2010

Assessing Genetic Differentiation Among Populations of the Invasive Plant *Impatiens glandulifera* in Maine

Jordan R. Schoonover
Colby College

Assessing Genetic Differentiation Among Populations of the Invasive Plant *Impatiens glandulifera* in Maine

An Honors Thesis

Presented to

The Faculty of The Department of Biology

Colby College

in partial fulfillment of the requirements for the

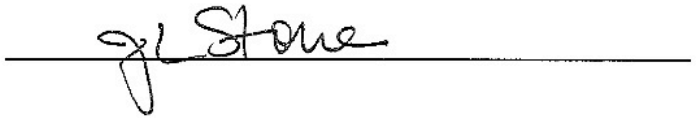
Degree of Bachelor of Arts with Honors

by

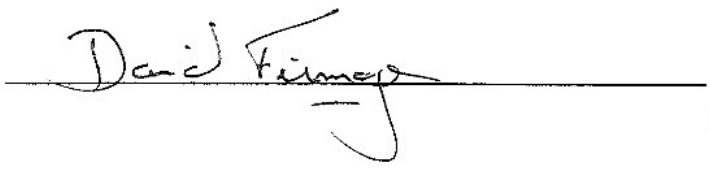
Jordan R. Schoonover

Waterville, Maine

May 18, 2010

Advisor: Judy L. Stone 

Reader: F. Russell Cole 

Reader: David H. Firmage 

Abstract

The annual herbaceous plant *Impatiens glandulifera* Royle is native to the Himalayas and is a significant invasive species in Europe. In the past century, it was introduced to the United States, where it has become established in 12 states. This study evaluated genetic differentiation among four Maine populations, to address a theory that posits hybridization of distinct lineages as a trigger for invasiveness. Regions of microsatellite repeats were evaluated at two polymorphic loci for 41 plants sampled from the four populations. A striking finding was that the observed heterozygosity was substantially higher than the heterozygosity expected from random combination of alleles. Our data suggest that the populations have already hybridized, either in North America or elsewhere in the introduced range, resulting in inflated heterozygosity due to hybrid vigor or fixed heterozygosity. We expect that the lag time following introduction is at an end and that *I. glandulifera* will soon become invasive and undergo significant expansion in the eastern United States.

Introduction

An invasive plant is one that is non-native to the ecosystem under consideration and that spreads rapidly, replacing native plants (USDA 2010b). Invasive plants are commonly introduced to a new habitat by human activity. The introduction may be intentional, such as for horticulture, or unintentional, such as when seeds are transported along with soil. The presence of an invasive plant usually has negative effects on the ecosystem. The invasive plant can compete with native plants for space and resources and alter the biotic and abiotic components of the entire ecosystem (Weidenhamer and Callaway 2010). The significant environmental and economic costs of invasive plants make them an important and timely topic of study.

The Evolution of Invasiveness

Many theories have been proposed to explain why certain introduced species establish and become invasive and others do not. In order to become established, an introduced species must form a self-sustaining population (Sakai et al. 2001). Superior competitive ability and high intrinsic growth rate are two characteristics that favor successful establishment (Sakai et al. 2001). Hayes and Barry (2008) reviewed 49 studies on the establishment of various invasive species and found that climate/habitat match between the native and the introduced range and a high number of arriving individuals consistently predicted a successful transition from introduction to establishment. For plants, length of juvenile period, ability to reproduce vegetatively, and length of flowering period also predicted successful establishment.

After the establishment of an introduced species, there is usually a lag time before the species begins to spread and is classified as invasive. This lag time has been attributed to the exponential growth process of an initially small population, the random extinction of propagules, or an evolutionary effect (Sakai et al. 2001, Suarez and Tsutsui 2008). An initially small population would expand slowly at first and then more rapidly as the number of individuals increased. This process could be hindered by the loss of some propagules to random extinction events. An established population may also require time to evolve and optimize the phenotype to the new environment before it becomes invasive. Ellstrand and Schierenbeck (2000) proposed one way in which this evolution could occur. They hypothesize that hybridization between species or between individuals of the same species from disparate source populations might lead to invasiveness.

Hybridization could stimulate invasiveness in four possible scenarios: 1) when genetic recombination creates a genetically distinct species that is more fit than either parent, 2) when hybridization increases genetic variation and thus fitness and resiliency, 3) when hybridization leads to fixed heterozygosity which is an advantage, or 4) when the integration of new alleles dilutes detrimental alleles that were prevalent in a previously isolated population (Ellstrand and Schierenbeck 2000). These four scenarios are not mutually exclusive. Ellstrand and Schierenbeck (2009) found 35 examples from 16 plant families in which hybridization preceded invasiveness. Most of these examples were herbaceous perennials because their life history traits often coincide with frequent, spontaneous hybridization (Ellstrand and Schierenbeck 2009).

In the past decade, there has been increased interest in and research in support of Ellstrand and Schierenbeck's hypothesis (Sakai et al. 2001, Lee 2002, Prentis et al. 2008, Suarez and Tsutsui 2008, Whitney and Gabler 2008, Ayres et al. 2009, Schierenbeck and Ellstrand 2009). Durka et al. (2005) found molecular evidence for multiple introductions of garlic mustard (*Alliaria petiolata*) into the United States and suggested that the genetic diversity resulting from multiple introductions contributed to the success of the invasion. Rieseberg et al. (2005) found that hybridization with a locally adapted species facilitated the invasion of Texas by the common sunflower (*Helianthus annuus*). They also found evidence that transgressive phenotypes generated by hybridization allowed three other sunflower species to colonize new habitats.

Experimental Design and Objectives

The goal of this study was to investigate genetic relatedness among populations of *Impatiens glandulifera* in Maine to determine whether the populations are the result of a single or of multiple introductions, whether they have the potential to hybridize and become increasingly invasive, or whether hybridization has already occurred. Microsatellite variation in three polymorphic loci, developed by Provan et al. (2006), was analyzed for four populations. If populations of *I. glandulifera* prove to be genetically distinct, then we can hypothesize that they resulted from the introduction of different lineages and may have the potential to hybridize in the future. Following Ellstrand and Schierenbeck (2000, 2009), this hybridization could result in increased invasiveness of *I.*

glandulifera in Maine and the spread of the plant far beyond its current, restricted range.

Description of Species

Habitat

Impatiens glandulifera Royle (synonym: *Impatiens roylei* Walp.) is an annual herbaceous plant commonly known as Himalayan balsam. Other common names include policeman's helmet, jewelweed, and touch-me-not. *I. glandulifera* is native to the Himalayan regions of India and Pakistan (Beerling and Perrins 1993). This species has been introduced to New Zealand, Canada, 18 countries in Europe, and the United States, where it is found in Alaska, California, Oregon, Washington, Idaho, Montana, Michigan, New York, Connecticut, Vermont, Massachusetts, and Maine (Beerling and Perrins 1993, Clements et al. 2007). In Maine, *I. glandulifera* is most prevalent along the coast, though it is also found in isolated inland populations (Tabak and von Wettberg 2008).

Beerling (1993) showed that the length of the growing season limits the northern distribution of *I. glandulifera* in Europe, with a current limit of 64° N latitude. The species is also constrained by its frost sensitivity and high soil moisture requirement (Beerling and Perrins 1993). *I. glandulifera* tolerates a variety of soil textures and nutrient levels and can grow in soils with pH ranging from 3.5 to 7.5 (Beerling and Perrins 1993). *I. glandulifera* usually requires disturbance to become established. The most common communities colonized by *I. glandulifera* are inundation communities, fens, mesotrophic grasslands, and

woodlands (Beerling and Perrins 1993). In addition to these communities, it is often found along roadsides, in ditches, and in vacant lots.

I. glandulifera most frequently grows in dense, monotypic stands, which result from the synchronous germination of seeds in the spring, though individual plants can be found growing alone or in stands of three or four in drier habitats (Beerling and Perrins 1993, Clements et al. 2007). This species is shade tolerant, though growth is adversely affected when the amount of light is less than 30% of full daylight (Beerling and Perrins 1993). Andrews et al. (2005) concluded that accumulation of NO_3^- in the stems allows *I. glandulifera* to achieve heights of up to 3 m in low-sunlight habitats such as below developing canopies. *I. glandulifera* is sensitive to drought (Beerling and Perrins 1993). In Europe, it is also sensitive to frost, though in the Himalayas the species grows up to altitudes of 4000 m and is frost tolerant (Beerling and Perrins 1993). Kollmann and Banuelos (2004) found that aboveground biomass, height, and time until flowering were all lower for northern European populations when plants were grown in a common garden, indicating local adaptation.

Morphology and Growth

Growth is directly proportional to available water in the soil (Beerling and Perrins 1993). Germination occurs in February or March, the cotyledon stage lasts until early to mid-April, and plants flower from July to October (Beerling and Perrins 1993). From germination to flowering takes 13 weeks, and plants usually flower for 12 weeks (Beerling and Perrins 1993). Adventitious roots growing from the base of the stem anchor the plant in the soil, particularly in sloping terrain (Beerling and

Perrins 1993, Ennos et al. 1993). No mycorrhiza has been recorded for this species (Clements et al. 2007). Stomata are present in similar quantities on both sides of the leaf (Beerling and Perrins 1993). Leaves are opposite or in whorls of three, lanceolate, and sharply serrated (Clements et al. 2007).

Reproduction

Flower color ranges from white to pink to purple (Clements et al. 2007). Flowers are protandrous and self-compatible (Beerling and Perrins 1993). The bilaterally symmetrical flowers are bell-shaped (Figure 1; Clements et al. 2007). This flower shape is ideal for pollination by bees, which are completely enclosed by the flower, bringing their backs in contact with the stamens and stigmas (Figure 1; Beerling and Perrins 1993). Nienhuis and Stout (2009) found that bumblebees are effective pollinators of *I. glandulifera* in England, depositing enough pollen in one visit for 100% seed set. The morphology of the flower contributes to its high pollination efficiency (Titze 2000). *I. glandulifera* attracts many pollinators in its introduced habitat due to the abundance of high-sugar nectar it produces. It is a particularly important food source in late fall when it is one of the few nectar sources available (Titze 2000).

Seed production per plant is inversely related to plant density. Salisbury (1961) found that plants growing in Britain at a density of 20 plants/m^2 produced 700-800 seeds per plant in 140 pods with 5-7 seeds/pod. Beerling (1990) recorded 500 seeds/plant in 95 pods with 3-5 seeds/pod at densities of 36 plants/m^2 in South Wales. Seeds are dispersed over short distances by the explosive dehiscence of the seed capsule, which can disperse seeds up to 5 m from the plant (Beerling and

Figure 1. *Impatiens glandulifera* flower with a bee inside.

Perrins 1993). Seeds are dispersed over long distances by flowing water or human transport. The spherical seeds are 4-7 mm in diameter, and they transition in color from green to brown to black at maturity. In experiments with one English population, Willis and Hulme (2004) found that seed mass increased with increasing resource limitation and at higher elevations. Seeds can persist in the seed bank for at least 18 months and can remain viable for three years when fully imbibed (Beerling and Perrins 1993). *I. glandulifera* seeds require a period of chilling to break dormancy (Mumford 1988). As the seed ages, the period of chilling

needed for dormancy break lessens (Mumford 1988). *I. glandulifera* is not capable of vegetative reproduction (Beerling and Perrins 1993).

Herbivory and Disease

Sheep and cattle graze on the leaves, stems, and flowers of *I. glandulifera* (Beerling and Perrins 1993). A few insects have been identified that feed on the plant, including two species of aphid and the larvae of the elephant hawk moth *Deilephila elpenor* (Beerling and Perrins 1993). Some species of epiphytic yeasts and rusts have been found on *I. glandulifera* in Europe. No bacteria have been recorded (Beerling and Perrins 1993). Kollmann et al. (2007) offer the first report of a viral infection of *I. glandulifera* from their common garden experiment in Europe. The infection significantly reduced aboveground biomass, but did not affect reproductive traits. The absence of reported viral infection previous to this study could mean that *I. glandulifera* has been released from the diseases that affected it in its native habitat, and this could contribute to its invasiveness. This virus may be newly introduced to Europe, and it could serve as a biocontrol.

Invasiveness

Many characteristics of *I. glandulifera* contribute to its invasiveness. The synchronous germination of a large seed bank and its rapid growth to great height enable it to shade out its competitors. The prolific production of seeds, which can be spread explosively by the plant, in flowing water, or by human transport, also contributes to the rapid spread of this plant. It is tolerant of nutrient deprivation, and Willis and Hulme (2004) found that seed mass increased with increasing

resource limitation and at higher elevations, an adaptation that maximizes the opportunity for colonization and establishment.

History and Extent of Invasion

I. glandulifera was introduced to Europe in 1839 when some seeds were sent to the Royal Botanical Garden in England (Beerling and Perrins 1993). The first recorded naturalizations occurred less than 20 years later, in Hertfordshire in 1855 and in Manchester in 1859. The species rapidly colonized almost all of the British Isles. It was introduced into the Czech Republic in the 19th century and escaped from cultivation around the turn of the century (Pysek and Prach 1995). The rate of spread in the Czech Republic has increased in recent years, and it is presently found along 56% of the total river length in the country. It was first recorded in Ontario, Canada in 1901 (Clements et al. 2007). It was then found in British Columbia and Nova Scotia in 1939, Quebec in 1940, and Washington State in 1944. The first recorded introduction of *I. glandulifera* in the United States occurred in 1883 in a Norwich, Connecticut garden (Tabak and von Wettberg 2008). In the early 1900s, it was cultivated in the New York Botanical Garden and also recorded in Southern Vermont, and Boston, Massachusetts. It is currently found in 12 states across the country. In Maine, *I. glandulifera* is mainly found in Knox County and other coastal areas, though there is one known inland population in Farmington (IPANE 2009). The current northern limit of *I. glandulifera* is 64° N latitude, but Beerling (1993) showed that a 1.5° C increase in global temperature would expand the northern range of *I. glandulifera* to 69° N latitude.

Methods of Control

I. glandulifera can be controlled with the herbicides 2,4 D-amine and glyphosate when the herbicide is applied two times, early in the season, prior to seed production (Beerling and Perrins 1993, Clements et al. 2007). However, the application of herbicides is limited in riparian environments. Mowing in mid to late July immediately following the appearance of flowers can be an effective means of control if the invasion is in an accessible area (Clements et al. 2007). Flaming of the stems with a hand-held propane flamer has been tried, but it was not found to be completely effective (Clements et al. 2007). Given the shallowness of the root system, uprooting the plant is usually not very difficult and may be more effective than the methods previously described, as long as care is taken to prevent colonization of the disturbed soil by other invasive plants (Clements et al. 2007). Currently, there are no approved biological control agents for *I. glandulifera*, but the thrips *Taeniothrips major* shows promise and has been assigned to further testing (Clements et al. 2007), while the new virus discovered by Kollmann et al. (2007) also has potential.

Impacts

Hejda and Pysek (2006) found no significant difference in species richness or diversity between invaded and uninvaded plots in riparian habitats in the Czech Republic. They noticed a small effect on species composition as *I. glandulifera* replaced tall, nitrophilous dominants. Hulme and Bremner (2006) found that removal of *I. glandulifera* from experimental riparian plots in England resulted in a significant increase in the number of species, particularly those with a high demand

for light. However, non-native species responded proportionally more to removal, which raises the concern that removal of *I. glandulifera* may benefit non-native species, defeating conservation goals (Hulme and Bremner 2006). Both Hejda and Pysek (2006) and Hulme and Bremner (2006) recommend the protection of habitat to prevent establishment of this species, rather than the widespread removal of the species, which can promote other invasions. Clements et al. (2007) observed that *I. glandulifera* does not prevent erosion due to its shallow root system. Chittka and Schürkens (2001) found that *I. glandulifera*'s abundance of nectar draws pollinators away from native plants. However, *I. glandulifera*'s abundant nectar has been shown to benefit some native bumblebees, particularly late in the season when other flowers have ceased to bloom (Clements et al. 2007).

Legislation

I. glandulifera is classified as a noxious weed in British Columbia, and it is on the Invasive Plant Alert List there (Clements et al. 2007). This species is listed as a Class B noxious weed in Washington State and as a 'B' designated weed in Oregon, which means it has been designated for control, containment, and prevention (WSNWCB 2010). It is quarantined in both states (USDA 2010a). It has been illegal to transport, sell, distribute, or cultivate the plant in Connecticut since 2004 (Clements et al. 2007). *I. glandulifera* is listed by the Invasive Plant Atlas of New England (IPANE 2009).

Materials and Methods

Sample Collection

Seeds and leaf tissue samples were collected from four of the six known populations of *Impatiens glandulifera* in Maine on September 19, 2009 (Table 1). A total of 43 plants were sampled from the four populations (Table 1). The minimum distance between sampled plants was 2 m to help ensure that genetically distinct plants were being sampled. All of the seeds from a single plant were placed into paper envelopes for transport back to the lab. Small pieces of young leaf tissue were collected from each plant and placed in paper envelopes with silica beads to promote desiccation for storage.

Table 1. Name, location, sample size, and latitude and longitude of the four populations of the invasive plant *Impatiens glandulifera* in Maine that were sampled. Seeds and leaf tissue samples were obtained from each population to investigate genetic relatedness among populations.

Population Name	Location	Sample Size	GPS Coordinates
Alford	Alford Lake Rd, Hope, ME	10	N44.223°, W69.205°
Augusta	Augusta Nature Center, Augusta, ME	10	N44.313°, W69.752°
Farmington	Univ. of Maine Farmington, Farmington, ME	12	N44.665°, W70.148°
Lubec	Lubec, ME	11	N44.855°, W66.993°

Common Garden Experiment

One or two seeds from each plant were planted immediately after collection. These seeds were placed on the mist bench in the Colby College greenhouse where they received regular water. After four months, these seeds showed no signs of germinating. The rest of the seeds were imbibed and stratified to hasten germination following the procedure elaborated in Mumford (1988). The seeds

were placed in an incubator at 35° C for 30 days. They were then transferred to vials filled with deionized water and allowed to imbibe for approximately 48 hours. The seeds were removed from the water and placed in sterile petri dishes with moistened vermiculite. The petri dishes were sealed in plastic bags and placed in the refrigerator for 40 days. The seeds were supposed to stratify for 48 days, but the presence of a fungus necessitated an earlier planting after only 40 days of stratification. The seeds were planted in five blocks. Up to ten seeds per plant were planted, however many plants had fewer than ten viable seeds remaining. The pattern of planting was randomized. 38 days after planting, 12 seeds had germinated. The seedlings were transplanted into pots with a seedling potting mixture, but none of the seedlings survived the transplant. The common garden experiment was discontinued.

Microsatellite Analysis

DNA was extracted from the leaf tissue samples using a DNeasy Plant Mini kit (QIAGEN). A spectrophotometer was used to quantitate the DNA, which was then diluted to a concentration of 20 ng/μL and stored in the AE buffer provided with the kit. Using the eight unlabeled primers identified by Provan et al. (2006), polymerase chain reactions (PCRs) were performed using the Augusta 1, 2, 3 and 4 DNA templates to determine which primers amplify the microsatellite regions. The PCR recipe was based on the recipe used by Provan et al. (2006). Reactions were carried out with a total volume of 25 μL containing: 17.6 μL water, 2.5 μL 10x Taq buffer with 15 mM $MgCl_2$, 1.0 μL $MgCl_2$, 0.5 μL 40 mM dNTPs, 1.2 μL 10 mM primer 1, 1.2 μL 10 mM primer 2, 0.5 μL Taq polymerase and 0.5 μL DNA template. The

touchdown PCR involved the following cycles: 95° C for 2.5 min.; 20 cycles of 95° for 20s, 60-50° for 30s (decreasing by 0.5° each time), and 72° for 30s; followed by 15 cycles of 95° for 20s, 50° for 20s, and 72° for 30s; and ending with 72° for 10 min. The PCR products were visualized on a 3% low melting agarose gel, and it was determined that primers 104, 203, and 210 most successfully amplified the microsatellite regions (Table 2).

Table 2. Primer sequences for loci 104, 203 and 210, which successfully amplified microsatellite regions in the DNA of the invasive plant *Impatiens glandulifera*.

Primer	Sequence
104L (labeled)	CCACCATACCTTCTTCTCCTG
104R	GTTGCCCGGAAGTAGACATT
203L (labeled)	CAAAGGGCGACGGTTTCT
203R	TTCCATGGACAATTCCTTCA
210L (labeled)	CCAGAGAGGTGGAGGTTCAA
210R	GAAAGCAGGTTCCGTCGATA

Fluorescently labeled versions of these three primers were used to perform PCRs on all the DNA templates from all 43 samples. The PCR recipe described above was used except the volume of water was decreased to 17.1 μL and the volume of DNA template was increased to 1 μL. Labeled fragments were run on the Applied Biosystems 3130 genetic analyzer to determine the length of the fragments. Fragment lengths were compared with the GeneScan-500 ROX size standard and analyzed using GeneMapper software. Two samples, Augusta 1 and Farmington 7, were eliminated from further consideration because they failed to amplify across the three loci.

For Locus 104, a total of five normally distributed peaks were observed in homozygotes. The middle peak, which was also the tallest, was considered to be the

true allelic peak. The expected peak pattern for heterozygotes with alleles that were 2 and 4 basepairs (bp) apart was calculated by superimposing two sets of the peaks from the homozygote. For heterozygotes at 2 bp apart, a total of six peaks were observed, with the middle two being the tallest and the same height. For heterozygotes at 4 bp apart, a total of five peaks were observed, with the second and fourth peaks being the tallest. When this pattern was observed, the second and fourth peaks were always called as the true alleles, even if the third and fourth peaks were similar in height.

For Locus 203, the same labeled PCR product was run with the genetic analyzer on two separate occasions 20 days apart. This was done to determine whether the samples, which had two peaks 1 bp apart, were heterozygous, as the result of a one-base addition or deletion, or due to the random addition of an extra adenine at the end of some fragments by the polymerase. These individuals were indeed heterozygous, and only the first run was used to score all individuals. If only one tall peak was observed or if there were two peaks but the shorter was less than 70% of the height of the taller, the individual was scored as homozygous with the tallest peak as the true allelic peak. If two peaks were observed only 1 bp apart and the shorter peak was greater than 70% of the height of the taller, the individuals was scored as a heterozygote, with both peaks representing true alleles. Finally, if three peaks were observed with the middle one being the tallest, the individual was scored as a heterozygote with alleles 2 bp apart.

For Locus 210, all but five of the individuals displayed two peaks 9 bp apart. The remaining individuals had one peak at the shorter fragment length. However,

there is no known theory of microsatellites that would explain this pattern, as the peaks were too far apart for microsatellite repeats. For this reason, Locus 210 was not included in the analysis.

Data Analysis

The allele lengths for each individual were entered into GenAlEx population genetics software (Peakall and Smouse 2006). Variation within and among populations was assessed by calculating the following parameters: number of alleles per locus, allele frequency for both loci in each population, expected heterozygosity (H_e), and observed heterozygosity (H_o). Chi-square values were calculated for each population at each locus to determine whether H_o was significantly different from H_e.

Wright's F-statistics are the classic population genetic statistics for characterizing population genetic structure. F-statistics describe the deviation from the expected heterozygosity within and among populations. In this study, R-statistics were calculated in addition to F-statistics because R-statistics are more appropriate for microsatellite data (Flanagan 2005). Like F-statistics, R-statistics compare the distribution of variance of allele frequencies. However, R-statistics take into account the length of the allele and the magnitude of the differences between alleles, whereas F-statistics only classify alleles as same or different, ignoring the degree of difference (Halliburton 2004, p.347). By using a step-wise model of mutation, R-statistics can account for the fact that mutations in microsatellite regions depend on the initial allele size (Slatkin 1995).

F-statistics and R-statistics were calculated by Analysis of Molecular Variance (AMOVA). AMOVA is a versatile approach that enables statistical testing by random permutation (Flanagan 2005). In AMOVA, a distance matrix is defined to measure the distance between any two haplotypes and analysis of variance is used to partition the total variation into variation within individuals, variation among individual, and variation among populations (Halliburton 2005, p.356). F_{ST} and R_{ST} measure the deviation in heterozygosity among populations relative to the region as a whole. This is a measure of how different the populations are and the extent of subdivision between populations. F_{IT} and R_{IT} measure the deviation in heterozygosity among individuals relative to the total population, and F_{IS} and R_{IS} measure the deviation in heterozygosity among individuals relative to their population (Halliburton 2004, p.319). R_{ST}, R_{IT}, and R_{IS} values do not sum to 1, rather $(1-R_{IT})=(1-R_{IS})(1-R_{ST})$, and the same is true for F-statistics (Halliburton 2004, p.320).

A Mantel test was performed to assess the correlation between geographic distance and genetic distance among all four populations. The Mantel test determines the significance of a correlation between two distance matrices. The geographic distance matrix, calculated using the latitude and longitude of each site, was compared to Nei's genetic distance. Nei's genetic distance assumes that differences in populations arise due to mutation and genetic drift (Nei 1972).

Results

Data were analyzed for 41 individuals at two loci. A total of four alleles ranging in size from 116 to 122 were identified for locus 104, and three alleles ranging in size from 141 to 143 were identified for locus 203. Allele frequencies for each population at each locus are shown in Figure 2. Each allele was not present in every population. In Farmington all individuals were homozygous for allele 120, and allele 116 was only found in Alford.

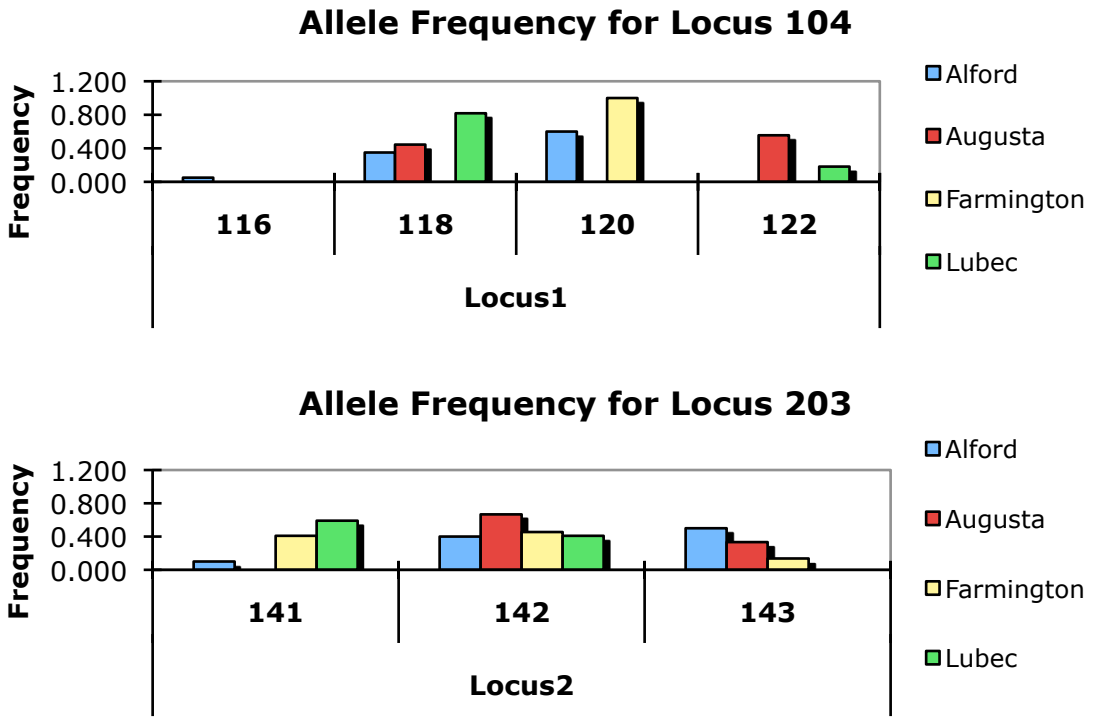


Figure 2. Allele frequencies for the four study populations of *Impatiens glandulifera* located in Alford, Augusta, Farmington, and Lubec, Maine at locus 104 and locus 203.

Table 3 shows the observed and expected heterozygosity by locus for each population, except for Farmington which is monomorphic at locus 104. Augusta at locus 104 is the only population and locus for which the difference between H_e and H_o is statistically significant (see Table 3). The results of the Chi-squared tests to

test the significance of the differences between H_e and H_o should be treated with caution as the sample size is less than 50 and there are fewer than five expected individuals in some categories.

Table 3. Observed and expected heterozygosity by locus for each population of *Impatiens glandulifera* that was studied. The population for which the observed and expected heterozygosity are significantly different ($X^2_{3, n=41}=5.76$, $p=0.02$) is shown in bold. The Farmington population is monomorphic at locus 104.

Population	Locus	H_o	H_e
Alford (n=10)	104	0.500	0.515
	210	0.700	0.580
Augusta (n=9)	**104**	**0.889**	**0.494**
	210	0.222	0.444
Farmington (n=11)	104	---	---
	210	0.727	0.607
Lubec (n=11)	104	0.364	0.298
	210	0.455	0.483
Mean	both	0.482	0.428

The distribution of variance used to calculate R-statistics and the distribution of variance used to calculate F-statistics are represented in Figure 3. In both cases, the majority of the variation occurred within individuals and the remainder of the variation occurred among populations. The following values were calculated for the R-statistics: $R_{ST}=0.226$, $R_{IS}=-0.306$, and $R_{IT}=-0.011$. A negative R_{IS} means that the average observed heterozygosity across loci for a population is greater than the average expected heterozygosity. A negative R_{IT} means that the observed heterozygosity for the total population is greater than the expected (Halliburton 2005, p. 320). The following values were calculated for the F-statistics: $F_{ST}=0.374$, $F_{IS}=-0.074$, and $F_{IT}=0.327$.

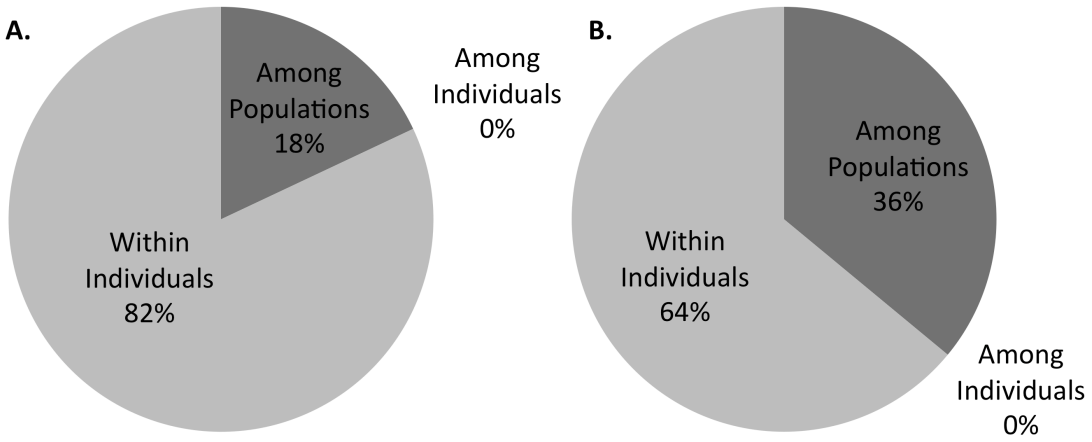


Figure 3. Distribution of variance used for calculating R-statistics by AMOVA (A) and distribution of variance used for calculating F-statistics by AMOVA (B) for the four study populations of *Impatiens glandulifera* in Maine.

The Mantel test did not find a significant correlation between geographic distance and Nei's genetic distance among populations (r=0.086, p=0.440). The results of the Mantel test are shown in Figure 4.

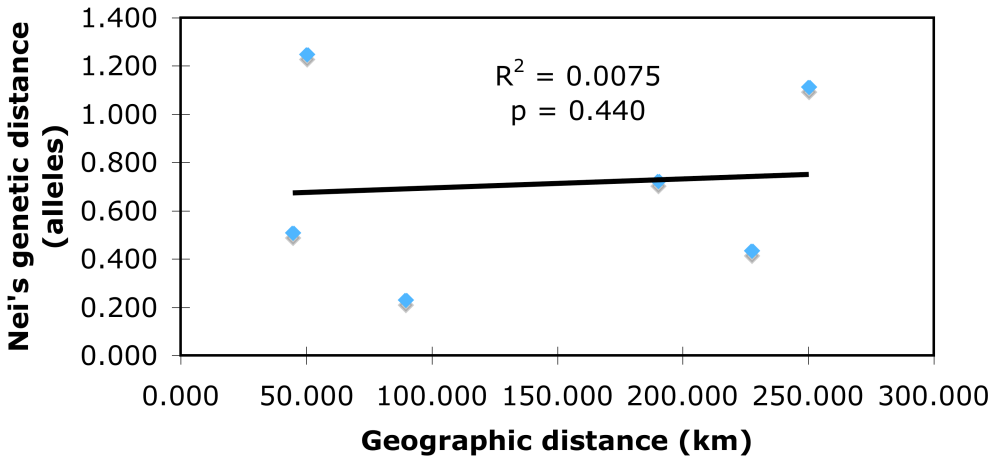


Figure 4. Results of the Mantel test for correlation between geographic distance and Nei's genetic distance between the four study populations of *Impatiens glandulifera* in Maine.

Discussion

In support of the theory that hybridization may enhance invasiveness, we found that invading populations of *I. glandulifera* in Maine have an unusually high level of heterozygosity. The extremely low value of R_{is} (-0.306, $p<0.02$) indicates that there are more heterozygotes in the population than expected, perhaps due to hybrid vigor. Hybrid vigor occurs when hybridization between two individuals with distinct genotypes creates a heterozygous individual whose genes make it more fit than either of the parents (Rieseberg et al. 2007). The hybrid individuals consistently outcompete the parental genotypes, leading to the appearance of fixed heterozygosity in the population. Ellstrand and Schierenbeck (2000) proposed hybrid vigor as one of the ways that hybridization could stimulate increased invasiveness, and it appears that this is the case with *I. glandulifera* in Maine.

In addition, the populations of *I. glandulifera* in Maine have an unexpectedly high degree of allelic diversity. Compared to the three populations in England sampled by Provan et al. (2006), the study populations had an equal or greater number of alleles at each locus, and a higher level of expected heterozygosity. We found four alleles at locus 104 and three alleles at locus 203, whereas Provan et al. found three alleles at both loci. Also, we found a mean H_e of 0.428 across populations and loci, whereas they found a mean H_e of 0.289 (Provan et al. 2006). Despite experiencing the demographic bottleneck that comes with introduction to a new location, *I. glandulifera* does not appear to have experienced a genetic bottleneck upon its introduction into Maine.

I. glandulifera did not show isolation by distance in Maine, and the Mantel test showed that there was no statistically significant correlation between the geographic distance between populations and their genetic relatedness. This is what we would expect from a recently introduced species. The geographically isolated populations are likely the result of separate introductions or human dispersal of seeds. If the seeds were dispersed naturally, we would expect populations that were closer to be more genetically similar.

Our results support the hypothesis of Ellstrand and Schierenbeck (2000) that hybridization may promote invasiveness, yet much more work would be required to determine which of their four scenarios are specifically applicable to this case. Very few studies have been published on the population genetics of *I. glandulifera.* Provan et al. (2006) studied two populations along river systems in Northern Ireland and Wales. Our finding of increased genetic variation applies only to this reference population. It would be interesting to compare genetic diversity in Maine with numerous other populations in the native range, in the introduced range, and in other North American populations.

It would also be of great interest to examine departures from expected heterozygosity across the native and introduced range. Walker et al. (2009) used genetic markers to investigate populations along three river catchments in England. They found significantly high F_{is} values and a deficiency of heterozygotes, which is what one would expect from a self-compatible species like *I. glandulifera*. This result makes our finding of elevated heterozygosity even more remarkable. Further genetic testing could ascertain whether the high heterozygosity found in Maine

populations is due to the temporary heterosis expected of recently-hybridized lineages, or to fixed heterosis, as suggested by Ellstrand and Schierenbeck (2000), stabilized by chromosomal rearrangements or agamospermy. Population genetic and common garden studies could also address the question of whether increased genetic variation, evolutionary novelty, or heterosis are the most important forces permitting the evolution of enhanced invasiveness.

Understanding the evolutionary trigger of invasiveness provides only limited practical information about how to prevent or control invasions - these procedures must also be informed by targeted applied studies. However, the knowledge that hybridization can stimulate invasiveness should inform management decisions. Vigilant efforts should be made to prevent introduction of an exotic species even after it is already present because multiple introductions may provide additional genetic diversity and enable hybridization. If it is known that a species has the potential to evolve increased invasiveness, it should be the focus of control efforts. Additionally, investigating the population genetics of a rapidly spreading invasive species provides a unique and valuable opportunity to study evolutionary change and adaptation in a condensed time frame. Studies such as this one can have both practical and theoretical value, and further investigation of the population genetics of *I. glandulifera* and other invasive species should be pursued.

Acknowledgments

I would like to thank my thesis advisor Dr. Judy Stone for her patient guidance throughout the project, and my thesis readers Dr. F. Russell Cole and Dr. David Firmage for their valuable comments on earlier drafts. I would also like to thank Dr. Herb Wilson for collecting seeds and leaf tissue samples from the Lubec population and Alex Twibell for assisting with the seed stratification process. Funding for this project was provided by the Colby College Biology Department.

Literature Cited

Andrews, M., H. G. Maule, J. A. Raven, and A. Mistry. 2005. Extension growth of *Impatiens glandulifera* at low irradiance: Importance of nitrate and potassium accumulation. Annals of Botany **95**:641-648.

Beerling, D. J. 1990. The ecology and control of Japanese knotweed (*Reynoutria japonica* Houtt) and Himalayan balsam (*Impatiens glandulifera*) on river banks in South Wales. PhD thesis, University of Wales, Cardiff.

Beerling, D. J. 1993. The impact of temperature on the northern distribution limits of the introduced species *Fallopia japonica* and *Impatiens glandulifera* in North-West Europe. Journal of Biogeography **20**:45-53.

Beerling, D. J. and J. M. Perrins. 1993. *Impatiens glandulifera* Royle (*Impatiens roylei* Walp.). Journal of Ecology **81**:367-382.

Chittka, L. and S. Schürkens. 2001. Successful invasion of a floral market: an exotic Asian plant has moved in on Europe's river-banks by bribing pollinators. Nature **411**:653.

Clements, D. R., K. R. Feenstra, K. Jones, and R. Staniforth. 2007. The Biology of Invasive Alien Plants in Canada. 9. *Impatiens glandulifera* Royle. Canadian Journal of Plant Science **88**:403-417.

Durka, W., O. Bossdorf, D. Prati, and H. Auge. 2005. Molecular evidence for multiple introductions of Garlic mustard (*Alliaria petiolata*, Brassicaceae) to North America. Molecular Ecology **14**:1697-1706.

Ellstrand, N. C. and K. A. Schierenbeck. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? PNAS **97**:7143-7050.

Ennos, A. R., M. J. Crook, and C. Grimshaw. 1993. A comparative study of the anchorage systems of Himalayan balsam *Impatiens glandulifera* and mature sunflower *Helianthus annuus*. Journal of Experimental Botany **44**:133-146.

Flanagan, N. 2005. A Guide to GenAlEx6. Australian National University, Canberra, Australia.

Halliburton, R. 2004. Introduction to Population Genetics. Pearson Prentice Hall, Upper Saddle River, NJ.

Hayes, K. R. and S. C. Barry. 2008. Are there any consistent predictors of invasion success? Biological Invasions **10**:483-506.

Hejda, M. and P. Pysek. 2006. What is the impact of *Impatiens glandulifera* on species diversity of invaded riparian vegetation? Biological Conservation **132**:143-152.

Hulme, P. E. and E. T. Bremner. 2006. Assessing the impact of *Impatiens glandulifera* on riparian habitats: partitioning diversity components following species removal. Journal of Applied Ecology **43**:43-50.

Invasive Plant Atlas of New England. 2009. University of Connecticut. < http://nbii-nin.ciesin.columbia.edu/ipane/>. [Accessed 1 May 2010].

Kollmann, J. and M. J. Banuelos. 2004. Latitudinal trends in growth and phenology of the invasive alien plant *Impatiens glandulifera* (Balsaminaceae). Diversity and Distributions **10**:377-385.

Kollmann, J., M. J. Bañuelos, and S. L. Nielsen. 2007. Effects of virus infection on growth of the invasive alien *Impatiens glandulifera*. Preslia **79**:33-44.

Lee, C. E. 2002. Evolutionary genetics of invasive species. Trends in Ecology and Evolution **17**:386-391.

Mumford, P. M. 1988. Alleviation and induction of dormancy by temperature in *Impatiens glandulifera* Royle. New Phytology **109**:107-110.

Nei, M. 1972. Genetic distance between populations. American Naturalist **106**:283-392.

Nienhuis, C. M. and J. C. Stout. 2009. Effectiveness of native bumblebees as pollinators of the alien invasive plant *Impatiens glandulifera* (Balsaminaceae) in Ireland. Journal of Pollination Ecology **1**:1-11.

Peakall, R. and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes **6**:288-295.

Prentis, P. J., J. R. U. Wilson, E. E. Dormontt, D. M. Richardson, and A. J. Lowe. 2008. Adaptive evolution in invasive species. Trends in Plant Science **13**:288-294.

Provan, H. M. L. and C. A. Maggs. 2006. Development of microsatellites for the invasive riparian plant *Impatiens glandulifera* (Himalayan balsam) using intersimple sequence repeat cloning. Molecular Ecology Notes **2007**:451-453.

Pysek, P. and K. Prach. 1995. Invasion dynamics of *Impatiens glandulifera* - a century of spreading reconstructed. Biological Conservation **74**:41-48.

Rieseberg, L. H., S. C. Kim, R. A. Randell, K. D. Whitney, B. L. Gross, C. Lexer, and K. Clay. 2007. Hybridization and the colonization of novel habitats by annual sunflowers. Genetica **129**:149-165.

Sakai, A. K., F. W. Allendorf, J. S. Holt, D. M. Lodge, J. Molofsky, K. A. With, S. Baughman, R. J. Cabin, J. E. Cohen, N. C. Ellstrand, D. E. McCauley, P. O'Neil, I. M. Parker, J. N. Thompson, and S. G. Weller. 2001. The Population Biology of Invasive Species. Annual Review of Ecology and Systematics. **32**:305-332.

Salisbury, E. 1961. Weeds and Aliens. Collins, London.

Schierenbeck, K. A. and N. C. Ellstrand. 2009. Hybridization and the evolution of invasiveness in plants and other organisms. Biological Invasions **11**:1093-1105.

Suarez, A. V. and N. D. Tsutsui. 2008. The evolutionary consequences of biological invasions. Molecular Ecology **17**:351-360.

Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics **139**:1436.

Tabak, N. M. and E. v. Wettberg. 2008. Native and introduced jewelweeds of the Northeast. Northeastern Naturalist **15:** 159-176.

Titze, A. 2000. The efficiency of insect pollination of the neophyte Impatiens glandulifera (Balsaminaceae). Nordic Journal of Botany **20**:33-42.

USDA. 2010a. *Impatiens glandulifera* Royle. PLANTS Database. Natural Resources Conservation Service. <http://plants.usda.gov/java/profile?symbol=imgl>. [Accessed 9 September 2009].

USDA. 2010b. National Invasive Species Information Center. <http://www.invasivespeciesinfo.gov/whatis.shtml>. [Accessed 1 May 2010].

Walker, N. F., P. E. Hulme, and A. R. Hoelzel. 2009. Population genetics of an invasive riparian species, *Impatiens glandulifera*. Plant Ecology **203**:243-252.

Washington State Noxious Weed Control Board. 2010. Class B Noxious Weeds. [Accessed 12 May 2010].

Weidenhamer, J. D. and R. M. Callaway. 2010. Direct and indirect effects of invasive plants on soil chemistry and ecosystem function. Journal of Chemical Ecology **39**:59-69.

Whitney, K. D. and C. A. Gabler. 2008. Rapid evolution in introduced species, 'invasive traits' and recipient communities: challenges for predicting invasive potential. Diversity and Distributions **14**:569-580.

Willis, S. G. and P. E. Hulme. 2004. Environmental severity and variation in the reproductive traits of *Impatiens glandulifera*. Functional Ecology **18**:887-898.