variation in breeding systems within the entire *Lobelia* genus.
- (3) Assess whether any patterns exist among the closest relatives of *L. spicata* in regards to pollination type, floral compatibility and pollination syndrome.

CLASSIFICATION AND PHYLOGENETIC RELATIONSHIPS

Classification of *Lobelia* - The taxonomic composition of Campanulales, an angiosperm order, has been highly variable among classification authorities (Lammers 1992), yet it has consistently retained a group of five key taxa including Campanulaceae and Lobeliaceae (Cosner et al. 1994). Debate exists within the Besseyan or "Big Four" (Takhtajan, Cronquist, Thorne and Dahlgren) systems of classification regarding the relationship of Campanulaceae to Lobeliaceae, in particular if Lobeliaceae should be assigned a family or subfamily taxonomic ranking. Two of the "Big Four" authorities place Lobeliaceae as subfamily Lobelioideae under Campanulaceae (Cronquist [1988] and

Thorne [1992]) while the remaining two recognize Campanulaceae and Lobeliaceae as closely related, yet distinct families (Takhtajan [1987] and Dahlgren [1980, 1983]). Morphological synapomorphies for Campanulaceae and Lobeliaceae include protandrous pollen presentation, possession of articulated lactifers, and comparably aggressive endosperm haustoria (Lammers 1992). However, Lobeliaceae's possession of zygomorphic corollas, 2-locular ovaries, and often bilobed stigmas and the complete or partial connation of filaments distinguish its members from those of Campanulaceae (Lammers 1992). An analysis of *rbcL* sequences of 44 species in 21 genera by Cosner et al. (1994) showed that the broad circumscription of Campanulaceae to include Lobeliaceae as a subfamily was paraphyletic, unless the genus *Nemacladus* (currently placed in Cyphiaceae) was also included (Figure 1.1). However, the bootstrap value for the clade containing Campanulaceae, Lobeliaceae and *Nemacladus* was not strong, whereas the individual Campanulaceae and Lobeliaceae clades were. This suggests that Campanulaceae and Lobeliaceae should be addressed as separate families. However Lammers (2007a), among others, still continue to address Lobeliaceae as subfamily Lobelioideae Burnett (1835) within Campanulaceae, claiming that the ranking issue is "strictly a matter of taste" (Lammers, pers. comm.). For the purposes of this paper, the subfamily designation will be assumed.

The family Campanulaceae, widely distributed across six continents (Lammers 2007a), is composed of five subfamilies including Lobelioideae Burnett, Campanuloideae Burnett, Cyphioideae Walp., Nemacladoideae Lammers and Cyphocarpoideae Miers (Lammers 2007b). The two most abundant subfamilies, Lobelioideae and Campanuloideae, contain over 96% of the 2,319 species assigned to Campanulaceae (Lammers 2007b). Little emphasis has been placed on structuring a reliable tribe and subtribe ranking system under Lobelioideae though numerous tribes have been proposed for Campanuloideae. Due to the lack of comprehensive molecular data and phylogenetic analysis for both subfamilies, genera are the next accepted level of classification. Nested within Lobelioideae are 29 different genera including the highly diverse and widespread genus *Lobelia* (Lammers 2007a). Of the 416 species in *Lobelia*, 5.3% and 3.4% of species are found in mainland North America and the Hawaiian Islands, respectively. The remaining species populate areas of Africa, Asia, Australia, South America and the Caribbean Islands (Lammers 2011).

Sectional Classification in *Lobelia* - The genus *Rapuntium*, described by Tournefort, was later renamed *Lobelia* by Linnaeus in 1735 (McVaugh 1936). Bentham and Hooker (1876) proposed one of the first classification schemes for *Lobelia*, claiming that the entire genus could be divided into eight distinct sections based on habit and a suite of morphological characters. The sections were designated as *Trimeris*, *Tupa*, *Tylomium*, *Rhynchopetalum*, *Homochilus*, *Eulobelia*, *Hemipogon* and *Holopogon* (Bentham and Hooker 1876). Under this classification system, the majority of North American *Lobelia* species fell within sections *Eulobelia* and *Hemipogon*, characterized by their "large short-pedicelled flowers in lax terminal racemes" and "slender, simple or branching stems and few flowers", respectively (Bentham and Hooker 1876).

McVaugh (1936) later declared the morphological features used by Bentham and Hooker (1876) for sectional differentiation to be insignificant. Based on examination of live and herbarium specimens, McVaugh concluded that

a more accurate section classification system of *Lobelia* could be constructed using seed morphology and geographical distribution patterns (McVaugh 1936). Consequently, McVaugh (1940) published a six section system in the form of a dichotomous key that could be used to classify the ninety *Lobelia* species native to North America. The sections were identified as *Holopogon* Benth. & Hook., *Eulobelia* Benth. & Hook., *Homochilus* A.DC., *Palmerella* A. Gray, *Hemipogon* Benth. & Hook., and *Tylomium* (Presl) Benth. & Hook. Each section was distinguished by seed shape and surface appearance (i.e. texture, luster), with supplementary information on corolla characteristics (i.e. pubescence on lower lip and length), anther/filament tube length, and geographical distribution (McVaugh 1940). With one exception, all *Lobelia* native to the eastern United States that had previously been assigned to section *Hemipogon* by Bentham and Hooker (1876) were now placed under section *Eulobelia* with *Lobelia spicata*. McVaugh (1936) also produced a tree depicting the probable phylogenetic relationships existing among 27 species and varieties of *Lobelia* (Figure 1.2). Characters used to construct the tree included basal leaf arrangement, absence of bracteoles and auricles, a fenestrate corolla and large flowers. The species were divided into two lines based on flower size, namely large and small. The two branches connected at the bottom of the diagram (*Lobelia cardinalis*, *L. siphilitica*, *L. amoena*, *L. elongata* and *L. glandulifera*) make up the large-flowered line. Alternatively, small flowers are characteristic of those species (e.g. *Lobelia spicata*) placed on the upper, multi-branched line, with the exception of *L. puberula*, *L. brevifolia* and *L. glandulosa*. *Lobelia dortmanna* and *L. kalmii*, two highly specialized species, are depicted as solitary arrows to represent their early divergence from the two main ancestral lines. Though helpful for visualizing relationships, the McVaugh (1936, 1940) classification scheme and phylogeny was based on only a few distinctive morphological traits. Additionally, its scope was limited to North American *Lobelia* species, making it unfit to supersede the Bentham and Hooker (1876) classification.

The next classification system for *Lobelia* was outlined by Wimmer (1953). However, the infrageneric scheme he proposed was highly complicated, consisting of three subgenera, ten sections and six subsections (Table 1.2). Differentiation between the taxonomic rankings was based predominantly on reproductive traits (i.e. flower shape, fruit type and dehiscence), however, like Bentham and Hooker (1876) and McVaugh (1940), geographic distribution and habit were also considered. Most of the North American *Lobelia*, including *Lobelia spicata*, were placed under subgenus *Lagotis* section *Hemipogon* subsection *Trachyspermae*.

Bowden (1959) made a slight revision to the Wimmer (1953) classification system by changing the name of section *Hemipogon* subsection *Trachyspermae* to section and subsection *Lobelia* in accordance with Article 22 of the Code (states that a section containing a genus type specimen should be named after the genus itself) (Table 1.2). In addition, Bowden (1959) created a phylogenetic tree for the twenty-one eastern North American species in *Lobelia* section *Lobelia* (Figure 1.3). Four distinct lines can be recognized in the diagram, three of which possess only a single, morphologically distinct species (i.e. *Lobelia dortmanna*, *L. kalmia* and *L. inflata*). The early evolutionary divergence of *L. dortmanna* and *L. kalmii* depicted is in agreement with the rationale of McVaugh (1936). From the fourth line arose three subgroups, referred to as the small-flowered species (*Lobelia spicata*, *L. appendiculata*, *L. gattingeri*, *L. boykinii*, *L. canbyi*, *L. nuttallii* and *L. feayana*), narrow-leaved species (*L. flaccidifolia*, *L. glandulosa*, *L. floridana* and *L. paludosa*) and medium to large flowered species (*L. georgiana*, *L. brevifolia*, *L. puberula*, *L. siphilitica*, *L. cardinalis*, *L. elongata* and *L. amoena*). As with his predecessors, Bowden

(1959) relied heavily on plant morphology and geographical distribution patterns to ascertain evolutionary relationships. All species found within *Lobelia* section *Lobelia* were predicted to have arisen from a diploid ($2n = 14$) ancestor.

More detailed examination of seed coat morphology by Murata (1992, 1995) led to a drastic alteration of the original classification scheme proposed by Wimmer (1953). Five distinct seed coat types were identified based on number of cell layers and a description of the lumen. After organizing all species in Wimmer's classification scheme, it was apparent that the type B seed coat appeared in species belonging to four different lineages (Murata 1995). Based on the concept of parsimony in phylogenetic relationships, multiple evolution events arriving at the same seed coat was highly unlikely. In response, Murata (1995) proposed a new classification system in which seed type B evolved from two rather than four ancestral lines. Three subgenera were recognized including *Lobelia*, *Mezleria* and *Tupa*, and many of the subsections in the Wimmer (1953) classification were given section status for a total of fourteen (Murata 1995; Table 1.2). Distinguishing features such as seed morphology, habit, corolla characteristics, chromosome number (Lammers 1993) and geographical distribution were used to create a dichotomous key that included each of the subgenera and sections in the Murata (1995) classification system. As with Wimmer (1953) and Bowden (1959), Murata (1995) assigned most of the mainland North American *Lobelia* (including *Lobelia spicata*) to subgenera *Lobelia* section *Lobelia*, while the Hawaiian lobeliads were placed in subgenera *Tupa* sections *Revolutella* and *Galeatella* (Table 1.2). Assignment of the Hawaiian endemics *Lobelia yuccoides* and *L. hypoleuca* to subgenus *Tupa* section *Revolutella* (Murata 1995) was supported based on analysis of *trnL-F*, *rbcL* and *ndhF* gene regions (Antonelli 2008; Figure 1.4). A phylogenetic tree generated from 132 cpDNA restriction site mutations, though it excluded subgenus *Mezleria* due to lack of molecular data, placed subgenus *Tupa* as a more recently derived evolutionary lineage than subgenus *Lobelia* (Murata 1995; Figure 1.5). A second phylogenetic tree based on sequence analysis and indel variation among gene regions *psbA-trnH*, *trnL-trnF*, *rpl16*, *trnT-trnL*, *trnV-trnK* and *atpB-rbcL* showed tight clustering of the Hawaiian *Lobelia* species (Givnish et al. 2009; Figure 1.6). However, this phylogeny also showed how far removed the Hawaiian endemics were from the eastern North American species *Lobelia cardinalis*, and thus from *L. spicata*.

The classification system within *Lobelia* was further refined by Lammers (2011) through the elimination of subgeneric rankings. Rather, an 18 section classification system was proposed based on phenotypic characters, biogeographical patterns and available molecular phylogenies (Lammers 2011; Table 1.3). Within this classification system, *Lobelia spicata* and the 21 other eastern North American species are included in section *Lobelia*, while the Hawaiian species are divided between sections *Revolutella* (nine species) and *Galeatella* (five species).

In short, the closest relatives of *Lobelia spicata* appear to be the 21 eastern North American *Lobelia* species grouped into *Lobelia* section *Lobelia*. These taxa have consistently been grouped together throughout the many revisions of sectional classification in *Lobelia*. Though the Hawaiian *Lobelia* are located geographically close to the eastern North American *Lobelia* relative to those species found in South America, Africa, Asia and Australia, the two groups do not appear to be closely related based on limited phylogenetic evidence. In the next section of this paper the breeding system distribution for *Lobelia* is discussed, and the pollination types, floral compatibility and

pollination syndromes of *L. spicata*'s closest relatives, the eastern North American species in *Lobelia* section *Lobelia*, are examined and compared to those found within the less closely related Hawaiian *Lobelia* species.

BREEDING SYSTEM DYNAMICS

Breeding Systems in *Lobelia* - *Lobelia* species are almost exclusively synoecious, but a few species in sections *Hypsela* and *Lobelia* do display a polymorphic breeding system (Lammers 2011; Table 1.3). In section *Hypsela*, species such as *Lobelia dioica*, *L. pedunculata* and *L. purpurascens* are dioecious (Murata 1995). Alternatively, section *Lobelia* contains two gynodioecious species, *Lobelia spicata* and *L. siphilitica* L. (Bowden 1959, Lammers 2007a, Molano-Flores 2002, Miller and Stanton-Geddes 2004, Mutikainen and Delph 1998; Table 1.4). Though these two species are both assigned to section *Lobelia*, the phylogenies put forth by McVaugh (1936) and Bowden (1959) place them on separate evolutionary lines predominantly due to flower size differences (Figures 1.2 and 1.3). As previously stated, these phylogenetic trees were based on a set of characters describing plant morphology, geographical distribution and chromosome number rather than molecular evidence. Morphologically the two species are quite distinct, with *L. spicata* displaying small blue to white flowers in a predominantly single spike inflorescence and *L. siphilitica* possessing large blue to white colored flowers in multiple crowded racemes (Gleason and Cronquist 1991). In any case, parsimony with respect to evolution of the gynodioecious gender system would only be reflected if both species were included on one ancestral line rather than two. To date no phylogenies based on molecular data have been published that include both *L. spicata* and *L. siphilitica* to resolve this evolutionary question.

It is somewhat unexpected that all thirteen Hawaiian *Lobelia* found in sections *Revolutella* and *Galeatella* exhibit a synoecious breeding system (Wagner et al. 1990; Table 1.5), since the Hawaiian Islands are known to have a higher incidence of dioecious species than other geographical locations (dioecy displayed by 14.7% of their angiosperms). Perhaps the group's synoecy is correlated to their avian pollination syndrome (Givnish et al. 2009; Wagner et al. 1990) since the evolution of dioecy has been tied to wind pollination in the Hawaiian Islands (Sakai et al. 1995b).

Pollination Types and Floral Compatibility within *Lobelia* - Only six of the twenty-two species of *Lobelia* section *Lobelia* have been examined to determine pollination type and floral compatibility (Table 1.4). Four of the six species (*L. boykinii*, *L. cardinalis*, *L. siphilitica* and *L. spicata*) were classified as xenogamous. Alternatively, *L. dortmanna* and *L. inflata* are autogamous, such that each flower will use its own pollen to form a zygote. In these two species, the stigma and style do not project beyond the anther tube like other *Lobelia* species, thereby preventing cross-pollination (Farmer 1989; Simons and Johnston 2000). Additionally, the submerged flowers from the aquatic *L. dortmanna* do not open but still produce viable fruit (Farmer 1989). Of the four xenogamous species,

three are self-compatible but have some other mechanism for minimizing geitonomy. Separation of the gender phases within and among flowers could be one potential explanation. The two autogamous species are, of course, self-compatible.

Pollination Syndromes within *Lobelia* - In the case of *Lobelia* section *Lobelia*, most species have a biotic pollination syndrome that utilizes either insect or avian vectors. Specifically, all of the eastern North American species in section *Lobelia* (including *Lobelia spicata*) are insect-pollinated with white, blue or purple corollas, with one exception, *Lobelia cardinalis*. *L. cardinalis* has an avian pollination syndrome, most likely due to its large, red flowers (Johnston 1991; Table 1.4). Originally *L. cardinalis* was predicted to have diverged early in evolutionary history (McVaugh 1936), however it has also been labeled as diverging recently from the same ancestral lineage as the gynodioecious species *L. siphilitica* (Bowden 1959). No molecular work has been published regarding how close of a relative *L. cardinalis* is to *L. siphilitica* or to any of the other eastern North American *Lobelia* species. The Hawaiian species in sections *Revolutella* and *Galeatella* are predominantly thought to be bird-pollinated and display a great range in corolla color from crimson to blue/violet to yellow/green (Table 1.5).

CONCLUDING STATEMENT

The classification of *Lobelia* has undergone multiple revisions throughout history to incorporate new information, techniques and species. Despite this, no molecular phylogenies have been published that include all species within the genus. If sequence analysis could be used to ascertain relationships among all species, it would be possible to assess the accuracy of past morphology-based classification systems and phylogenies. Expansion of the research completed on *Lobelia* species breeding systems combined with more complete phylogenies, particularly of the North American species, will allow further study into the evolutionary history behind plant gender systems and pollination syndromes.

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TABLE 1.1 Summary of gender system classification, including both monomorphic and polymorphic strategies

Gender System	Description	Percent Occurrence*
Monomorphic		
Synoecious	Plants have exclusively perfect flowers	72.0%
Monoecious	Staminate and carpellate flowers are located on the same plant	5.0%
Gynomonoecious	Carpellate and hermaphrodite flowers are located on the same plant	2.8%
Andromonoecious	Staminate and hermaphrodite flowers are located on the same plant	1.7%
Trimonoecious	Staminate, carpellate and hermaphrodite flowers are located on the same plant	Very rare
Polymorphic		
Dioecious	Staminate and carpellate flowers are located on separate plants	4.0%
Gynodioecious	Carpellate and hermaphroditic flowers are located on separate plants	7.0%
Androdioecious	Staminate and hermaphroditic flowers are located on separate plants	Very rare
Trioecious	Staminate, carpellate and hermaphroditic flowers are located on separate plants	Very rare

*Percentage of angiosperms utilizing each sex system as documented in a large scale study by Yampolsky and Yampolsky (1922)

TABLE 1.2 Classification systems proposed by Wimmer (1953), Bowden (1959) and Murata (1995) for *Lobelia*, including subgenus, section and subsection divisions.

Wimmer (1953)	Bowden (1959)	Murata (1995)
Subgenus <i>Lagotis</i> Section <i>Hemipogon</i> Subsection <i>Trachyspermae</i> Subsection <i>Leiospermae</i> Section <i>Holopogon</i> Subsection <i>Cryptostemon</i> Subsection <i>Delostemon</i>	Subgenus <i>Lobelia</i> Section <i>Lobelia</i> Subsection <i>Lobelia</i> Subsection <i>Leiospermae</i> Section <i>Holopogon</i> Subsection <i>Cryptostemon</i> Subsection <i>Delostemon</i>	Subgenus <i>Lobelia</i> Section <i>Lobelia</i> Section <i>Heyneana</i> Section <i>Cryptostemon</i> Section <i>Delostemon</i>
Subgenus <i>Mezleria</i> Section <i>Eumezleria</i> Section <i>Paramezleria</i>	Subgenus <i>Mezleria</i> Section <i>Eumezleria</i> Section <i>Paramezleria</i>	Subgenus <i>Mezleria</i> Section <i>Dioica</i> Section <i>Pratia</i> Section <i>Paramezleria</i> Section <i>Isolobus</i>
Subgenus <i>Tupa</i> Section <i>Isobulus</i> Section <i>Eutupa</i> Subsection <i>Primanae</i> Subsection <i>Haynaldianae</i> Section <i>Rhynchopetalum</i> Section <i>Homochilus</i> Section <i>Revolutella</i> Section <i>Galeatella</i>	Subgenus <i>Tupa</i> Section <i>Isobulus</i> Section <i>Eutupa</i> Subsection <i>Primanae</i> Subsection <i>Haynaldianae</i> Section <i>Rhynchopetalum</i> Section <i>Homochilus</i> Section <i>Revolutella</i> Section <i>Galeatella</i>	Subgenus <i>Tupa</i> Section <i>Tupa</i> Section <i>Colensoa</i> Section <i>Rhynchopetalum</i> Section <i>Homochilus</i> Section <i>Revolutella</i> Section <i>Galeatella</i>

TABLE 1.3 Number of species, geographic distribution, breeding system and corolla color characteristic of the 18 *Lobelia* sections recognized by Lammers (2011) (information summarized from Lammers [2011])

Section	Number of Species	Geographic Distribution	Breeding System	Corolla Color
<i>Delostemon</i>	44	Southern Africa, Tropical Africa, Southern and eastern Asia, South America	Synoecious	Shades of blue or purple; sometimes pink or white
<i>Holopogon</i>	14	Africa	Synoecious	Shades of blue or purple; sometimes marked with yellow
<i>Colensoa</i>	1	New Zealand	Synoecious	Purple or blue (sometimes pale)
<i>Cryptostemon</i>	9	New World (Southwestern United States to Panama and Peru)	Synoecious	Shades of blue or purple; rarely white
<i>Stenotium</i>	144	Continental North America, Caribbean, South America, Africa, Madagascar and the Mascarenes, Southern and Eastern Asia	Synoecious	Shades of blue, purple, pink or white
<i>Lobelia</i>	22	Eastern and Central North America	Synoecious Gynoecious	Shades of blue or purple, often with a white eye; rarely white or red
<i>Hypsela</i>	43	Southern and Eastern Asia, Australia, New Zealand, Southern South America	Synoecious Dioecious	Blue, purple, magenta, pink or white
<i>Mezleriopsis</i>	7	Cape Provinces of South Africa	Synoecious	Blue, purple, pink or white
<i>Jasionopsis</i>	1	Cape Provinces of South Africa	Synoecious	White or purple with several red or purple marks on each corolla lobe
<i>Tylomium</i>	38	North America (Greater and Lesser Antilles and Southern Mexico to Panama)	Synoecious	Magenta, pink, red, orange, yellow, green or white
<i>Homochilus</i>	6	New World (Southern Arizona to Southwestern Columbia and Peru)	Synoecious	Magenta, purple, red, pink, orange or yellow
<i>Tupa</i>	4	Central Chile	Synoecious	Red, pink or purple; rarely yellow
<i>Trimeris</i>	1	St. Helena	Synoecious	White
<i>Speirema</i>	4	Southeastern Asia	Synoecious	Greenish, bluish lilac, red-purple or dark violet
<i>Plagiobotrys</i>	2	Malesia (Borneo, Sulawesi and Flores)	Synoecious	Purple or red-purple, red or white
<i>Rhynchopetalum</i>	62	Southern and Eastern Asia, Tropical Africa, South America	Synoecious	Shades of blue, purple, red, yellow, green or white
<i>Revolutella</i>	9	Hawaiian Islands	Synoecious	Blue (when tree habit) and magenta (when shrub habit)
<i>Galeatella</i>	5	Hawaiian Islands	Synoecious	Pale purple, red, yellow or white
Total	416			

TABLE 1.4 Summary of available information on the current name, breeding system (S = synoecious, G = gynodioecious), pollination type, compatibility (SI = self-incompatible, SC = self-compatible), pollination syndrome and corolla color of the 22 eastern North American species in *Lobelia* section *Lobelia*.

Current Name	Breeding System	Pollination Type	Compatibility	Pollination Syndrome	Corolla color
<i>L. amoena</i> Michx.	S	---	---	---	Blue [24]
<i>L. appendiculata</i> A. DC.	S	---	---	---	Light violet blue, lilac [24]
<i>L. boykinii</i> Torr. & A. Gray ex A. DC.	S [32]	Xenogamous [32]	SI [32]	Insect [32]	Blue, white center [12]
<i>L. brevifolia</i> Nutt. ex A. DC.	S	---	---	---	Pale blue to azure [24]
<i>L. canbyi</i> A. Gray	S	---	---	---	Blue [12, 24]
<i>L. cardinalis</i> L.	S [15]	Xenogamous [27]	SC [16]	Avian [15, 38]	Scarlet [12]
<i>L. dortmanna</i> L.	S [10]	Autogamous [10]	SC [10]	Insect [10]	Pale blue or white [12, 24]
<i>L. elongata</i> Small	S	---	---	---	Blue [12]
<i>L. feayana</i> A. Gray	S	---	---	---	Blue, white eye with two green tubercles [24]
<i>L. flaccidifolia</i> Small	S	---	---	---	Blue [24]
<i>L. floridana</i> Chapm.	S	---	---	---	Blue [24]
<i>L. gattingeri</i> A. Gray	S	---	---	---	Light violet-blue or lilac [24]
<i>L. georgiana</i> McVaugh	S	---	---	---	Violet
<i>L. glandulosa</i> Walter	S	---	---	---	Blue with a white eye [12]
<i>L. inflata</i> L.	S [35]	Autogamous [35]	SC [35]	Insect [38]	Blue, white [12]
<i>L. kalmii</i> L.	S	---	---	Insect [38]	Blue, white center [12, 24]
<i>L. nuttallii</i> Schult.	S	---	---	---	Blue, white center with 2 greenish spots [12, 24]
<i>L. paludosa</i> Nutt.	S	---	---	---	Light blue, white [24]
<i>L. puberula</i> Michx.	S	---	---	---	Blue [12, 24]
<i>L. reverchonii</i> B. L. Turner	S	---	---	---	Violet
<i>L. siphilitica</i> L.	G [27]	Xenogamous [27]	SC [27]	Insect [15, 16]	Blue, occasionally white [12]
<i>L. spicata</i> Lam.	G [27]	Xenogamous [27]	SC [27]	Insect [27]	Blue to white [12]

TABLE 1.5 Summary of available information on the current names, pollinator (insect or avian) and corolla color of the Hawaiian *Lobelia* species of in sections (A) *Revolutella* and (B) *Galeatella*.

(A) Current Name	Pollinator	Corolla Color
<i>L. dunbariae</i> Rock	---	Lilac, blue [31, 39]
<i>L. grayana</i> E. Wimm.	---	Blue [39]
<i>L. hillebrandii</i> Rock	---	Blue to lilac [31, 39]
<i>L. hypoleuca</i> Hillebr.	Avian [11, 23]	Blue [31, 39]
<i>L. monostachya</i> (Rock) Lammers	---	Magenta [39]
<i>L. niihauensis</i> H. St. John	Avian [11]	Magenta [39]
<i>L. oahuensis</i> Rock	---	Pale blue [31, 39]
<i>L. remyi</i> Rock	---	Purplish pink [31]
<i>L. yuccoides</i> Hillebr.	Avian [11, 23]	Blue to lilac [31, 39]

(B) Current Name	Pollinator	Corolla Color
<i>L. gaudichaudii</i> A. DC.	---	Crimson, rarely greenish or yellowish white [31, 39]
<i>L. gloria-montis</i> Rock	Avian [11]	Yellowish or greenish white with purple stripes [8, 31,39]
<i>L. koolauensis</i> (Hosaka & Fosberg) Lammers	---	White [21]
<i>L. villosa</i> (Rock) H. St. John & Hosaka	Avian [11]	Yellowish to greenish white with purple veins [39]
<i>L. wahiawa</i> Lammers	---	Pale purple with dark purple longitudinal stripes [21]

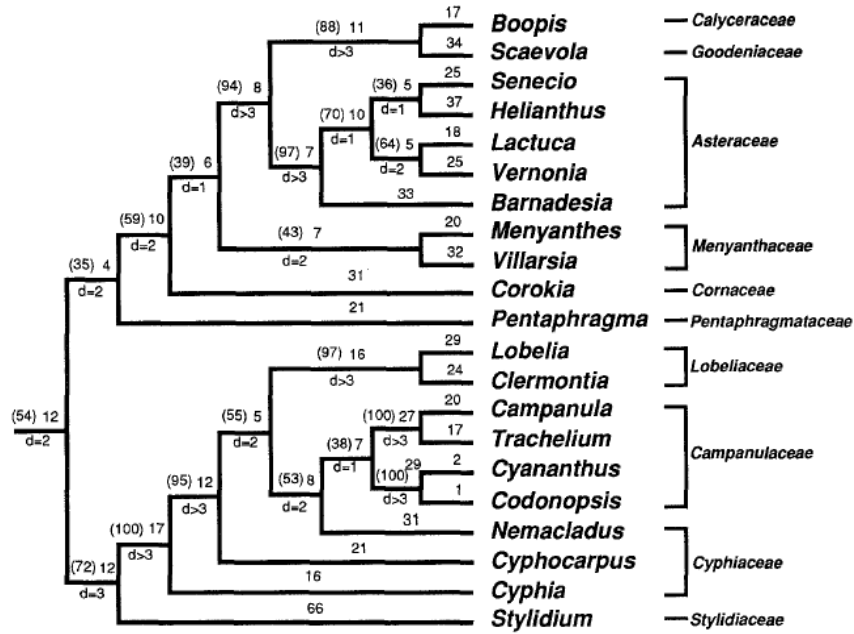


FIGURE 1.1 Phylogenetic tree constructed by analyzing *rbcL* sequences of 44 species (in 21 genera) in the Campanulales/Asterales clade. Numbers above the branches include bootstrap values (in parentheses) and changes at each node, and numbers below the branches are decay indices (Figure reproduced from Cosner et al. 1994)

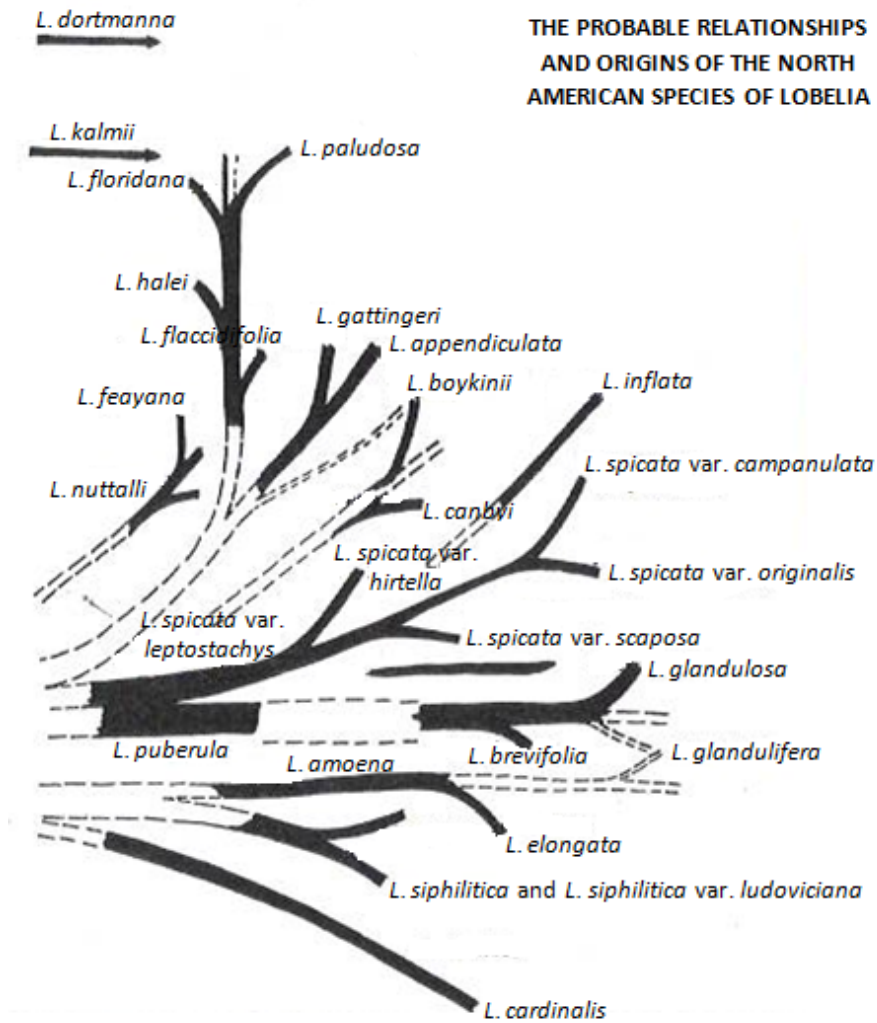


FIGURE 1.2 Early hypothesis of phylogenetic relationships of North American *Lobelia* species generated using seed morphology and geographical distribution patterns (Figure reproduced from McVaugh 1936)

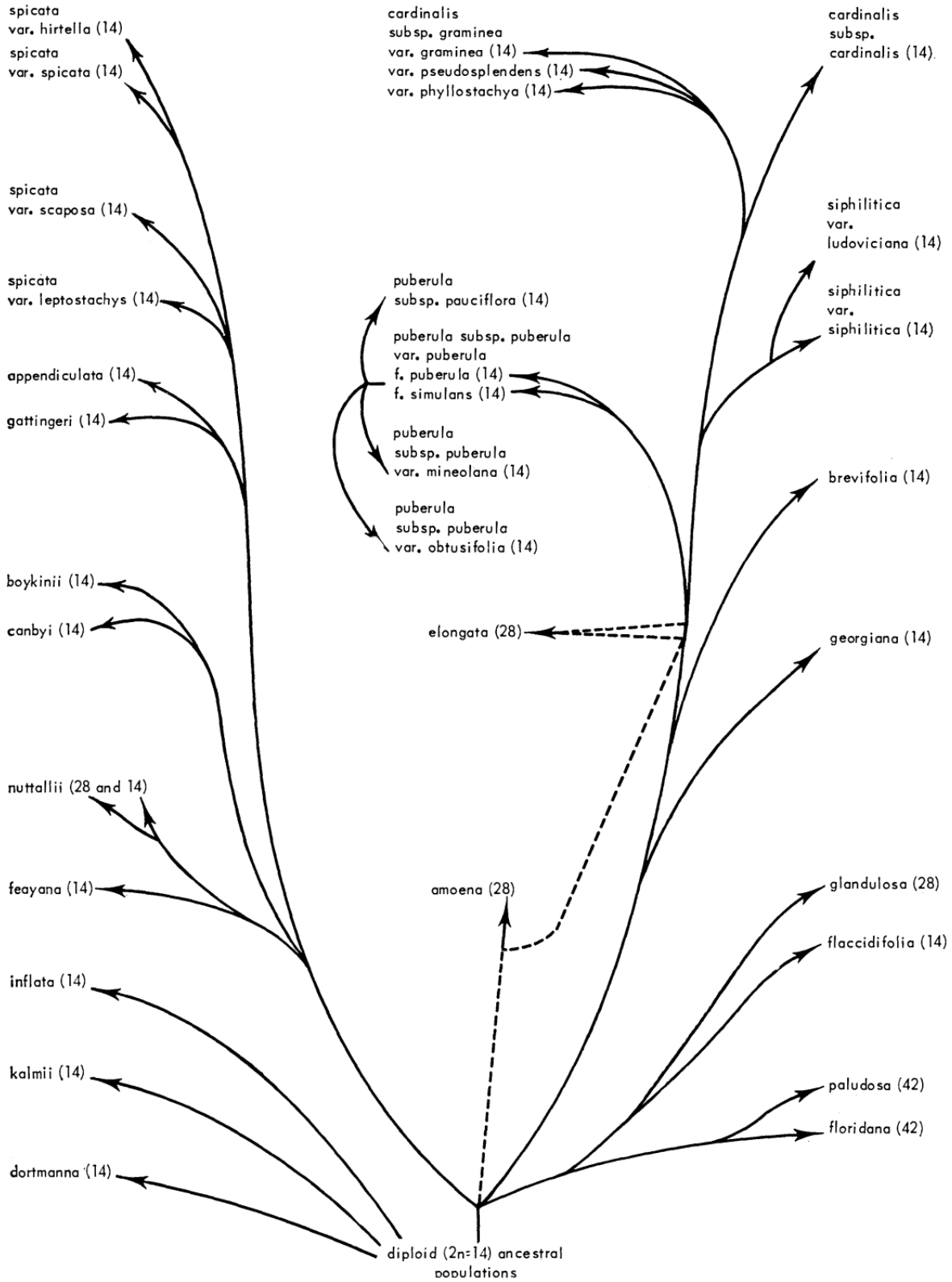


FIGURE 1.3 Hypothesis of phylogenetic relationships for 21 species in *Lobelia* section *Lobelia* generated using plant morphology, geographical distribution patterns and somatic chromosome numbers (presented in parentheses; 2n) (Figure reproduced from Bowden 1959)

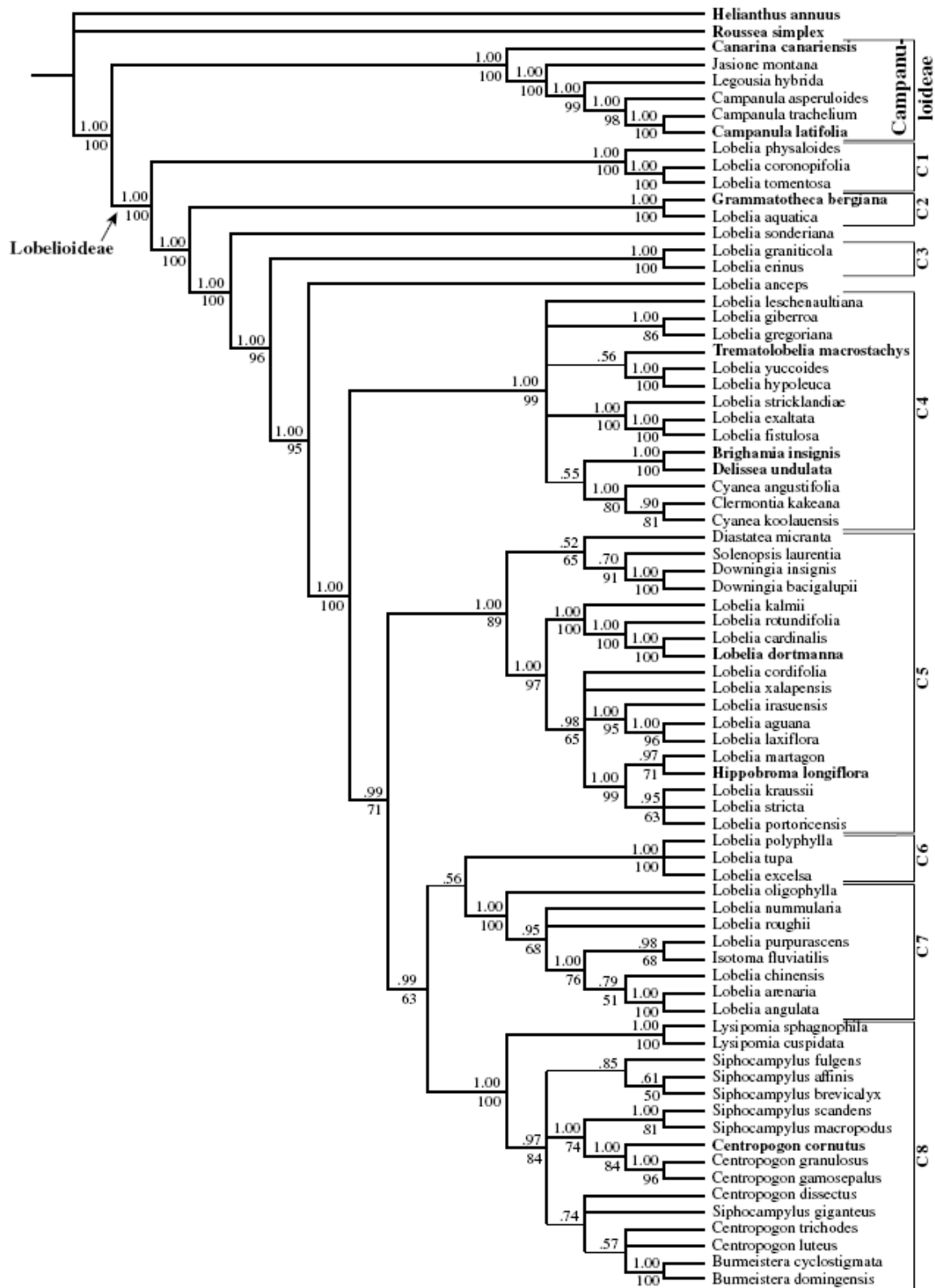


FIGURE 1.4 Phylogenetic relationships between species in Campanulaceae subfamily Lobelioideae generated by Bayesian inference using *trnL-F*, *rbcL* and *ndhF* gene regions. Number above branch indicates posterior probability values and numbers below each branch are jackknife support values; major clades are designated by brackets and are labeled C1 – C8 (Figure reproduced from Antonelli 2008).

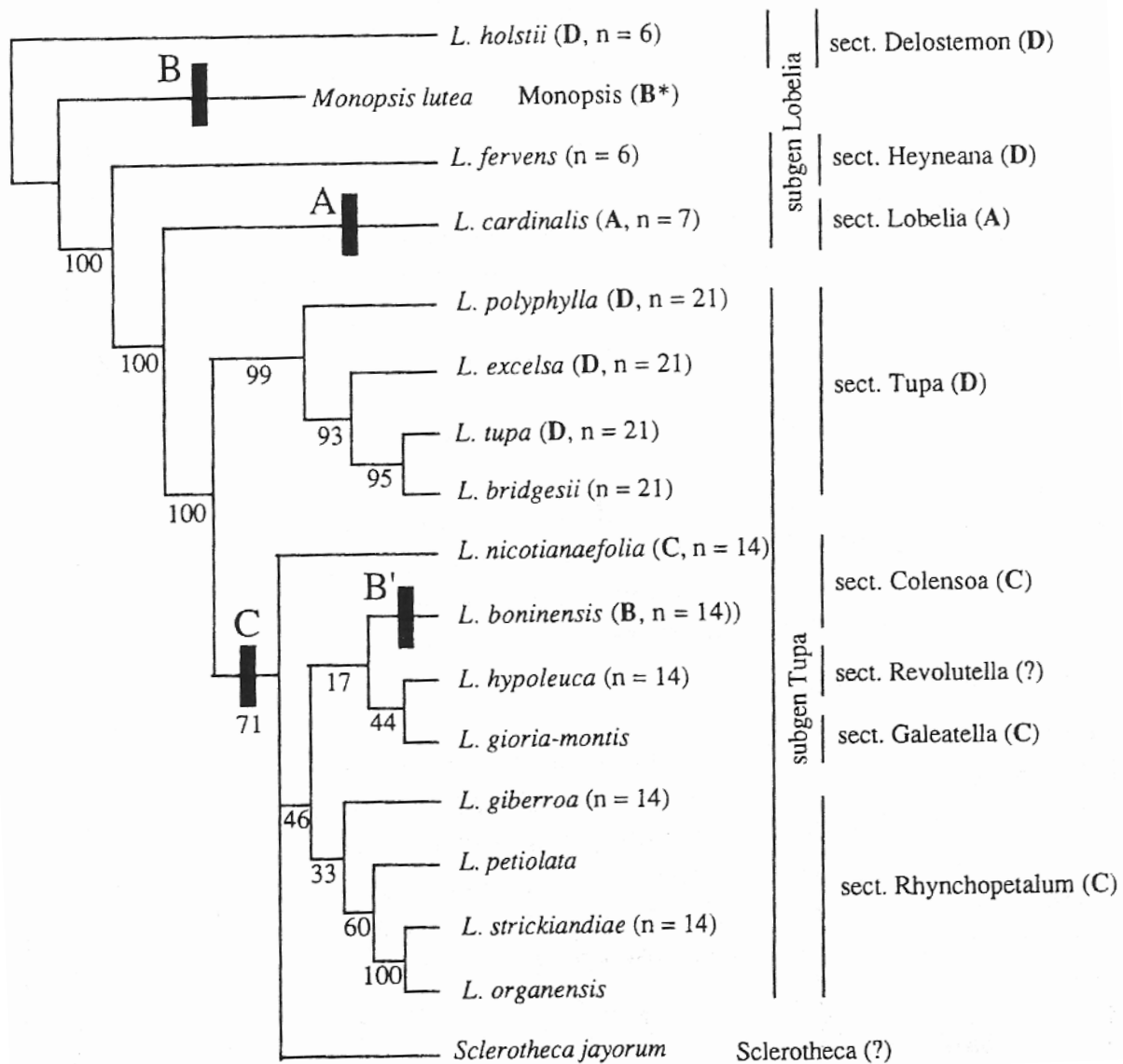


FIGURE 1.5 Phylogenetic tree for select subgenera and sections of *Lobelia* under the Murata (1995) classification system based on 132 cpDNA restriction site mutations. Major seed coat types (A-D) and chromosome numbers follow species names (Lammers 1993), and bootstrap values (under branches) are also indicated (Figure reproduced from Murata 1995)

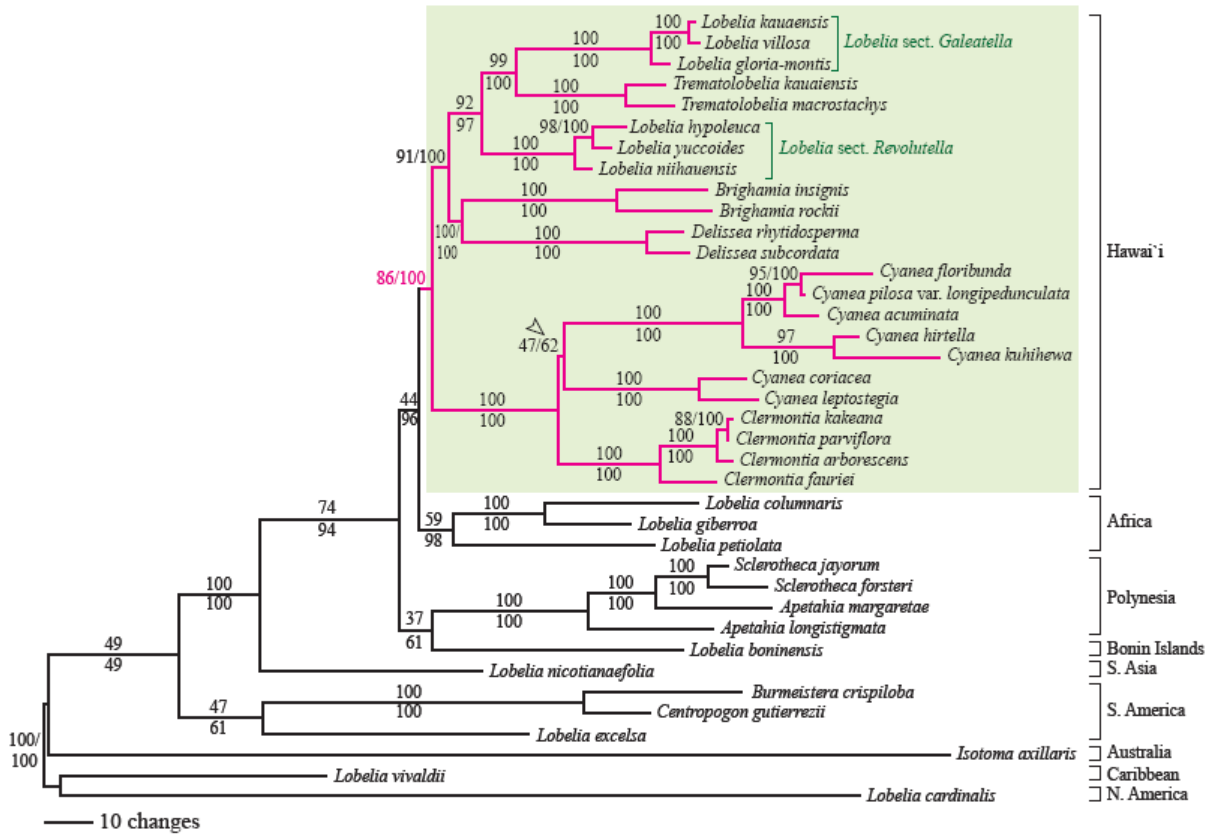


FIGURE 1.6 Phylogenetic relationships among the Hawaiian lobeliads as determined by sequence analysis and indel variation of *psbA-trnH*, *trnL-trnF*, *rpl16*, *trnT-trnL*, *trnV-trnK*, *atpB-rbcL* and *rbcL* DNA sequences. Jackknife value is indicated above branches and posterior probabilities written as percentages are below the branches. *Lobelia cardinalis* was used as an outgroup. (Figure reproduced from Givnish et al. 2009)

THE EFFECTS OF POPULATION SIZE AND DENSITY ON REPRODUCTIVE SUCCESS OF THE PRAIRIE SPECIES *Lobelia spicata* Lam. (CAMPANULACEAE)

ABSTRACT

The expansion and dispersal limitations placed on plant populations through habitat fragmentation have increased the relative importance of intra-population dynamics (e.g. population size and density) in determining species persistence. Both population size and density have been linked to individual reproductive success and thus may influence the overall viability of existing plant populations. In this study, the impact of population size and density on reproductive output and fitness measurements were examined for the prairie species *Lobelia spicata* Lam. (Campanulaceae). During the summers of 2008 and 2009, data on population size, population density, and reproductive output (i.e. fruit set, seed number per fruit, seed biomass) and fitness (i.e. percent seed germination) were collected for 11 *L. spicata* populations across Illinois. In 2008 and 2009, no relationship was found between population size or density and any of the four reproductive measurements with three exceptions. Population size exhibited a marginally significant negative correlation with fruit set in 2008 and a positive correlation with seed number per fruit in 2009, while population density was positively correlated to seed number per fruit in 2008. Size of *L. spicata* populations may be having an indirect impact on fruit set and seed number per fruit during intermittent years due to its influence on the availability of compatible, out-crossed pollen. Alternatively, high plant density may be linked to increased visitation of flowers by pollinators, causing an increase in the number of seeds produced per fruit. Thus, population dynamics such as size and density could impact future reproductive success and population persistence in *L. spicata*.

INTRODUCTION

Human-mediated habitat fragmentation poses a major threat to the persistence of plant species due to their sessile nature and limited seed dispersal capabilities (e.g. Honnay et al. 1999). When fragmentation occurs, established plant populations become surrounded by a matrix of unsuitable and/or impassable habitat. This matrix prevents the flow of genetic information between populations by acting as a barrier to either pollinator movement (Jennersten 1988; Rathcke and Jules 1993; Kwak et al. 1998) or seed dispersal (Benitez-Malvido 1998; Santos et al. 1999; Herrera and García 2010). Consequently, persistence of currently established populations through continued reproductive success is vital for species preservation (Fischer and Stöcklin 1997). By examining intra-population characteristics linked to plant reproduction such as population size (Lamont et al. 1993; Kéry et al. 2000; Jacquemyn et al. 2002; Vergeer et al. 2003; Hansen and Totland 2006) and density (Kunin 1997; Roll et al. 1997; Molano-Flores and Hendrix 1999; Ågren et al. 2008; Klank et al. 2010), predictions about long-term population viability can be made.

Population size often indirectly influences plant reproductive success through its impact on genetic variability (Ellstrand and Elam 1993; Young et al. 1996; Reed and Frankham 2003) and/or plant-pollinator interactions (Jennersten and Nilsson 1993; Kolb 2008). In general, as population size decreases, genetic diversity is reduced via random genetic drift or inbreeding (Young et al. 1996; Reed and Frankham 2003; Spigler and Chang 2009). Low genetic diversity has been linked to reduced reproductive output and offspring fitness in a number of species (e.g. Menges 1991; Dole and Sun 1992; Widen 1993; Heschel and Paige 1995; Fischer and Matthies 1998a), particularly self-incompatible species which are obligatory outcrossers and must receive pollen from genetically compatible mates in order to reproduce (Byers 1995; Mustajärvi et al. 2001; Leimu et al. 2006). Population size may also influence plant-pollinator interactions and thus the quantity and/or quality of pollen plants receive. Pollinators are typically attracted to the more visually apparent floral displays of large populations for foraging (e.g. Sih and Baltus 1987; Jennersten 1988; Ågren 1996). By attracting more pollinators, a large population may increase the probability of each individual plant (and flower) receiving sufficient amounts of compatible pollen, thus increasing overall reproductive output (Jennersten 1988; Matsumura and Washitani 2000; Kolb 2008). However, if population size is large enough for pollinator saturation (Steven et al. 2003), intraspecific competition among plants may reduce pollinator visitation rates per plant (Söber et al. 2009). In this scenario, population size may not exhibit a positive correlation with reproductive output (Mustajärvi et al. 2001). Though population size has been linked to plant reproductive success, this relationship is highly dependent on species and the reproductive measurement type.

The impact of population density on plant reproduction is also highly species and habitat-dependent. In high density populations, some species have greater reproductive output due to either their attractiveness to pollinators (Klinkhamer et al. 1989; Kunin 1993; Roll et al. 1997) or establishment in resource-rich environments (Obeso 2002; Dainese 2011). Pollinator abundance, and thus quantity of pollen deposition, has been cited to increase with population density due to greater visual appeal and potential for rewards (Dreisig 1995; Nattero et al. 2011). Further, the minimal foraging distance between plants in high-density populations encourages xenogamous pollination (Krueger and Knapp 1991; van Treuren et al. 1993; Karron et al. 1995), and thus higher pollen quality and reproductive success, through increased pollinator movement among plants. Alternatively, pollinators in sparse populations reduce their foraging distance by visiting more flowers on the same plant (Bateman 1956; Field et al. 2005), thereby increasing geitonogamy and potentially decreasing pollen quality and reproductive output. The covariance of population density with high resource availability may also explain why reproduction can increase with conspecific plant density. Regardless of density, plants located in high quality, nutrient-rich environments are cited to have higher reproductive output because they can invest energy into reproduction without becoming resource-limited (Breen and Richards 2008; Dainese 2011). Reproduction in other plant species may not be density-dependent at all due to the overriding effects of intraspecific competition. As with population size, if population density becomes too great an individual plant's pollinator visitation rate (Mustajärvi et al. 2001) and probability of growth and survival (Feldman and Morris 2011) may decrease due to competition with conspecifics.

Lobelia spicata Lam. (Campanulaceae) is a gynodioecious, self-compatible species (Molano-Flores 2002) commonly found in prairies. Currently the variation as well as the impact of population size and density on reproductive output and fitness for the species is unknown. This study has three main objectives:

- (1) Examine differences in *Lobelia spicata* reproductive output (i.e. fruit set, seed number per fruit and seed biomass) and fitness (i.e. seed germination) among populations and between years.
- (2) Assess relationships existing among *L. spicata*'s reproductive output and fitness measurements.
- (3) Determine if population size and/or population density influences reproductive output and fitness of *L. spicata* plants.

MATERIALS AND METHODS

Study system – *Lobelia spicata* is a short-lived, herbaceous perennial found throughout eastern North America (McVaugh 1936) in loess, sandy and loam prairies (Byers et al. 2005). As previously mentioned, it is a gynodioecious species (Molano-Flores 2002) like another member of its genus (i.e. *Lobelia siphilitica*). Female and hermaphrodite *L. spicata* plants produce single, terminal raceme inflorescences from May to June (Swink and Wilhem 1994). These racemes contain 2 to 70+ zygomorphic flowers that are approximately 7 to 13mm long (Molano-Flores 2002). Hermaphroditic flowers are protandrous and self-compatible (Molano-Flores 2002). After being pollinated by small bees in the *Augochlorella* genus (Molano-Flores 2002), *L. spicata* plants produce capsule fruits containing few to over 200 very small seeds.

Population Surveys – During the summers of 2008 and 2009, comprehensive searches were conducted during peak flowering time to determine the total number of individuals in 11 Illinois *L. spicata* populations (Table 2.1). Population density was calculated for each of the 11 populations by dividing total population size by the area of each population in square meters using ArcMap 9.3.1 (ESRI 2011). Forty individual plants (both hermaphrodite and female of approximately equivalent height) per population were randomly tagged and collected to examine reproductive success between years and among populations. The fruit set of each infructescence was determined by dividing the number of mature fruits by the total number of flowers. Five unopened fruits in the center of each infructescence were selected to determine the mean seed number per fruit for each individual. Seed collected from each individual was pooled, and due to the seeds' small size (< 1mm diameter), 200 were counted out and weighed to obtain an estimate of seed biomass. All seed produced was pooled by population for a total of 11 seed samples. Ten petri dishes per population were lined with moist filter paper and 20 seeds were placed in each. Seed germination in a growth chamber set at 21.1°C with a 14 hour photoperiod was recorded three times a week for 12 weeks. Total percent seed germination was calculated for each dish and mean values were calculated for each population.

Statistical analyses – All statistical analyses were performed using Sigma Stat 3.1 (Systat Software, Inc.). To determine differences between years and among populations in reproductive output (i.e. fruit set, seed number per fruit, seed biomass) and fitness (i.e. percent seed germination), multiple 2-way ANOVAs were performed. A log-transformation of the seed biomass data allowed it to pass the normality assumption for ANOVA, however none of

the transformations (i.e. $\log(10)$, $\log(n+1)$ and $\arcsin\sqrt{x}$) used for fruit set, seed number per fruit and percent seed germination resulted in normally distributed data with homogeneity of variances. For these variables, we performed 2-way ANOVAs with ranked values to determine differences between years and among populations (Zar 1996). Pearson correlations were used to examine whether population size and/or density was related to the reproductive output or fitness variables.

RESULTS

Reproductive Success – There was a significant interaction between population and year for fruit set, seed number per fruit, seed biomass and percent seed germination, indicating that the differences among populations for these variables were not consistent between 2008 and 2009 (Table 2.2; Figure 2.1). Mean fruit set for the *L. spicata* populations surveyed was higher in 2008 ($\bar{x} = 0.78 \pm 0.07$) than in 2009 ($\bar{x} = 0.65 \pm 0.14$). Alternatively, the number of seeds produced per fruit was slightly lower on average in 2008 ($\bar{x} = 54.3 \pm 11.7$) than in 2009 ($\bar{x} = 62.8 \pm 13.3$). Seed biomass and percent seed germination were both slightly greater in 2008 ($\bar{x} = 4.4\text{mg} \pm 0.9$ and $19\% \pm 19\%$, respectively) than in 2009 ($\bar{x} = 3.8 \pm 0.6$ and $13\% \pm 51\%$, respectively).

Lobelia spicata fruit set, seed number per fruit and seed biomass were not correlated to one another (Table 2.3). Alternatively, percent seed germination was negatively correlated with fruit set and seed number per fruit in 2009 ($r = -0.592$ and -0.612 , respectively, $P < 0.05$) though not in 2008 ($r = -0.273$ and -0.175 , respectively, $P > 0.010$). Further, percent seed germination was positively correlated to seed biomass in 2008 ($r = 0.761$, $P < 0.01$) and marginally so in 2009 ($r = 0.566$, $P < 0.10$).

Population Size and Density – In the 11 *L. spicata* populations surveyed, population size ranged from 203 to 3478 and from 215 to 5322 plants in 2008 and 2009, respectively (Table 2.1). There was a significant change in population size from 2008 to 2009, with the direction of the change varying among populations ($\chi^2 = 1398.99$, $df = 10$, $P < 0.01$). Population density ranged from 0.01 to 2.66 plants per square meter in 2008 and from 0.02 to 3.64 plants per square meter in 2009 (Table 2.1). There was not a significant relationship between population size and population density in 2008 or 2009 ($r = 0.144$ and 0.132 , respectively, $P > 0.05$).

Size and density of the *L. spicata* populations surveyed were not related to any of the reproduction measurements (fruit set, seed number per fruit, seed biomass and percent seed germination) in 2008 or 2009 with three exceptions (Table 2.4). A marginally significant negative correlation existed between population size and fruit set in 2008 ($r = -0.562$, $P < 0.10$). In 2009, a positive correlation was found between population size and seed number per fruit ($r = 0.660$, $P < 0.05$). Lastly, in 2008 population density was positively correlated to seed number per fruit ($r = 0.658$, $P < 0.05$).

DISCUSSION

Reproductive Success – In this study, the reproductive success and fitness of *Lobelia spicata* was highly variable among populations and between sampling years. Site characteristics could potentially be having a large impact on the amount of resources plant populations have available for reproduction. Specifically, those populations in resource-poor environments may be unable to expend as much energy on reproduction as those populations in resource-rich environments (Obeso 2002), independent of population dynamics. Likewise, yearly variation in climatic conditions (i.e. temperature and precipitation) may influence resource availability for growth and reproduction or pollinator behavior (Vaughton and Ramsey 2010) and thus indirectly affect plant reproductive success.

Relationships existing between select reproductive measurements indicate trade-offs in resource investment. Though not significant, there was a trend in *L. spicata* toward larger seed biomass in populations with lower mean fruit set and fewer seeds per fruit during intermittent years. Consequently, plants may invest more resources per seed if they are not expending as much on producing high numbers of fruits or seeds (Kosiński 2010). Greater seed biomass is advantageous as it has been linked to higher percent seed germination in many species (e.g. Oostermeijer et al. 1994; Kéry et al. 2000). In *L. spicata*, percent seed germination was negatively correlated to fruit set and seed number per fruit in one sampling year, but positively correlated to seed biomass in both 2008 and 2009. Thus, it is likely that *L. spicata* populations producing high fruit set or seed number per fruit will have more seeds available to germinate, but those populations that produce low fruit sets or fewer seeds per fruit will exhibit higher percent seed germination due to greater maternal resource investment per seed.

Population Size and Density – Variation in population size and density existed among populations and across years in *Lobelia spicata*. As with reproductive output, population size and density may vary with habitat quality (Dainese 2011) or display yearly variation due to climatic conditions. Alternatively, it is important to consider that when conducting population surveys for this species, only flowering individuals were counted due to the difficulty of locating sterile rosettes. Thus yearly variation in population size and density could be a product of population age structure, where younger individuals have not begun producing a raceme inflorescence, or mature individuals choose to flower only during intermittent years.

Overall, the size and density of *L. spicata* populations did not impact their levels of reproductive output or fitness, with three notable exceptions. *L. spicata* population size exhibited a marginally significant, negative correlation with fruit set in 2008, but only a non-significant, negative trend in 2009. Though many species have been cited to produce higher fruit sets in large populations (e.g. Lamont et al. 1993; Molano-Flores and Hendrix 1999; Kéry et al. 2000; Ågren et al. 2008), presumably due to increased pollinator attraction (Sih and Baltus 1987; Jennersten 1988; Ågren 1996), other species like *L. spicata* only demonstrate a weak or non-existent relationship between these two variables (e.g. Jennersten and Nilsson 1993; Fischer and Matthies 1998b; Molano-Flores et al. 1999). It is possible that individuals in large *L. spicata* populations experience increased intraspecific competition

for environmental resources (Mustajärvi et al. 2001) or pollinators (Söber et al. 2009) that could mask the positive effects on reproduction generally associated with large population size.

Larger *L. spicata* populations were shown to produce significantly more seeds per fruit in 2009, with a positive, though non-significant trend existing between the two variables in 2008. A number of other species are cited to produce more seeds per fruit in large populations (e.g. Fischer and Matthies 1998b; Jacquemyn et al. 2002; Vergeer et al. 2003; Johnson et al. 2004; Waites and Ågren 2004; Hansen and Totland 2006; Winter et al. 2008) though no relationship between population size and seeds per fruit has been found for others (e.g. Lamont et al. 1993; Kunin 1997; Weber and Kolb 2011). This disparity in seed production between populations of different sizes has often been attributed to pollen quantity and quality. Due to the greater attraction of pollinators (Jennersten and Nilsson 1993; Kolb 2008) and higher genetic variability (Ellstrand and Elam 1993; Young et al. 1996; Reed and Frankham 2003) associated with large populations, the probability of receiving compatible, genetically diverse pollen is greater. This is particularly important in self-incompatible species (Byers 1995; Mustajärvi et al. 2001; Leimu et al. 2006), since they are required to outcross. Though *L. spicata* is a self-compatible species, its gynodioecious breeding system necessitates outcrossing for female plants at least. Thus, it is highly likely that the quantity and quality of pollen are influencing the amount of seeds produced per fruit in this species.

Density of *L. spicata* populations was positively correlated to fruit set in 2008, but the two variables displayed only a positive trend in 2009. Positive correlations between population density and fruit set have been found for a number of other species (e.g. Platt et al. 1974; Roll et al. 1997; Kéry et al. 2000) due to greater pollinator abundance (Dreisig 1995; Nattero et al. 2011) and movement among plants (Krueger and Knapp 1991; Van Treuren et al. 1993; Karron et al. 1995). Increased inter-plant versus intra-plant visitation by pollinators leads to more out-crossed, and thus higher quality pollen. Due to the small flowers and slim raceme inflorescences associated with *L. spicata*, density may be particularly important in the initial attraction of pollinators.

Seed biomass and percent seed germination were not correlated to population size or density in *L. spicata* populations in either year. It could be that seed biomass only experiences slight, indirect effects of population size and density due to their impact on fruit set and seed number per fruit. Alternatively, percent seed germination was low overall and may not show population size and density effects due to the benign laboratory conditions the seeds were grown under.

In conclusion, though population size and density do appear to have some impact on *Lobelia spicata*'s reproductive success, other population factors may be more influential due to the species' gynodioecious breeding system. For instance, female plant frequency within gynodioecious populations has been cited to influence the level of reproductive advantage (Delph 1990; McCauley and Brock 1998) that females often exhibit over their hermaphroditic counterparts (reviewed in Shykoff et al. 2003). Further, gender-based density measurements may have a more pronounced effect on reproduction (Williams et al. 2000) than overall population density. Thus, additional research is required to determine if other population dynamics (i.e. female frequency, female density and hermaphrodite density) unique to the gynodioecious breeding system are better predictors of the continued reproductive success of *L. spicata* (see Chapter 3).

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TABLES AND FIGURES

TABLE 2.1 Population size and density (plants per m²) in 2008 and 2009 for 11 *Lobelia spicata* populations

Site Code	Population	Population size		Population density	
		2008	2009	2008	2009
BEA	Beaverville Prairie	541	747	0.27	0.37
BEL	Belmont Prairie	445	909	0.02	0.05
FD1	Falling Down Prairie Site #1	530	724	2.66	3.64
FD2	Falling Down Prairie Site #2	965	1244	1.36	1.75
FUL	Fulton Prairie	3478	2999	0.13	0.11
LOD	Loda Cemetery Prairie	203	215	0.01	0.02
MID	Midewin National Tallgrass Prairie	222	300	0.02	0.03
PAX	Paxton Cemetery Prairie	1233	2508	0.57	1.16
PEL	Pelville Railroad Prairie	1976	1005	0.55	0.28
PRO	Prospect Cemetery Prairie	209	591	0.03	0.07
SOM	Somme Prairie	3076	5322	0.04	0.07

TABLE 2.2 Results of 2-Way ANOVAs examining the effects of year and population on *Lobelia spicata* fruit set, seed number per fruit, seed biomass and percent seed germination.

Variable	Source of variation	df	F	P
Fruit set	Year	1	14.329	< 0.001
	Population	10	56.637	< 0.001
	Year × Population	10	5.408	< 0.001
Seed number per fruit	Year	1	4.372	< 0.001
	Population	10	8.960	0.003
	Year × Population	10	2.208	0.016
Seed biomass	Year	1	14.676	< 0.001
	Population	10	43.101	< 0.001
	Year × Population	10	3.080	< 0.001
Percent seed germination	Year	1	41.138	< 0.001
	Population	10	18.816	< 0.001
	Year × Population	10	2.707	0.004

TABLE 2.3 Pearson correlation coefficients among the four reproduction measurements examined for plants in *Lobelia spicata* populations during summer 2008 and 2009. P < 0.10 designated by *, P < 0.05 by ** and P < 0.01 by ***.

Reproduction Measurements	Seed number per fruit		Seed biomass		Percent seed germination	
	2008	2009	2008	2009	2008	2009
Fruit set	0.163	0.132	-0.504	-0.032	-0.273	-0.592**
Seed number per fruit	---	---	-0.095	-0.483	-0.175	-0.612**
Seed biomass	---	---	---	---	0.761***	0.566*

TABLE 2.4 Pearson correlation coefficients between population size or population density and the four reproduction measurements collected in 2008 and 2009 for 11 *Lobelia spicata* populations. P < 0.10 designated by * and P < 0.05 by **.

Reproduction Measurements	Population Size		Population Density	
	2008	2009	2008	2009
Fruit set	-0.562*	-0.358	0.658**	0.323
Seed number per fruit	0.303	0.660**	0.053	0.049
Seed biomass	0.241	-0.324	-0.399	-0.130
Percent seed germination	-0.225	-0.155	-0.209	-0.153

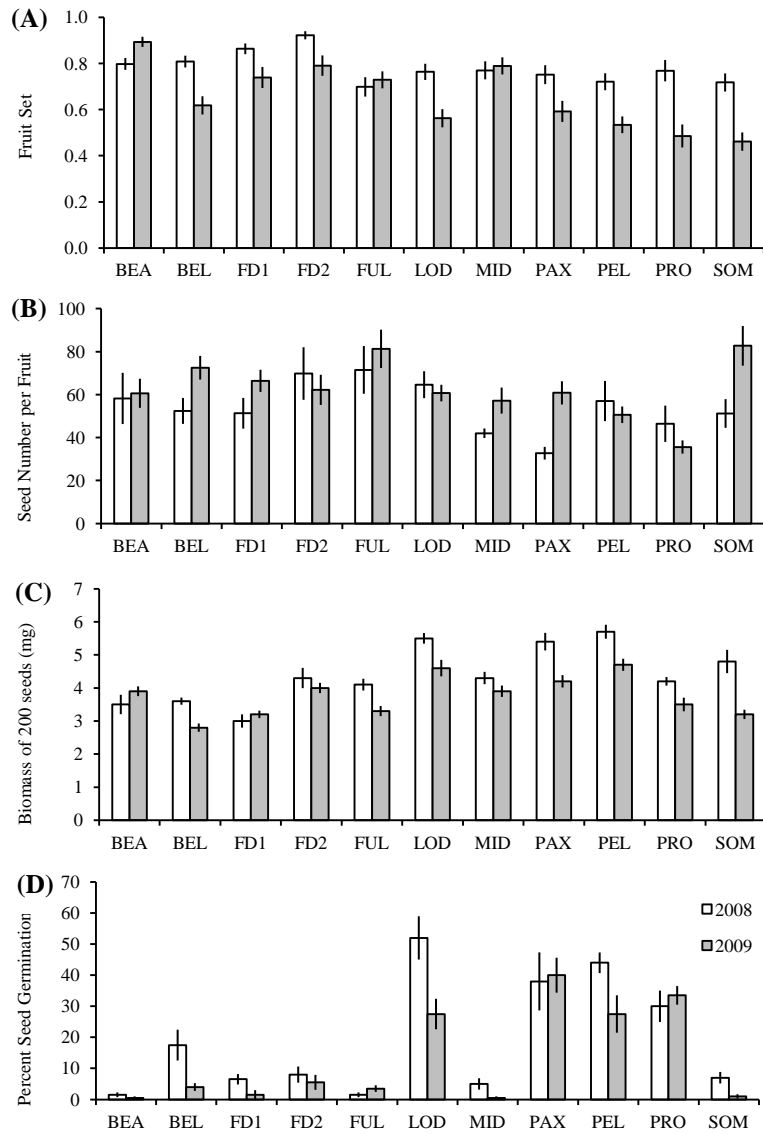


Figure 2.1 Mean reproductive output and fitness measurements (\pm SE) during summer 2008 and 2009 for 11 *Lobelia spicata* populations (refer to Table 2.1 for population abbreviations). (A) Fruit set, (B) Seed number per fruit, (C) Seed biomass for 200 seeds in milligrams and (D) Percent seed germination.

THE IMPACT OF FEMALE FREQUENCY AND GENDER DENSITY ON REPRODUCTIVE GENDER DIFFERENCES IN *Lobelia spicata* Lam. (CAMPANULACEAE), A GYNODIOECIOUS PRAIRIE SPECIES

ABSTRACT

Gynodioecious species are characterized by having female and hermaphrodite plant morphs coexisting within populations. Female plants are often at a disadvantage due to their reliance on hermaphroditic pollen donation and lower attractiveness to pollinators. In some gynodioecious species, females compensate for these disadvantages through greater reproductive output and progeny fitness. However, the level of female advantage in reproduction may vary based on population dynamics including female frequency and the density of plant genders. Thus, the objective of this study was to determine if reproductive differences exist between plant genders in the gynodioecious prairie species *Lobelia spicata* Lam. (Campanulaceae), and if so how those differences are impacted by female frequency and/or gender density. During the summers of 2008 and 2009, population measurements (i.e. female frequency, female density and hermaphrodite density) and reproductive output (i.e. fruit set, seed number per fruit, seed biomass) and fitness (i.e. percent seed germination) measurements for both plant genders were obtained for 11 *L. spicata* populations located in northern and central Illinois. Female plants were found to produce greater fruit sets, seed numbers per fruit and percent seed germination than hermaphrodites, though these reproductive gender differences varied among populations and between sampling years. Female frequency, female density and hermaphrodite density did impact the gender-based reproductive measurements, particularly fruit set, presumably due to their influence on pollen quantity and quality. Future studies should examine how resource availability and pollinator behavior varies among gender morphs and populations of varying female frequencies and densities.

INTRODUCTION

Gynodioecy, a plant breeding system where populations are composed of female and hermaphrodite plant morphs, has been documented in only 543 plant species across 50 families (Jacobs and Wade 2003). Unlike other breeding systems, gender dimorphism in gynodioecious systems occurs at the plant rather than flower level (Shykoff et al. 2003; Miller and Stanton-Geddes 2007), with nucleo-cytoplasmic gene interactions controlling gender inheritance. Specifically, the presence and/or absence of cytoplasmic male sterility (CMS) mutations and nuclear restorers are responsible for the occurrence of female and hermaphrodite plants within populations (Dudley et al. 2001). Female plants will contain at least one CMS mutation, while hermaphrodite plants will either not have a CMS mutation, or if they do they will also have the corresponding nuclear restorer to restore male function.

Though gender dimorphism in gynodioecious plant species does have a genetic basis, ecological factors may also aid in the maintenance of two plant genders. Gynodioecious populations can derive a number of potential benefits from the inclusion of female plants due to the females' ability to produce more flowers (López-

Villavicencio et al. 2005), larger fruit and seed sets (e.g. Delph 1990; Widén and Widén 1990; Sugawara and Horii 1995; Koelewijn 1996; Sakai et al. 1997; Gigord et al. 1999), and larger, more quickly-germinating seeds (Dulberger and Horovitz 1984; Koelewijn 1996; Manicacci et al. 1998; Gigord et al. 1999) than hermaphrodites (reviewed in Shykoff et al. 2003). Despite these advantages, a female plant's inability to produce pollen leaves its reproductive output entirely dependent upon pollen donation by hermaphrodites. In addition, lack of nectar and smaller flower size (Caruso et al. 2003; reviewed in Shykoff et al. 2003) in female plants may result in reduced pollinator visitation (Eckhart 1991; Johnson et al. 1995; Williams et al. 2000), which could limit reproductive success (Jennersten 1988; Matsumura and Washitani 2000; Asikainen and Mutikainen 2005; Kolb 2008). Thus, the degree to which female's superior reproductive success aids in maintenance of their gender is unclear.

In some species the level of reproductive superiority exhibited by females may vary with female frequency (Delph 1990; McCauley and Brock 1998) and to a lesser extent female or hermaphrodite density (Williams et al. 2000) within a population. Since female plants are required to outcross with hermaphrodites, high female frequency and density or low hermaphrodite density may reduce the number of hermaphrodites (i.e. pollen donors) available or in proximity to a female plant, reducing pollination levels and thereby their reproductive success. Variation in female frequency across populations is often high for many gynodioecious species (Koelewijn and Van Damme 1996; Webb 1999; Alonso et al. 2007; Caruso and Case 2007; Landry et al. 2009) and thus female advantage in reproduction may vary greatly among populations.

Lobelia spicata Lam. (Campanulaceae) is cited as a gynodioecious species (Molano-Flores 2002), however it is currently unknown if females possess a reproductive advantage over hermaphroditic plants and if so, how this advantage varies based on population dynamics (i.e. female frequency, female density and hermaphrodite density). The two objectives of this study are to:

- (1) Examine differences in reproductive output (i.e. fruit set, seed number per fruit and seed biomass) and fitness (i.e. seed germination) between female and hermaphrodite plants in *Lobelia spicata* populations.
- (2) Determine if *L. spicata* female frequency, female density or hermaphrodite density influences (A) female or hermaphrodite reproductive output and fitness or (B) female advantage in reproduction.

MATERIALS AND METHODS

Study system – *Lobelia spicata* is a short-lived, herbaceous perennial that is common in loess, sandy and loam prairies (Byers et al. 2005) throughout eastern North America (McVaugh 1936). It produces a single terminal raceme inflorescence that matures acropetally, with flowering and fruiting occurring from May to August (Swink and Wilhem 1994). Each inflorescence contains 2 to 70+ zygomorphic flowers that are approximately 7 to 13mm long (Molano-Flores 2002). The principal pollinators of *L. spicata* are small bees, the most common being members of the *Augochlorella* genus (Molano-Flores 2002). It has been cited as a gynodioecious species like another member of its genus (i.e. *Lobelia siphilitica*), and the hermaphrodite plant flowers are protandrous and self-compatible (Molano-Flores 2002). *L. spicata* produces few to over 200 tiny seeds in capsule fruits.

Population Dynamics – During the summers of 2008 and 2009, comprehensive searches were performed in Illinois during peak flowering time to determine the population size and female frequency of 11 *L. spicata* populations (Table 3.1). ArcMap 9.3.1 (ESRI 2011) was used to find the area (in square meters) of land that the *L. spicata* population occupied, and female and hermaphrodite plant density was calculated by dividing the number of female or hermaphrodite plants, respectively, by the area of each population.

Reproductive Output and Fitness – Twenty individual plants per gender per population of approximately equivalent height were randomly tagged and collected in order to examine reproductive success between genders and among populations. Fruit set was obtained for each individual by dividing the number of mature fruits by the total number of flowers. Seed counts were performed for five unopened fruits in the center of each infructescence to determine average seed number per fruit. Seed was pooled by individual and 200 seeds were counted out and weighed to obtain seed biomass due to the small size of the seeds (< 1mm diameter). Within the 11 populations, all produced seed was pooled by maternal parent (female or hermaphrodite) for a total of 22 seed samples. Five petri dishes per gender per population were lined with moist filter paper and 20 seeds were placed in each. Seed germination in a growth chamber set at 21.1°C with a 14 hour photoperiod was monitored three times a week for 12 weeks. Mean percent seed germination values were calculated for seed produced by female and hermaphrodite plants within each population. The level of female advantage (FA) in fruit set, seed number per fruit and seed biomass was calculated for each population using Equation 1. FA was not calculated for percent seed germination due to the extremely low (or non-existent) levels of germination exhibited by both plant genders across the populations.

Equation 1 Female Advantage (FA) = $\frac{\text{Female Reproductive Measurement Mean}}{\text{Hermaphrodite Reproductive Measurement Mean}}$

Statistical analyses – All statistical analyses were performed using Sigma Stat 3.1 (Systat Software, Inc.). A chi-square test was performed to determine if gender ratio was independent of sampling year and population. To determine differences between genders, among populations and between years in reproductive output (i.e. fruit set, seed number per fruit, seed biomass) and fitness (i.e. percent seed germination), multiple 3-way ANOVAs were performed. A log(10)-transformation of the seed number per fruit data and a log(n+1)-transformation of the percent seed germination data allowed them to pass the equal variance and normality assumptions, respectively, for ANOVA. None of the transformations (i.e. log(10), log(n+1) and arcsinsqrt) used for fruit set and seed biomass resulted in normally distributed data with homogeneity of variances. For these two variables, we performed 3-way ANOVAs with ranked values to determine differences between genders, among populations and between years (Zar 1996). Pearson correlations were used to examine whether female frequency, female density or hermaphrodite density were related to reproductive output and fitness measurements or female advantage in reproduction.

RESULTS

Female Frequency and Gender Density – The frequency of female plants in *Lobelia spicata* populations ranged from 12.8 to 63.4% in 2008 and from 12.2 to 72.8% in 2009 (Table 3.1). There was not a significant change in female frequency from 2008 to 2009 ($\chi^2 = 13.21$, $df = 10$, $P > 0.05$). Female plant density ranged from 0.01 to 0.71 and from 0.01 to 1.05 plants/m² in 2008 and 2009, respectively, while hermaphrodite plant density ranged from 0.01 to 1.96 and from 0.01 to 2.59 plants/m² in 2008 and 2009, respectively. Female and hermaphrodite densities were positively correlated in 2008 and 2009 ($r = 0.829$ and 0.849 , respectively, $P < 0.01$). In 2008 and 2009, there was not a significant relationship between female frequency and female density ($r = -0.107$ and -0.189 , respectively, $P > 0.10$), but there was a marginally significant, negative correlation between female frequency and hermaphrodite density ($r = -0.535$ and -0.521 , respectively, $P < 0.10$).

Reproductive Output and Fitness – Female fruit set was on average 1.2 and 1.4 times higher than that of hermaphrodites in 2008 and 2009, respectively (Table 3.2). Females also produced 1.4 and 1.8 times more seeds per fruit than hermaphrodites in 2008 and 2009, respectively (Table 3.2). Though females did produce a significantly greater fruit set and number of seeds per fruit than hermaphrodites overall, the interactions between gender and population, gender and year, and population and year were significant, suggesting that female advantage in fruit set and seed number per fruit varies among populations and between years (Figure 3.1A and 3.1B; Table 3.3). Seed biomass did not statistically differ between the plant genders; however it did vary among populations and between years (Figure 3.1C; Table 3.3).

Percent seed germination levels were very low overall in both 2008 and 2009, with only four out of eleven populations having any gender/population/year category exceed 40% germination (Figure 3.1D). Seed produced by female plants did germinate at a significantly greater frequency than seed from hermaphrodites (Table 3.3). There was, however, a significant interaction between gender, population and year for percent seed germination, suggesting that the degree and direction of the difference between the genders is highly dependent on site and the collection year.

Regardless of gender, fruit set was not related to number of seeds per fruit, seed biomass or percent seed germination nor was the number of seeds per fruit related to seed biomass (Table 3.4). For females the number of seeds per fruit had a marginally significant, negative relationship with the percent of seeds that germinated in 2009 ($r = -0.572$, $P < 0.10$), but not in 2008. Additionally, female seed biomass had a significant and marginally significant positive correlation with percent seed germination in 2008 ($r = 0.630$, $P < 0.05$) and 2009 ($r = 0.535$, $P < 0.10$), respectively. For hermaphrodites, higher seed biomass did tend to result in higher seed germination in 2008 ($r = 0.389$, $P > 0.05$), and this relationship was significant in 2009 ($r = 0.638$, $P < 0.05$).

Impact of Female Frequency and Gender Density on Reproduction – Female frequency was negatively correlated with female fruit set in 2009 ($r = -0.750$, $P < 0.01$) and with hermaphrodite fruit set in 2008 and 2009 ($r = -0.663$ and -0.665 , respectively, $P < 0.05$; Table 3.5). Additionally, an increase in female frequency resulted in a significant

decrease in the number of seeds per female-produced fruit in 2008 and 2009 ($r = -0.662$ and -0.598 , respectively, $P < 0.05$), and a significant increase in biomass of female-produced seed in 2008 ($r = 0.608$, $P < 0.05$), but not in 2009 ($r = 0.108$, $P > 0.05$). There was a strong positive correlation between female frequency and percent seed germination for both genders during intermittent years, however, this may be a product of the extremely low germination levels in 7 out of 11 *L. spicata* populations (Table 3.5).

Female and hermaphrodite plant densities were not significantly correlated to female or hermaphrodite seed number per fruit, seed biomass, or percent seed germination in 2008 and 2009 (Table 3.5). Female density exhibited a marginally significant positive relationship with female fruit set in 2008 ($r = 0.546$, $P < 0.10$), and but not in 2009 ($r = 0.475$, $P > 0.10$). Hermaphrodite density was positively correlated with female fruit set in 2008 and 2009 ($r = 0.706$ and 0.637 , respectively, $P < 0.05$) and with hermaphrodite fruit set in 2008 ($r = 0.600$, $P < 0.05$) but not in 2009 ($r = 0.084$, $P > 0.10$). These results suggest that fruit set, particularly of female plants, is relatively greater in denser plant populations.

Overall, female frequency and the density of female and hermaphrodite plants within populations had little impact on female advantage in the four reproduction measurements collected (Table 3.6). However, female advantage in seed number per fruit was lower in populations with high female frequency in 2009 ($r = -0.646$, $P < 0.05$), though only a non-significant negative trend existed between the variables in 2008 ($r = -0.462$, $P > 0.10$). Female density was positively correlated to female advantage in fruit set in 2009 ($r = 0.697$, $P < 0.05$), and exhibited a positive, non-significant trend in 2008 ($r = 0.323$, $P > 0.10$).

DISCUSSION

Female Frequency and Gender Density – Like many other gynodioecious species (e.g. *Plantago coronopus* L. [Koelewijn and Van Damme 1996], *Daphne laureola* L. [Alonso et al. 2007] and *Lobelia siphilitica* L. [Caruso and Case 2007]), female frequency was highly variable across *Lobelia spicata* populations. However, female frequency within populations remained relatively constant from summer 2008 to 2009 despite significant changes in overall population size between the two years (see Chapter 2). Thus, though *L. spicata* may experience slight variation in intra-population female frequency, these data suggest that the species possesses a stable gynodioecious breeding system at least in the short term (Asikainen and Mutikainen 2003). Inter-population variation and intra-population consistency in female frequency could be attributed to genetic and/or environmental causes. Due to the complicated nucleo-cytoplasmic gene interactions controlling gender inheritance in gynodioecious species, genetic diversity and thus population size (Spigler and Chang 2009) can have a large impact on female frequency within populations. A select number of studies have shown female frequency to increase with population size (e.g. *Plantago maritima* L. [Nilsson and Ågren 2006]) presumably due to an increase in cytoplasmic male sterility (CMS) gene diversity. However, *L. spicata* female frequency and population size were not correlated in 2008 or 2009 ($r = -0.021$ and -0.138 , respectively, $P > 0.10$; Ruffatto, unpublished data), suggesting that its populations possess the nuclear

restorer genes corresponding to the represented CMS genes. Alternatively, environmental factors may be more influential in determining *L. spicata* female frequency by promoting the success of a particular gender morph. Female plants are often reproductively superior to hermaphrodites under resource-poor conditions (Barrett 1992; Costich 1995; Sakai and Weller 1991), and thus their frequency may increase with environmental stress levels.

Both female and hermaphrodite plant densities were highly variable among populations; however the two density measures were positively correlated such that populations with high female density also had high hermaphrodite density. Further, female frequency for *L. spicata* was not correlated with female or hermaphrodite plant density in 2008 or 2009. These results suggest that female and hermaphrodite plants are comparable in their ability to withstand the effects of intraspecific competition for environmental resources, or that resources are not limited in these populations making intraspecific competition negligible even under high density conditions. By contrast, other polymorphic species, such as *Silene alba* (Mill.) Krause, have found female frequency to increase with plant density (Doust et al. 1987). Thus, the relationship between female frequency and gender-based density measurements in plants with gynodioecious breeding systems could be both species and habitat quality dependent.

Reproductive Output and Fitness – Female *Lobelia spicata* plants produced greater fruit sets and a larger number of seed per fruit overall than did hermaphrodites. These trends are consistent with a number of other gynodioecious species including *Hebe strictissima* (Kirk) L.B. Moore (Delph 1990), *Glechoma hederacea* L. (Widén and Widén 1990), *Plantago coronopus* L. (Koelewijn 1996), *Schiedea adamantis* H. St. John (Sakai et al. 1997) and *Thymus vulgaris* L. (Gigord et al. 1999). Two different rationales could explain female advantage for these reproductive measurements. First, female plants are able to devote their resources exclusively to fruit and seed production, whereas hermaphrodites must allocate some resources away from the manufacture of fruit and seeds in favor of pollen production (Delph 1990). Thus, hermaphrodite fruit and seed production would be lower overall and may display a more pronounced decline under resource-poor conditions than females. Conversely, inbreeding avoidance by females due to required xenogamous pollination and/or inbreeding depression in hermaphrodites due to their potential for geitonogamous pollination may increase the reproductive advantage females have over hermaphrodites. Though the plant genders did differ in fruit and seed production, the amount varied among populations and between years. It is probable that variation in environmental conditions across sites and sampling years indirectly alters female reproductive advantage through its impact on resource availability and/or pollinator behavior (Vaughton and Ramsey 2010).

Seed biomass did not differ between the gender morphs, however it did vary among populations and between years presumably because of environmental conditions influencing the amount of resources available for reproductive investment. Female advantage in seed biomass has been found for some species (e.g. *Plantago coronopus* L. [Koelewijn 1996], *Thymus vulgaris* L. [Gigord et al. 1999]), however others like *L. spicata* have exhibited no difference between female and hermaphrodite seed biomass (e.g. *Silene vulgaris* (Moench) Garcke [Jolls and Chenier 1989], *Echium vulgare* L. [Klinkhamer et al. 1991], *Raphanus sativus* L. [Miyake et al. 2009]). Interspecies variation of female advantage in seed biomass has been tied to whether or not the species produces a finite number of seeds. Generally, gynodioecious species that produce large, variable amounts of ovules will exhibit

a female advantage in seed set (e.g. *Lobelia spicata*), whereas species that produce small, finite amounts of ovules will display a female advantage in either fruit production or seed biomass since they have an upper limit on seed production per fruit. In *L. spicata*, a slight tradeoff was detected between the number of seeds produced per fruit by females and female seed biomass. These trends provide further evidence that female *L. spicata* plants gain an advantage over hermaphrodites in superior seed production than through better seed provisioning.

Females did exhibit higher percent seed germination than did hermaphrodites under laboratory conditions in *L. spicata* populations, as with other gynodioecious species (e.g. *Thymus vulgaris* and *T. zygis* [Manicacci et al. 1998]). However, differences among the genders varied significantly among populations and across years. Further, percent seed germination was very low overall, with only 4 of 11 *L. spicata* populations exhibiting over 40% germination for either gender. Germination chamber conditions may not have been optimal for seed germination in all populations (see Chapter 4), thereby contributing to inconsistent population and sampling year trends as well as overall low germination rates. Alternatively, differences in seed biomass among populations may have led to germination differences in both genders, as seed biomass was positively correlated with percent seed germination in *L. spicata* and was highest in those 4 populations with more substantial germination. Percent seed germination has been cited to increase with seed biomass in a number of other studies (e.g. Oostermeijer et al. 1994; Kéry et al. 2000), due to the better seed provisioning.

Impact of Female Frequency and Gender Density on Reproduction – High female frequency in *L. spicata* populations was linked to a reduction in female and hermaphrodite fruits sets, as well as the number of seeds in female-produced fruits. Further, female advantage in the number of seeds produced per fruit decreased with increasing female frequency in 2009, though there was only a non-significant trend for this in 2008. As previously mentioned, in gynodioecious species hermaphrodites are the only pollen donors and thus a relative reduction in their number or density can result in a reduction in pollen quantity or quality. For *L. spicata* females, this could be particularly detrimental as they need outcrossed, hermaphroditic pollen in sufficient quantities (McCauley and Taylor 1997) or their fruit and seed production will suffer. Alternatively, a reduction in available pollen may not be as critical to *L. spicata* hermaphrodites since they have the option of selfing, (i.e. geitonogamy) though receiving quality (i.e. xenogamous) pollen often results in higher reproductive output.

Female frequency was positively correlated with female seed biomass and percent seed germination of both gender morphs during intermittent years. As previously mentioned, only 4 of the *L. spicata* populations surveyed exhibited over 40% seed germination and those populations also had the highest percent of females within populations as well as the greatest seed biomass. Since larger seeds have been linked to greater percent seed germination in many species, female frequency may be indirectly influencing percent seed germination through its impact on seed biomass.

Female advantage in fruit set increased with female density in 2009, with a non-significant positive trend between the two variables in 2008. It is probable that superior fruit set production by females is resulting in a significantly greater amount of female progeny being produced, since *L. spicata* females have been cited to produce

approximately 50% female offspring whereas hermaphrodite maternal parents average around 10% female offspring (Byers et al. 2005). Thus, female advantage in fruit set may be increasing female plant density, rather than female density itself driving changes in female advantage.

In summary, *Lobelia spicata* does exhibit reproductive differences between gender morphs; specifically females have greater fruit sets, numbers of seeds per fruit and percent seed germination than hermaphrodite plants though variability does exist among populations and between sampling years. Additionally, female frequency and to a lesser extent gender-based density within populations influences the degree of difference between the two gender morphs in reproduction. Further study on environmental conditions (see Chapter 4) and pollinator behavior among *L. spicata* populations will allow more precise explanations of population dynamics and reproductive gender differences.

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TABLES AND FIGURES

TABLE 3.1 Female frequency and female and hermaphrodite density (plants per m²) in 2008 and 2009 for 11 *Lobelia spicata* populations

Site Code	Population	Female frequency (%)		Female density		Hermaphrodite density	
		2008	2009	2008	2009	2008	2009
BEA	Beaverville Prairie	25.5	25.7	0.07	0.09	0.20	0.27
BEL	Belmont Prairie	36.0	40.2	0.01	0.02	0.02	0.03
FD1	Falling Down Prairie Site #1	26.6	28.7	0.71	1.05	1.96	2.59
FD2	Falling Down Prairie Site #2	12.8	12.2	0.17	0.21	1.18	1.54
FUL	Fulton Prairie	31.6	33.8	0.04	0.04	0.09	0.07
LOD	Loda Cemetery Prairie	51.2	60.9	0.01	0.01	0.01	0.01
MID	Midwin National Tallgrass Prairie	35.1	38.3	0.01	0.01	0.02	0.02
PAX	Paxton Cemetery Prairie	63.4	53.6	0.36	0.62	0.21	0.54
PEL	Pelville Railroad Prairie	50.3	52.9	0.28	0.15	0.27	0.13
PRO	Prospect Cemetery Prairie	63.2	72.8	0.02	0.05	0.01	0.02
SOM	Somme Prairie	44.1	38.3	0.02	0.03	0.02	0.04

TABLE 3.2 Female advantage in fruit set, seed number per fruit and seed biomass in 2008 and 2009 for 11 *Lobelia spicata* populations

Site Code	Fruit set		Seed number per fruit		Seed biomass	
	2008	2009	2008	2009	2008	2009
BEA	1.21	1.05	2.21	1.54	0.71	1.11
BEL	1.21	1.48	0.80	1.41	1.18	1.15
FD1	1.21	1.79	1.57	1.32	0.82	1.28
FD2	1.09	1.41	1.76	3.17	0.78	1.05
FUL	1.12	1.08	1.64	2.28	0.89	1.20
LOD	1.16	1.20	0.92	1.12	1.08	0.98
MID	1.10	1.27	0.96	1.92	0.89	1.24
PAX	1.40	1.86	1.31	1.96	0.84	0.81
PEL	1.27	1.70	1.90	1.44	0.98	1.14
PRO	1.13	1.49	0.98	1.25	0.91	1.12
SOM	1.35	1.43	1.18	2.19	1.46	0.97

TABLE 3.3 Results of 3-Way ANOVAs examining the effects of gender, population and year on *Lobelia spicata* fruit set, seed number per fruit, seed biomass and percent seed germination

Variable	Source of variation	df	F	P
Fruit set	Gender	1	141.849	<0.001
	Population	10	14.381	<0.001
	Year	1	76.134	<0.001
	Gender × Population	10	2.532	0.005
	Gender × Year	1	5.495	0.019
	Population × Year	10	6.564	<0.001
	Gender × Population × Year	10	1.270	0.243
Seed number per fruit	Gender	1	58.535	<0.001
	Population	10	5.370	<0.001
	Year	1	3.547	0.060
	Gender × Population	10	2.516	0.006
	Gender × Year	1	6.542	0.011
	Population × Year	10	2.696	0.003
	Gender × Population × Year	10	1.519	0.129
Seed biomass	Gender	1	0.219	0.640
	Population	10	17.800	<0.001
	Year	1	49.299	<0.001
	Gender × Population	10	1.553	0.118
	Gender × Year	1	7.251	0.007
	Population × Year	10	3.431	<0.001
	Gender × Population × Year	10	2.858	0.002
Percent seed germination	Gender	1	5.093	0.025
	Population	10	83.458	<0.001
	Year	1	30.435	<0.001
	Gender × Population	10	3.583	<0.001
	Gender × Year	1	12.919	<0.001
	Population × Year	10	5.899	<0.001
	Gender × Population × Year	10	22.253	<0.001

TABLE 3.4 Pearson correlation coefficients among the four reproduction measurements examined for (A) female and (B) hermaphrodite plants in *Lobelia spicata* populations during summer 2008 and 2009. P < 0.10 designated by * and P < 0.05 by **.

(A)	Seed number per fruit		Seed biomass		Percent seed germination	
	2008	2009	2008	2009	2008	2009
Fruit set	0.019	0.096	-0.401	0.086	-0.073	-0.433
Seed number per fruit	---	---	-0.233	-0.507	-0.332	-0.572*
Seed biomass	---	---	---	---	0.630**	0.535*

(B)	Seed number per fruit		Seed biomass		Percent seed germination	
	2008	2009	2008	2009	2008	2009
Fruit set	0.244	-0.024	-0.265	-0.157	-0.250	-0.493
Seed number per fruit	---	---	-0.203	-0.307	0.255	-0.209
Seed biomass	---	---	---	---	0.389	0.638**

TABLE 3.5 Pearson correlation coefficients between female frequency, female density or hermaphrodite density and the eight gender-specific (F = female; H = hermaphrodite) reproduction measurements obtained in 2008 and 2009 for 11 *Lobelia spicata* populations. P < 0.10 designated by *, P < 0.05 by ** and P < 0.01 by ***.

Reproduction Measurements	Female Frequency		Female density		Hermaphrodite density	
	2008	2009	2008	2009	2008	2009
F Fruit set	-0.398	-0.750***	0.546*	0.475	0.706**	0.637**
H Fruit set	-0.663**	-0.655**	0.148	-0.149	0.600**	0.084
F Seed number per fruit	-0.662**	-0.598**	-0.001	-0.063	0.301	0.028
H Seed number per fruit	-0.179	-0.026	-0.535*	0.116	-0.197	-0.011
F Seed biomass	0.608**	0.180	-0.291	-0.092	-0.513	-0.071
H Seed biomass	0.462	0.330	-0.016	-0.018	-0.262	-0.168
F Percent seed germination	0.762***	0.650**	0.089	0.026	-0.224	-0.195
H Percent seed germination	0.534*	0.768***	-0.293	0.097	0.293?	-0.225

TABLE 3.6 Pearson correlation coefficients between female frequency, female density or hermaphrodite density and female advantage in four reproduction measurements obtained in 2008 and 2009 for 11 *Lobelia spicata* populations. $P < 0.05$ by **.

Female Advantage in:	Female frequency		Female density		Hermaphrodite density	
	2008	2009	2008	2009	2008	2009
Fruit set	0.475	0.230	0.323	0.697**	-0.099	0.454
Seed number per fruit	-0.462	-0.646**	0.349	-0.082	0.374	0.188
Seed biomass	0.302	-0.285	-0.367	0.052	-0.406	0.253
Percent seed germination	0.196	-0.415	0.212	-0.057	0.072	0.498

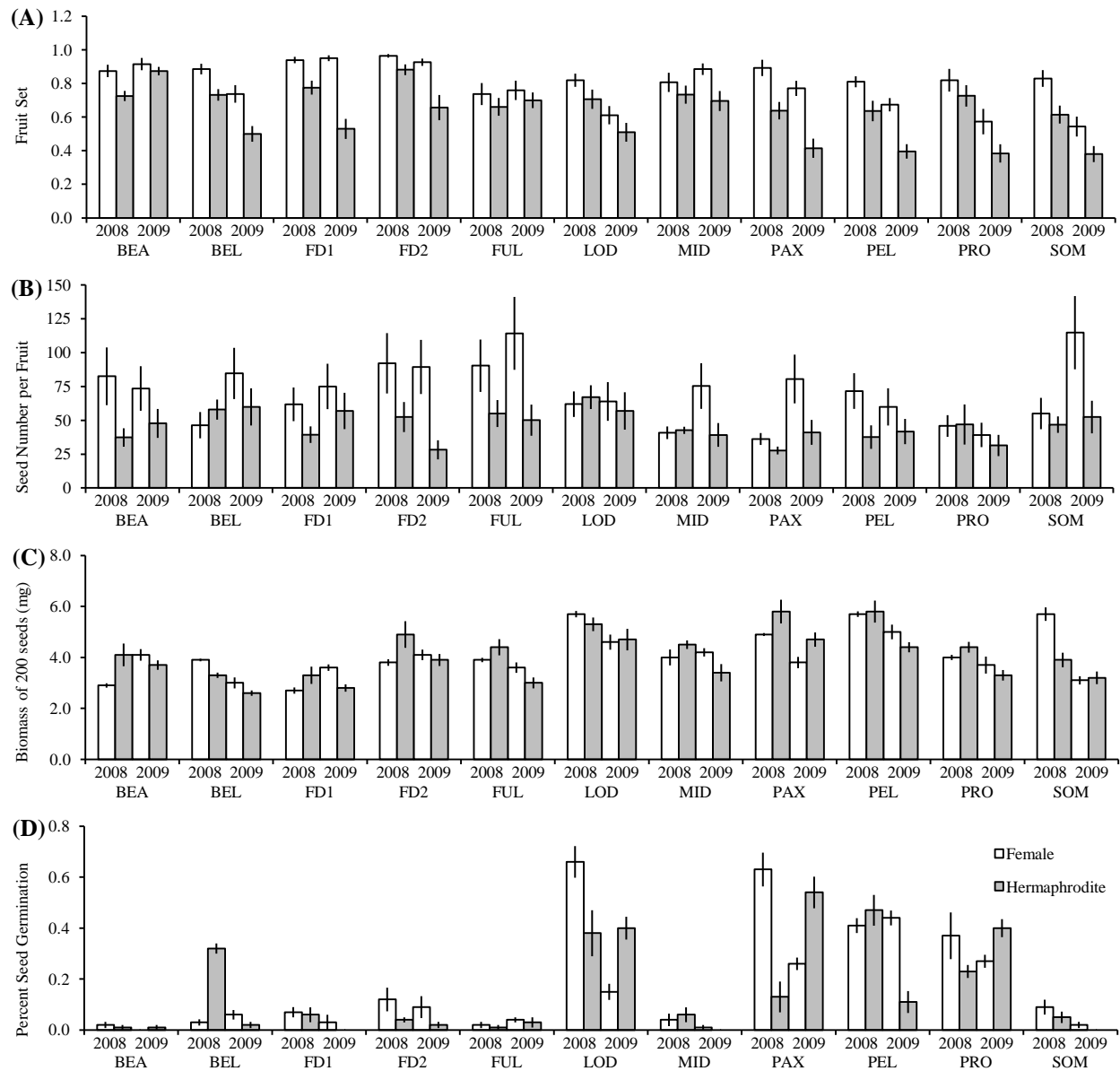


Figure 3.1. Mean reproductive output and fitness measurements (\pm SE) during summer 2008 and 2009 for 11 *Lobelia spicata* populations (refer to Table 3.1 for population abbreviations). (A) Fruit set, (B) Seed number per fruit, (C) Seed biomass for 200 seeds in milligrams and (D) Percent seed germination

**FEMALE FREQUENCY AND REPRODUCTIVE SUCCESS OF THE GYNODIOECIOUS PRAIRIE
SPECIES *Lobelia spicata* Lam. (CAMPANULACEAE) ACROSS A LATITUDINAL GRADIENT**

ABSTRACT

The high variability in female frequency observed for gynodioecious species has been linked to genetic, biotic and abiotic factors among populations and across a latitudinal gradient. This study focuses on assessing whether two abiotic factors, temperature and precipitation, are driving the relationship between female frequency and reproduction measurements across a latitudinal gradient for the gynodioecious prairie species *Lobelia spicata* Lam. (Campanulaceae). Data on latitude, longitude, mean temperature and mean precipitation from May to July (growing season), female frequency, female density, hermaphrodite density, reproductive output (i.e. fruit set, seed number per fruit, seed biomass) and fitness (i.e. percent seed germination), and female advantage in reproduction were collected for 11 populations across Illinois during the summer of 2008 and 2009. Female frequency within *L. spicata* populations was negatively correlated with latitude, such that there were higher percentages of females in southern than in northern populations. Temperature is likely the driving force behind the latitude/female frequency relationship because it negatively correlated with latitude and positively correlated with temperature across sampling years. However, less precipitation in southern populations during intermittent years may also account for some of the latitudinal variation in female frequency. Gender-based reproductive success measurements and female advantage did not correlate with geographic location or abiotic conditions with few exceptions. Both hermaphrodite and female seed biomass and percent seed germination were negatively correlated with latitude and positively correlated with temperature. Thus, stressful conditions such as high temperatures and low precipitation may somehow favor the success of female plants within populations of *L. spicata*, while high temperatures result in the production of larger seeds, better germinating seeds by hermaphrodite and female plants.

INTRODUCTION

Gynodioecy, or the classification assigned to all joint female and hermaphrodite plant breeding systems, has been documented in only 7% of angiosperms (Yampolsky and Yampolsky 1922). Unlike other breeding systems, gender dimorphism in gynodioecious systems occurs at the plant rather than flower level, such that there are separate female and hermaphrodite plants found within populations. Female plants within populations are unable to produce pollen necessitating their dependence on hermaphroditic pollen donation, and may suffer from lower pollinator visitation due to their lack of nectar incentives and smaller mean flower size (Asikainen and Mutikainen 2005). It has been suggested that female plants may compensate for these deficiencies by producing more flowers, larger fruit and seed sets, and larger, more quickly-germinating seeds than their hermaphroditic counterparts (Ashman 1992; Kohn 1989). The degree to which females' superior reproductive success aids in maintenance of

their gender is unclear, especially since the level of reproductive superiority may change according to the female frequency (Delph 1990b; McCauley and Brock 1998; Shykoff et al. 2003), female density or hermaphrodite density (Doust et al. 1987; Williams et al. 2000) of the given population (see Chapter 3).

Female frequency and gender morph density is often highly variable in gynodioecious populations (Alonso et al. 2007; Caruso and Case 2007; Koelewijn and Van Damme 1996; Landry et al. 2009; Webb 1999). Though the genetics behind gender inheritance in gynodioecious species (i.e. nucleo-cytoplasmic gene interactions) has been suggested to cause female frequency and gender morph density variation, ecological factors may also play a role. For instance, abiotic factors such as precipitation and temperature may promote the success of one gender morph over the other in different environments, thereby altering its abundance or density. Female plants have often been found to be reproductively superior to hermaphrodites when in stressful, resource poor environments (Barrett 1992; Costich 1995; Sakai and Weller 1991). One potential reason for this is differential resource allocation between the two genders. While hermaphrodites must use their resources to produce both pollen and fruit, females only need resources to produce fruit, making them less susceptible to resource limitation than hermaphrodites (Delph 1990a). As a result, female frequency is often higher in areas with low precipitation (Ashman 1999; Costich 1995; Cuevas et al. 2005; Wolfe and Shmida 1997). However, the definition of stressful conditions in regards to temperature has been found to vary depending on the life history of the gynodioecious species in question. Some species display a positive correlation between the percent of females in a population and temperature where warmer temperatures are more stressful and thus better suited to female plants (Alonso and Herrera 2001; Caruso and Case 2007; Vaughton and Ramsey 2004). Others display a strong negative relationship such that colder temperatures favor female success and/or superiority over hermaphrodites (Asikainen and Mutikainen 2003, Puterbaugh et al. 1997).

Abiotic factors such as temperature and precipitation are known to vary in predictable ways across the geographic landscape. Thus, predictions about female frequency within populations of a gynodioecious species could be made strictly by geographic location if the study populations are spread over a sufficient latitudinal or longitudinal range (Cuevas et al. 2008). A number of studies have been conducted on individual polymorphic species (e.g. *Lobelia siphilitica* [Caruso and Case 2007]) to examine the relationship between latitude and female frequency (Alonso et al. 2007; Van Rossum and Prentice 2004). The latitudinal ranges of these studies vary anywhere from 1.9° to 12° latitude and are mostly restricted to a particular country.

Lobelia spicata Lam. (Campanulaceae) is a gynodioecious prairie species (Molano-Flores 2002) that exhibits high variation in female frequency across populations (2-85%, Byers et al. 2005). Currently it is unknown if female frequency or gender morph density variation in the species is related to geographic location and/or abiotic conditions. Since female frequency and to some extent female and hermaphrodite plant density have been linked to reproductive success in both *L. spicata* gender morphs (see Chapter 3), it is necessary to determine what factors are most influential in determining these population dynamics. Thus, this study has three main objectives:

- (1) Examine the strength of the relationship between geographic location (i.e. latitude and longitude) and abiotic conditions (i.e. temperature and precipitation) in Illinois.

- (2) Determine if female frequency, female density or hermaphrodite density of *Lobelia spicata* populations are correlated to geographic location (i.e. latitude and longitude) and/or abiotic conditions (i.e. temperature and precipitation).
- (3) Assess whether *L. spicata* reproductive output and fitness measurements are correlated to abiotic conditions (i.e. temperature and precipitation).

METHODS

Study Species – *Lobelia spicata* is a short-lived, herbaceous perennial that is common in loess, sandy and loam prairies (Byers et al. 2005) throughout eastern North America (McVaugh 1936). Its terminal raceme inflorescence matures acropetally, with flowering and fruiting occurring from May to August (Swink and Wilhem 1994). Each inflorescence will contain 2 to 70+ zygomorphic flowers that are approximately 7 to 13mm long (Molano-Flores 2002). The principal pollinators of *L. spicata* are small bees, the most common being members of the *Augochlorella* genus (Molano-Flores 2002). Molano-Flores (2002) determined that this species is gynodioecious like another member of its genus (i.e. *Lobelia siphilitica*). Flowers on hermaphroditic plants are protandrous and self-compatible (Molano-Flores 2002). Few to over 200 tiny seeds are encased within *L. spicata*'s capsule fruits.

Data Collection – During the summer of 2008 and 2009, comprehensive searches were conducted to obtain total population size and female frequency for 11 *Lobelia spicata* populations across Illinois (Table 4.1). Female and hermaphrodite density were calculated for each population by dividing the number of female or hermaphrodite plants, respectively, by the area of each population in square meters using ArcMap 9.3.1 (ESRI 2011). Mean temperature and precipitation measurements during the growing season (May through July) were obtained for each population from the Illinois State Water Survey weather station in the closest proximity to the site (<http://www.isws.illinois.edu/data/climatedb/>). Twenty infructescences for each gender per population were collected and examined to obtain fruit set, seed number per fruit and seed biomass measurements (see Chapter 3). Percent seed germination was determined at 21.1°C with a 14 hour photoperiod for seed produced by female and hermaphrodite plants within each population. The level of female advantage (FA) in fruit set, seed number per fruit, seed biomass and seed germination was calculated for each population using Equation 1.

Equation 1 Female Advantage (FA) =
$$\frac{\text{Female Reproductive Measurement Mean}}{\text{Hermaphrodite Reproductive Measurement Mean}}$$

Statistical Analysis – Sigma Stat 3.1 (Systat Software, Inc.) was used to conduct all statistical analyses. A chi-square test was performed to determine if female frequency was independent of population and sampling year. Pearson correlations were used to detect relationships among latitude, longitude, mean temperature and mean precipitation (May to July), population size, female frequency, female density, hermaphrodite density, reproductive

output (i.e. fruit set, seed number per fruit, seed biomass) and fitness (i.e. percent seed germination) measurements and female advantage in reproduction.

RESULTS

Geographic Location and Abiotic Conditions - There was a 1.78° difference in latitude between the northernmost (Falling Down Prairie Site 2) and southernmost (Paxton Railroad Prairie) *Lobelia spicata* populations surveyed (Table 4.1). In May to June, mean temperature ranged from 18.37 to 21.02°C and from 18.09 to 20.74°C in 2008 and 2009, respectively, while mean precipitation ranged from 8.70 to 16.48 and from 9.29 to 15.72 centimeters in 2008 and 2009, respectively. Latitude had a strong negative correlation with temperature in both 2008 and 2009 ($r = -0.822$ and -0.896 , respectively, $P < 0.01$), but was not correlated with precipitation in 2008 or 2009 ($r = -0.453$ and -0.456 , respectively, $P > 0.10$). Longitude was not significantly correlated with temperature or precipitation in 2008 and 2009 (Pearson, $P > 0.10$). Temperature and precipitation exhibited a marginally significant positive correlation in 2008 ($r = 0.573$, $P < 0.10$), but not in 2009 ($r = 0.382$, $P > 0.10$).

Female Frequency and Gender Morph Density - The frequency of female plants in *L. spicata* populations ranged from 12.8 to 63.4% and from 12.2 to 72.8% in 2008 and 2009, respectively (Table 4.2). No significant change in female frequency within populations was found from 2008 to 2009 ($\chi^2 = 13.21$, $df = 10$, $P > 0.05$). The correlation coefficients between latitude, longitude, temperature or precipitation and female frequency, female density and hermaphrodite density are summarized in Table 4.3. Latitude was negatively correlated with female frequency in both 2008 and 2009 ($r = -0.764$ and -0.781 , respectively, $P < 0.01$), such that relatively higher proportions of females are found in southern as opposed to northern sites. Longitude had a marginally significant, negative correlation with female frequency in 2008 and 2009 ($r = -0.556$ and -0.559 , respectively, $P < 0.10$). Female frequency was positively correlated with temperature in both 2008 and 2009 ($r = 0.832$ and 0.791 , respectively, $P < 0.01$), but was not correlated to precipitation in either year. Female and hermaphrodite densities were not related to any of the geographic location or abiotic condition variables in 2008 or 2009.

Impact of Geographic Location and Abiotic conditions on Reproductive Output and Fitness – Geographic location (i.e. latitude and longitude) and abiotic conditions (i.e. temperature and precipitation) were not correlated with *L. spicata* hermaphrodite and female reproductive output with a few exceptions (Table 4.4). In 2008 and 2009, hermaphrodite seed biomass was negatively correlated with latitude ($r = -0.682$ and -0.693 , $P < 0.05$) and positively correlated with temperature ($r = 0.755$ and 0.738 , $P < 0.01$, respectively). Further, hermaphrodite percent seed germination was negatively correlated with latitude ($r = -0.770$, $P < 0.01$) and positively correlated with temperature ($r = 0.868$, $P < 0.01$) in 2009, but only marginally so in 2008 ($r = -0.575$, $P < 0.10$ and $r = 0.488$, $P > 0.10$, respectively).

Female seed biomass had a marginally significant, negative correlation with latitude in 2009 ($r = -0.579$, $P < 0.10$) as well as a marginally significant, positive correlation with temperature in 2008 and 2009 ($r = 0.592$ and

0.549, respectively, $P < 0.10$). In both 2008 and 2009 female percent seed germination was negatively correlated with latitude ($r = -0.780$ and -0.722 , respectively, $P < 0.05$) and positively correlated with temperature ($r = 0.895$ and 0.766 , respectively, $P < 0.01$). Additionally, female percent seed germination was positively correlated with precipitation in 2008 ($r = 0.664$, $P < 0.05$), and marginally so in 2009 ($r = 0.529$, $P < 0.10$).

Female advantage in the reproductive output and fitness measurements was not significantly correlated to latitude, longitude, temperature or precipitation in 2008 and 2009 with one exception (Table 4.4). Female advantage in number of seeds per fruit was positively correlated with longitude in 2009 ($r = 0.762$, $P < 0.01$), such that there was an increase in female advantage in number of seeds per fruit moving from east to west.

DISCUSSION

Female Frequency and Gender Morph Density - The results of this study support the assertion that female frequency in the gynodioecious prairie species *Lobelia spicata* varies along a latitudinal gradient. More specifically, female frequency in *L. spicata* populations was lower at northern as opposed to southern latitudes, and thus followed a similar pattern as a related species, *Lobelia siphilitica* L. (Caruso and Case 2007). Though only a small latitudinal range was examined in this study (1.78°), the observed patterns were very strong. Further, Miller and Stanton-Geddes (2007) found female frequency to be low in two Massachusetts *L. spicata* populations (12.3 and 12.4% females). Thus, it is expected that if the latitudinal range of this study were expanded, a negative correlation between latitude and female frequency would still be found. Female frequency also displayed a marginally significant, negative correlation with longitude across sampling years. This pattern is likely a byproduct of the uneven sampling of populations longitudinally across Illinois; the majority of *L. spicata* populations surveyed were located in central to eastern Illinois, while the two most northern populations (FD1 and FD2) were on the western side of the state.

Two abiotic factors that generally vary with latitude, namely temperature and precipitation, were put forth as possible explanatory factors for the observed gender ratio variation across latitudes. Mean precipitation from May to July did not decrease in the predicted fashion moving from north to south, however temperature varied such that southern sites experienced warmer temperatures during the growing season of *L. spicata* (May through July). In both 2008 and 2009 the percent of females in *L. spicata* populations displayed a strong positive correlation with temperature, indicating that the warmer temperatures of southern sites might promote the success and increase the relative amount of female plants in populations through increased photosynthate production. Of the two abiotic climate factors examined, temperature is likely driving the negative relationship between percent females and latitude since it had a strong positive relationship with gender ratio across sampling years, while precipitation did not. A number of other gynodioecious species display this trend including *Lobelia siphilitica* (Caruso and Case 2007), *Daphne laureola* L. (Alonso and Herrera 2001) and *Wurmbea biglandulosa* (R.Br.) T.D. Macfarl. (Vaughton and Ramsey 2004). Less precipitation in southern populations during intermittent years may also account for some of the latitudinal variation in female frequency within *Lobelia spicata* populations. Though the negative trends with

precipitation were not strong, the periodic stress caused by lack of precipitation may be enough to either promote female plant success or be detrimental to hermaphrodite survival and reproduction.

Female and hermaphrodite densities did not vary with geographic location, temperature or precipitation in *L. spicata* populations, suggesting that both gender morphs are not limited by these resources or are tolerant of some intraspecific competition. However, it must be noted that calculation of gender morph density at the population level may not provide an accurate representation of density conditions in *L. spicata* populations due to their patchy plant distribution. Further, female *L. spicata* plants have often been observed to grow in unisexual patches, whereas hermaphrodites will co-occur in patches with scattered female plants (Ruffatto, per. obs.). In this case microhabitat conditions (i.e. soil nutrients and moisture) within plant patches in addition to seed dispersal patterns may be more influential in determining gender morph density.

Impact of Geographic Location and Abiotic Conditions on Reproduction – In *L. spicata*, the relationships between geographical location, abiotic conditions and reproduction measurements of the two genders were almost non-existent except for seed biomass and percent seed germination. Further, the level of female advantage in the reproduction measurements also did not show a trend with geographic location or abiotic conditions with one exception. Female advantage in number of seeds per fruit did increase moving from east to west, possibly due to differences in resource availability or pollinator behavior. Overall though, mean temperature and mean precipitation during the growing season are not affecting reproductive output in *L. spicata*. The gender morphs may not be resource limited under these abiotic conditions or they could be able to withstand some intraspecific competition for resources without showing significant reductions in reproductive success.

However, hermaphrodite seed biomass was negatively correlated with latitude and positively correlated with temperature across sampling years, such that seed biomass increased moving from cooler, northern latitudes to warmer, southern latitudes. Female seed biomass also exhibited a negative trend with latitude and a marginally significant, positive correlation with temperature. These relationships are consistent with other studies (e.g. Dainese 2011) including a meta-analysis on latitude and seed biomass, where seed biomass was found to increase closer to the tropics (Moles and Westoby 2003). It may be that higher temperatures incur greater metabolic costs, necessitating larger, better provisioned seeds to ensure successful germination (Lord et al. 1997). Alternatively, higher temperatures may result in the production of more photosynthates by hermaphrodites and females, resulting in increased resource availability for the production of larger seeds (Murray et al. 2004). Hermaphrodite percent seed germination increased with decreasing latitude and increasing temperature in only 2008. Alternatively, female percent seed germination was negatively correlated with latitude and positively correlated with temperature in both sampling years. It is probable that latitude and temperature have an indirect effect on hermaphrodite and female seed germination through their impact on seed biomass, as heavier seeds often exhibit greater germination in *Lobelia spicata* (see Chapter 3) and other species (e.g. *Festuca hallii* (Vasey) Piper [Qiu et al. 2010]).

In conclusion, this study has shown that the percent of females in *Lobelia spicata* populations increases with decreasing latitude, presumably due to temperature. Further, though female advantage in reproduction does not vary

with latitude, production of larger seeds (particularly by hermaphroditic plants) does appear to be common among populations located in more southern, warmer latitudes.

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TABLES AND FIGURES

TABLE 4.1 Site code and geographic location (i.e. latitude and longitude) of the 11 *Lobelia spicata* populations used in this study. Arranged in order of decreasing latitude.

Site Code	Site	Latitude	Longitude
FD2	Falling Down Prairie Site #2	42.2017	-90.2063
FD1	Falling Down Prairie Site #1	42.2004	-90.2072
SOM	Somme Prairie	42.1403	-87.8297
FUL	Fulton Prairie	41.8460	-90.1010
BEL	Belmont Prairie	41.7990	-88.0470
MID	Midwin National Tallgrass Prairie	41.3550	-88.1574
BEA	Beaverville Prairie	40.9875	-87.5706
LOD	Loda Cemetery Prairie	40.5272	-88.0759
PEL	Pelville Railroad Prairie	40.4611	-87.9246
PRO	Prospect Cemetery Prairie	40.4447	-88.0973
PAX	Paxton Cemetery Prairie	40.4179	-88.1137

TABLE 4.2 Female frequency, female density (plants per m²) and hermaphrodite density (plants per m²) in 2008 and 2009 for 11 *Lobelia spicata* populations. Arranged in order of decreasing latitude.

Site Code	Female frequency		Female density		Hermaphrodite density	
	2008	2009	2008	2009	2008	2009
FD2	12.8	12.2	0.17	0.21	1.18	1.54
FD1	26.6	28.7	0.71	1.05	1.96	2.59
SOM	44.1	38.3	0.02	0.03	0.02	0.04
FUL	31.6	33.8	0.04	0.04	0.09	0.07
BEL	36.0	40.2	0.01	0.02	0.02	0.03
MID	35.1	38.3	0.01	0.01	0.02	0.02
BEA	25.5	25.7	0.07	0.09	0.20	0.27
LOD	51.2	60.9	0.01	0.01	0.01	0.01
PEL	50.3	52.9	0.28	0.15	0.27	0.13
PRO	63.2	72.8	0.02	0.05	0.01	0.02
PAX	63.4	53.6	0.36	0.62	0.21	0.54

TABLE 4.3 Pearson correlation coefficients between latitude, longitude, temperature or precipitation and female frequency, female density or hermaphrodite density of *Lobelia spicata* populations during summer 2008 and 2009. P < 0.10 designated by *, P < 0.05 by ** and P < 0.01 by ***.

	Latitude		Longitude		Temperature		Precipitation	
	2008	2009	2008	2009	2008	2009	2008	2009
Female frequency	-0.764***	-0.781***	-0.556*	-0.559*	0.832***	0.791***	0.306	0.353
Female density	0.139	0.167	0.023	-0.002	-0.018	-0.029	0.390	-0.135
Herm. density	0.489	0.486	0.424	0.411	-0.346	-0.321	0.309	-0.231

TABLE 4.4 Pearson correlation coefficients among latitude, longitude, temperature or precipitation and the eight gender-specific (F = Female; H = Hermaphrodite) reproductive output (i.e. fruit set, seed number per fruit, seed biomass) and fitness (i.e. percent seed germination) measurements of *Lobelia spicata* populations during summer 2008 and 2009. P < 0.10 designated by *, P < 0.05 by ** and P < 0.01 by ***.

	Latitude		Longitude		Temperature		Precipitation	
	2008	2009	2008	2009	2008	2009	2008	2009
F Fruit set	0.317	0.380	0.564*	0.373	-0.391	-0.487	0.383	-0.251
H Fruit set	0.397	0.260	0.741***	0.217	-0.464	-0.408	0.187	-0.264
F Seeds/fruit	0.332	0.711**	0.497	0.149	-0.238	-0.580	0.102	-0.305
H Seeds/fruit	0.235	0.242	0.176	-0.563*	-0.096	-0.283	-0.069	0.318
F Seed biomass	-0.397	-0.579*	-0.144	0.120	0.592*	0.549*	0.009	0.579*
H Seed biomass	-0.682**	-0.693**	0.151	0.138	0.755***	0.738***	0.377	0.531*
F Per. seed germ.	-0.780***	-0.722**	-0.133	-0.076	0.895***	0.766***	0.664**	0.529*
H Per. seed germ.	-0.575*	-0.770***	-0.238	-0.190	0.488	0.868***	0.259	0.258