REPRODUCTIVE ECOLOGY AND POPULATION GENETICS OF *BESSEYA BULLII*, A RARE SPECIES

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Project Objective(s) as Described in Application:

(1) Survey populations of *Besseya bullii* in Illinois
(2) Observe pollinator visitation to *B. bullii* plants
(3) Determine if there are fitness differences between selfed vs. outcrossed seeds
(4) Collect plant material for a population genetic study of this species

Completed Project Description:

Besseya bullii (Eaton) Rydb. (Plantaginaceae), commonly called kittentails or bull's coraldrops, is a plant species endemic to the Midwestern United States, found in savannahs, open woods, and gravel/sand prairies. Urbanization and agriculture has restricted populations of *B. bullii* to small, highly isolated remnant habitats where landscape factors could restrict pollinator movement and gene flow, potentially affecting the reproductive output of this species. In Summer 2008, over 20 populations were visited according to state records of occurrence. At each site, data was collected on population size, habitat characteristics, and the presence of pollinators. From these populations, 8 were selected for a study on the reproductive ecology and population genetics of the species. At each population, 20 infructescences were collected to determine fruit/seed set and seed germination. In addition to these measures of reproductive success and fitness, a hand-pollination study was conducted at one site, the Lost Mound Field Station, to determine potential fitness differences between selfed and outcrossed seeds. Results indicate that there is a significant relationship between fruit set and population size, and a weak relationship between seed set and population size. There were also significant differences in seed germination among sites. The hand-pollination study yielded poor results, but provided insight into improving protocols for future studies. This study demonstrates that there are potentially consequences of small population size on the ability of *B. bullii* to successfully reproduce.

Summary of the Project Accomplishments: (See attachment)

Budget: (See attachment)

Reproductive Ecology and Population Genetics of *Besseya bullii*, a Rare Species

INTRODUCTION

Current land use practices frequently alter or diminish the available habitat for native species, often leaving remnant populations small and highly isolated. Although these remnant populations seem secure in protected areas, they may eventually suffer declines due to a combination of small population size, low genetic diversity, and inability to outcross (Oostermeijer et al. 2003). Habitat fragmentation is expected to have severe consequences on the reproductive ecology of plant species, limiting the ability of pollen or seeds to disperse between populations (Trapnell et al. 2004). Reduction in gene flow is predicted to result in two primary concerns: 1) inbreeding depression and genetic degradation, which could ultimately inhibit successful recruitment in an already rare species (Young et al. 1996), and 2) inability of species to adapt to stress and disturbances, a special concern now that few species can escape such factors as global climate change and habitat degradation (Heard et al. 1999).

While studies of rare species are useful for constructing management plans, conservation studies with an emphasis on these effects of fragmentation and small population size can aid in determining the viability of remnant populations. For my study species, I chose *Besseya bullii* (Eaton) A. Rydberg (Family Plantaginaceae), a rare plant species endemic to the Midwestern United States. This species is characterized by a large basal rosette and a spiked inflorescence (Gleason et al. 1991). The flowers of *B. bullii* are protogynous (i.e., stigmas are receptive before pollen sheds), and the inflorescence flowers acropetally (i.e., maturing from the base towards the top) (McKone et al. 1995).

Currently *B. bullii* is classified as a species of concern in all states within its range: extirpated in Ohio, imperiled in Minnesota, endangered in Michigan and Indiana, and threatened in Illinois, Wisconsin, and Iowa (NatureServe 2007); it was also a candidate for federal listing as an endangered species in the 1980s (Fish and Wildlife Service 1985). Since its removal from the candidate list in 1993, likely because of changes to the C2 designation under the Endangered Species Act (McKenzie et al. 2009), little research has been conducted on this species. Prior to this study, the most recent surveys of many populations were over 20 years old. Additionally, most of the studies involving *B. bullii* have occurred in Minnesota, leaving other populations unexplored.

During the summers of 2008 and 2009, I conducted a preliminary study on the reproductive ecology and population genetics of *B. bullii*, with specific interests on potential impacts of small population size and habitat fragmentation on the species.

Although some research has been conducted on this species (McKone et al. 1995, Cholewa 1991), much is still unknown regarding its reproductive ecology and population genetics, which could be contributing to its current rarity. Moreover, assessing the potential impacts of small population size and habitat fragmentation on *B. bullii* could better inform future management for this species.

METHODS

For my project in 2008 and 2009, I had 5 research objectives: (1) conduct surveys of Illinois populations of *B. bullii* with the intention of updating state records, (2) monitor populations of the species and record pollinator visitation, (3) determine if fitness differences exist between seeds produced from self-pollen versus outcross-pollen, (4) collect leaf tissue from several populations of *B. bullii* and determine through preliminary laboratory research if a full population genetics study of the species is viable, and (5) assess differences in reproductive success and fitness between populations of *B. bullii*.

Population Surveys: Historic populations of *B. bullii* were surveyed using occurrence records retrieved from the Illinois Natural Heritage Database. Additionally, some populations without previous state record were surveyed according to anecdotal accounts from land managers and other scientists. Sites were surveyed for plants by visual search. At each population, data was gathered on total number of individuals, the number of flowering vs. non-flowering individuals, and the number of inflorescences produced by flowering individuals. Local habitat conditions and current management strategies were also noted.

Pollinator Observations: At each site, plants were monitored to determine the primary pollinators of *B. bullii*. Each cluster of plants was watched for 5 minutes to detect arrival of pollinators. Observations were recorded on any pollinator that made contact with flowers.

Fitness Differences Between Selfed and Outcrossed Seeds: A hand-pollination study was conducted using a population at the Lost Mound Field Station near Savanna, IL. Pollen for the outcross treatment was collected from another population at the Mississippi Palisades State Park, approximately 10 miles from the study site, and transferred to the Lost Mound population. At the site, 20 plants were randomly selected for both an outcross and selfing treatment. Pollen for both treatments was applied using a cotton swab to rub collected pollen against newly-emerged stigmas. Flowers in an inflorescence that received either self or outcross pollen were appropriately tagged with colored string for later identification. Inflorescences were then bagged with bridal veil tied at the base of the inflorescence to prevent contamination during the hand-cross study.

Population Genetics: Leaf samples were collected from each of 20 individuals at 8 sites for a preliminary population genetics study. Samples were dried and stored in coin envelopes before being transported back to the laboratory. Leaves were ground manually and DNA extracted according to protocols for the Qiagen DNeasy Plant Mini Kit. We then tested for polymorphism at 8 loci using cross-species amplification of microsatellite primers developed for *Collinsia verna* (Plantaginaceae). Primers that amplified visibly on gels and displayed polymorphism for those loci were then noted.

Reproductive Success and Fitness: In addition to the original objectives stated in the proposal, reproductive success (e.g., fruit set and seed set) and fitness (i.e., seed germination) were measured for 8 Illinois populations. Infructescences from 20 plants were randomly collected at each population and taken to the laboratory to assess fruit set and seed set. In addition, from each of these populations, seeds were collected and grown in a greenhouse under 3 treatments (greenhouse with no cold stratification, growth chamber with no cold stratification, and growth chamber with cold stratification) to test for germination.

PRELIMINARY RESULTS

Population Surveys

In 2008, I searched for populations of *B. bullii* across Illinois using state records and anecdotal information. Of the 24 populations visited, 11 populations were found at 8 sites, including 5 new populations without previous state records (Table 1). Although some new populations were found, 9 historic populations could not be located, and an additional 3 populations are presumed extirpated (Table 1). Populations were visited again in 2009 to monitor any changes to the sites.

Pollinator Observations

During 2008 and 2009, sites were visited to conduct pollinator observations. Unfortunately, weather conditions were not favorable (i.e., too windy, overcast) for pollinator activity either year when we visited the sites. In April 2009, some bees were observed visiting *B. bullii* (Figure 1), though their effectiveness was questionable as they seemed sluggish from the cold weather.

Fitness Differences Between Selfed and Outcrossed Seeds

Attempts to hand-pollinate and bag inflorescences in May 2009 yielded poor results. After inflorescences were pollinated, tagged, and then bagged, they were visited again the following week. The inflorescence was discovered to be an extremely sensitive structure, as every bagged individual dropped its inflorescence. No data could be collected.

Population Genetics

Preliminary work has been conducted in the laboratory to determine if microsatellite markers developed for *Collinsia verna* (Plantaginaceae) could be cross-amplified and used for a study on genetic diversity in *S. bullii*. Of the markers tested from *C. verna*, five appeared to be polymorphic and capable of being used in future studies (Table 2).

Reproductive Success and Fitness:

At 10 sites in Illinois, I collected infructescences to measure fruit/seed set. Analysis of data in 2008 showed that fruit set differed significantly among sites (Figure 2, $F=10.309$, $p<0.001$), but fruit set was not significantly correlated with population size (Figure 3, $R^2=0.60$, $p=0.11$). Seed set also differed significantly among sites (Figure 2, $F=7.14$, $p<0.001$), and a significant positive correlation was found between seed set and population size (Figure 3, $R^2=0.90$, $p=0.03$).

Seeds were gathered from the same 10 sites in Illinois to test germination viability in the greenhouse and growth chamber. This study involved germinating *S. bullii* seeds using the following treatments: 1) greenhouse without stratification, 2) growth chamber without cold stratification, and 3) growth chamber with cold stratification. Seed germination was significantly different among treatments (Figure 4, $F=197.977$, $p<0.001$). Seed germination was significantly lower in the greenhouse treatment (0.265 ± 0.02) compared to the cold stratification treatment (0.785 ± 0.02) and the no cold stratification treatment (0.748 ± 0.02). Also, significant differences were found for seed germination among sites (Figure 4, $F=2.364$, $p=0.028$). Seed germination was significantly higher at the Nachusa Kit site (0.690 ± 0.03) compared to the Palisades Oak site (0.524 ± 0.03). No other comparisons were significant among sites.

PRESENTATION / PUBLICATION OF RESULTS

Preliminary results have been given by oral presentation at the 101st Annual Meeting of the Illinois State Academy of Science at Southern Illinois University. These results have also been presented in poster form at the Chicago Botanic Gardens' "Global Plant Conservation Science & Outreach Symposium" and the "Graduate Student Symposium" for University of Illinois's Graduate Student Organization in Ecology, Evolution, and Conservation.

In agreement with receiving special use permits for conducting research at the Lost Mound Field Station and Nachusa Grasslands, annual reports have also been submitted to the Fish and Wildlife Service and The Nature Conservancy (respectively).

FUTURE WORK

Using protocols developed during the previous 2 years, surveys of populations and reproductive/fitness data will be collected for an additional 3 years in Illinois. Because of the lack of success in pollinator observations and cross-pollination, the protocols for these studies will be re-evaluated and adjusted for the future. Pollinator observations will need to be performed only on sunny days with minimal wind. Also, a different design will be developed for bagging individuals in the cross-pollination study to prevent unintended loss of inflorescences.

For a complete assessment of the current genetic diversity among populations, additional leaf samples will be required from study sites. In addition to this research on current genetic diversity, historic genetic diversity will also be evaluated using herbarium specimens to determine if diversity has been lost in populations since the time of fragmentation. Herbaria specimens of *B. bullii* will be located with the assistance of herbarium curators and online searches of herbarium records available through both universities and botanical gardens.

Additionally, several parts of the project, including population surveys, reproductive success/fitness, and population genetics, will be expanded to incorporate populations across the range of the species. Currently, records of occurrence for *B. bullii* have been obtained from Michigan, Indiana, and Wisconsin, and permits are being requested from these states.

CITATIONS

Cholewa, AF. 1991. *Besseya bullii*: Breeding systems, pollinators, and preliminary genetic analyses. Unpublished report and data, The Nature Conservancy.

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Trapnell, DW, JL Hamrick. 2004. Partitioning nuclear and chloroplast variation at multiple spatial scales in the neotropical epiphytic orchid, *Laelia rubescens*. *Molecular Ecology* 13: 2655-2666.

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Table 1. Populations known to have occurred in Illinois based on records from the Illinois Natural Heritage Database.

Population ID	Description	Historic Size (Date)	Population Size (2008)	Population Size (2009)
Hanover Bluff Nature Preserve	River bluff	"Small population" (2001)	Not found	Not found
Silver Creek Prairie	Gravel prairie	"Small population" (1985)	Extirpated	Extirpated
Colored Sands Nature Preserve	Sand prairie	>8 (1995)	Not found	Not found
Sugar River Forest Preserve	Sand forest	>10 (1985)	Not found	Not found
Grand Detour Botanical Area	River bluff	>500 (1991)	Not found	Not found
Camp Ralston Girl Scout Camp	Sand savanna	"Locally dense" (1985)	Extirpated	Extirpated
Castle Rock State Park	Sand prairie	8 (1995)	Not found	Not found
Devil's Backbone	Sand prairie	36 (1993)	Not found	Not found
Lowden-Miller Forest	Sand forest	12 (1994)	Not found	Not found
Manito Prairie Nature Preserve	Gravel prairie	31 (2001)	Not found	Not found
Beloit Gravel Prairie	Gravel prairie	"Large population" (1999)	Extirpated	Extirpated
Lost Mound F-105	Sand prairie	Not surveyed	Not surveyed	1893
LoMo Mound Rail	Sand prairie	>100 (1998)	762	1407
Nachusa Kittentail	Oak savanna	924 (1992)	1000	1485
Nachusa Tellabs	Oak savanna	Not surveyed	389	96
Harlem Hills	Gravel prairie	Not surveyed	209	431
Fulton	Cedar forest	Not surveyed	472	972
Palisades Mush	Cedar forest	Not surveyed	635	350
Palisades Ozzy	Cedar forest	94 (1992)	40	68
Palisades Oak	Cedar forest	87 (1992)	451	357
BigRiver State Park	Sand forest	>5 (1985)	Not surveyed	30
Shirland RR W	Sand prairie	70 (1993)	Not surveyed	19
Shirland RR H	Sand prairie	Not surveyed	Not surveyed	10
Kinn Creek	Oak savanna	Not surveyed	Not surveyed	170
Daysville Cementary	Sand prairie	789 (1994)	Not surveyed	149

Table 2. Microsatellite primers that have been cross-amplified with *B. bullii*.

Primer ID	PCR Results
Cove A107	Amplified at all temperatures; appears polymorphic
Cove A119	Bright bands for 3 temperatures; clearly polymorphic
Cove A125	Requires standardization for clearer results
Cove A134	Amplified at all temperatures; not certain if polymorphic
Cove B2	Requires standardization for clearer results
Cove B105	Amplified at almost all temperatures; polymorphic
Cove B116	Amplified at all temperatures; not certain if polymorphic
Cove C8	Amplified at all temperatures; not certain if polymorphic

Figure 1. Bees observed visiting flowers of *B. bullii* at the Nachusa Grasslands.

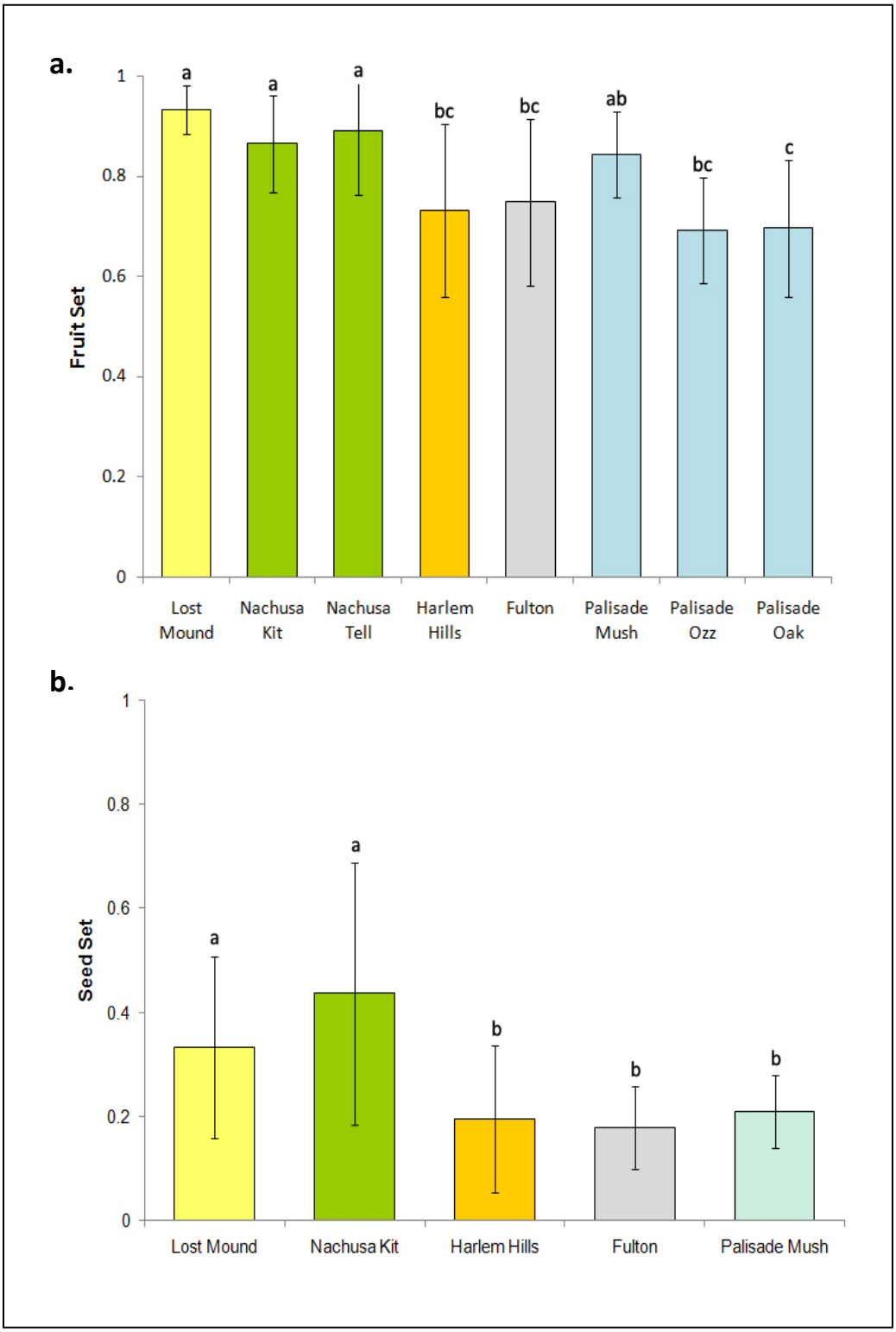

Figure 2. (a) Differences in fruit set among 8 populations ($F=10.309$, $p<0.001$). (b) Differences in seed set among 5 populations (Figure 2, $F=7.14$, $p<0.001$

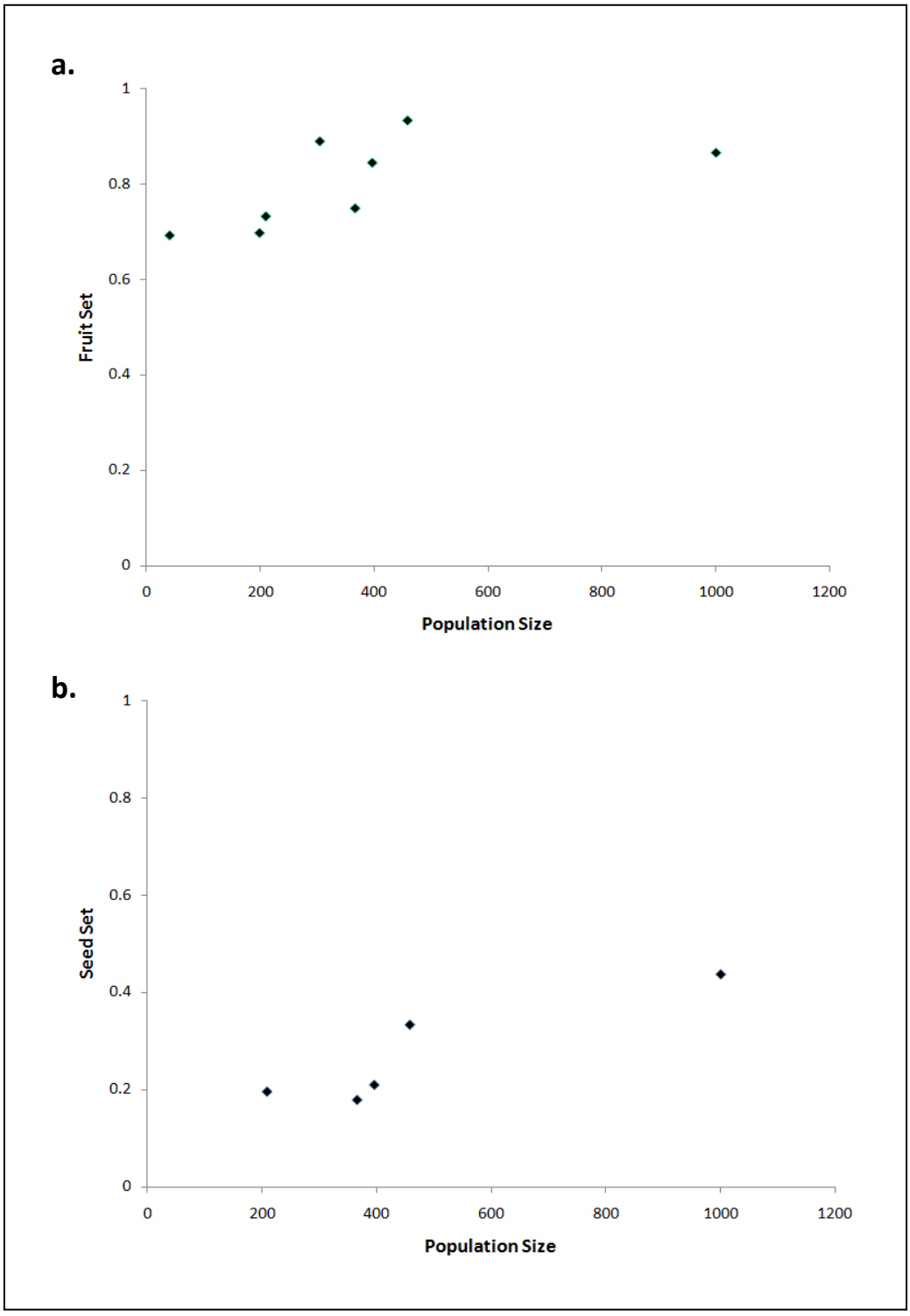

Figure 3. (a) Fruit set as a function of population size for 8 populations (R^2=0.60, p=0.11). (b) Seeds set as a function of population size for 5 populations (R^2=0.90, p=0.03).

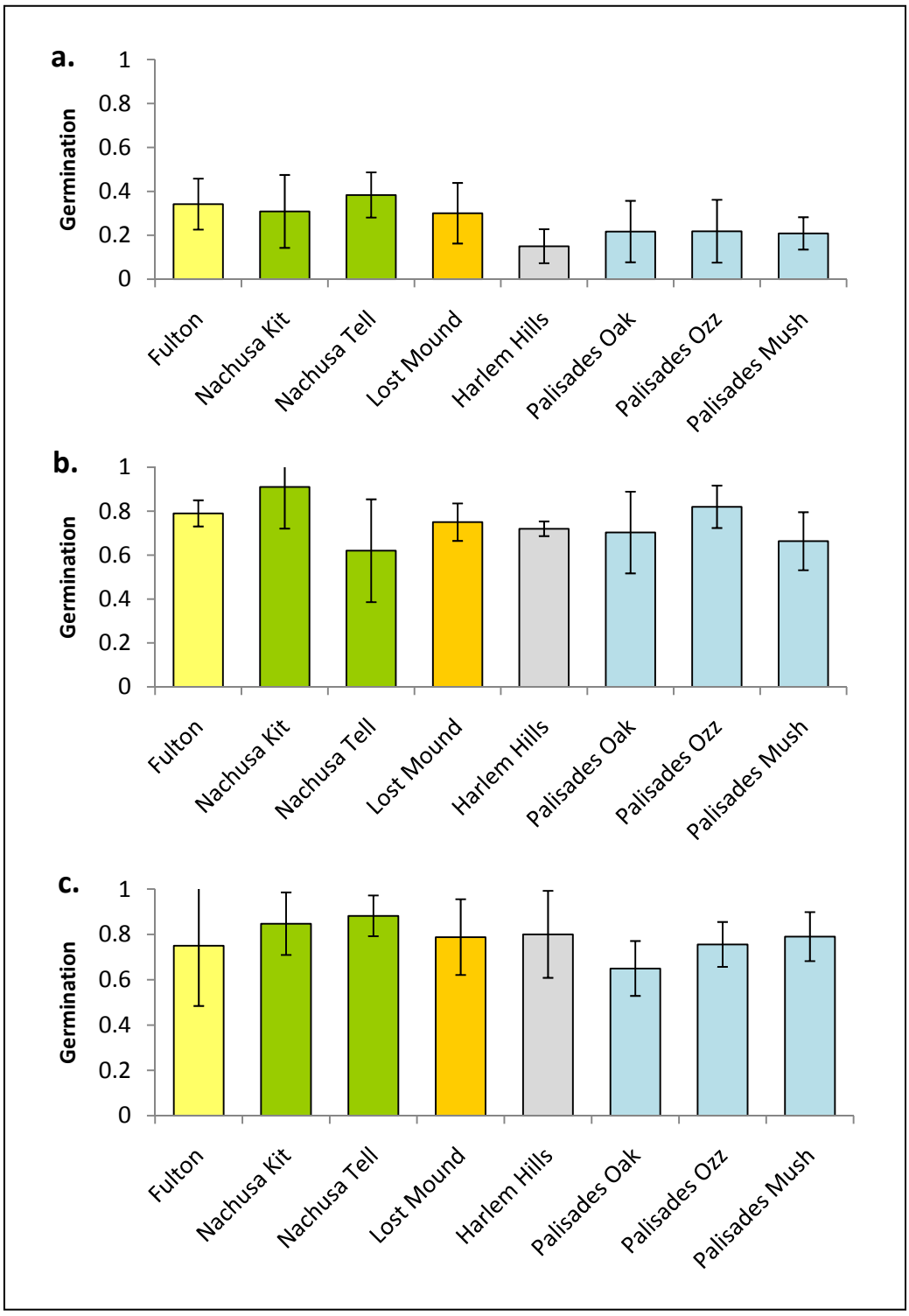

Figure 4. Seed germination for (a) greenhouse treatment, no cold stratification, (b) growth chamber treatment, no cold stratification, and (c) growth chamber treatment, cold stratification.