REPRODUCTION AND POPULATION GENETICS OF INVASIVE PLANTS: THE

ROLE OF ENVIRONMENTAL VARIATION

by

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A dissertation submitted to the Graduate School – Newark

Rutgers, The State University of New Jersey

In partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Graduate Program in Biology – Ecology and Evolution

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Newark, New Jersey

October, 2016

ABSTRACT OF THE DISSERTATION:

Reproduction and population genetics of invasive plants: the role of

environmental variation

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Reproductive versatility of plants is an important part of their success. We

investigated environmental factors that influence the reproductive strategies of

three non-native plants and investigate the larger impact of these factors at the

population level.

Initial work consisted of two studies with *Trifolium repens*. A two month

experiment was conducted under a factorial design, testing a combination of

resource abundance and heterogeneity. Resource distribution had no effect on

reproductive strategy. Resource abundance increased the amount of biomass

allocated to horizontal stem development and foraging, but showed no effect on

flowering at two months. A four month study showed that fertilizer does increase

inflorescence development. The conflict between the two studies is evidence that reproductive strategy and flowering cues in *Trifolium repens* are strongly influenced by density depended effects.

Two studies were performed with *Ailanthus altissma*, a high impact invasive tree species. A germination study was conducted on seeds from Ailanthus altissima based on human land use legacy, in the form of brownfield and non-brownfield sites. Brownfield sites showed a significant reduction in seed germination, this difference in germination was independent of initial seed mass. These two factors indicate that individuals from a brownfield site have a reduced fitness benefit for the same amount of resources invested in seeds.

Population genetics of *Ailanthus altissima* were surveyed across six sites, using a set of eight microsatellite loci. Despite a strong propensity for clonal growth, the microsatellites revealed no evidence of clonal reproduction at the population level. Geneflow between sites was found to be independent of geographic distance, rather, geneflow was linked to the level of human traffic at a site. Evidence was also found that land management practices were effective at inhibiting geneflow into managed sites.

Finally, an investigation was conducted into the possibility of ecotypes and reproductive isolation in *Schismus arabicus* in the Sonoran and Mojave deserts, based on the extreme heterogeneity of the nurse plant dominated landscape.

Results from the ITS marker showed no evidence of ecotypes at either site.

Number of SNPs between individuals were not correlated with distance,

indicating that high internal geneflow prevents the level of isolation necessary for

the formation of ecotypes. MCMC modeling also showed a small, but consistent,

unidirectional geneflow from the Mojave to the Sonoran desert site. This was

taken as evidence of anthropogenic geneflow.

Ultimately, it is shown that reproductive strategy and population structure are

strongly influenced by anthropogenic factors such as nutrient level, land-use

legacy, management practices, and anthropogenic transport.

Dedicated to:

My parents, Bernard and Monica Brusa,

for all they have done for me.

To my friends,

for their continuing support though out the years.

...and to all those willing to sacrifice comfort and complacency in the pursuit of

knowledge.

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Chapter 1: Resource Abundance and Distribution: Sexual vs. Asexual Reproductive Allocation in *Trifolium repens*

Introduction

Although it may not appear so at first glance, plants constantly react to their environment to optimize their fitness. The real world has a large number of varying and contrasting environments, and response to these differences is vital to survival. The sessile nature of plants limits the type of response that is viable, as plants cannot simply move to a location with better resources, avoid a hazard, or travel to find potential mates. Instead, plants can alter their resource allocation between different tissue types in order to react to environmental shifts. These environmental shifts can be thought of as forms of heterogeneity, either spatial or temporal. Spatial heterogeneity is driven by uneven distribution of resources across the environment, while temporal heterogeneity consists of variations in the availability of resources/habitat across a span of time.

These heterogeneous environments can take many forms. Examples of spatial heterogeneity include soil composition (D. K. Wijesinghe & Hutchings, 1997), light availability (Kanno & Seiwa, 2004; Josef F Stuefer, During, & de Kroon, 1994), water stress (Peterson & Chesson, 2002; Schenk, 1999), nutrient abundance (F. Liu, Chen, & Wang, 2008; Price & Marshall, 1999) and soil salinity (Shumway, 1995). With their inability to move directly, a plant must be able to deal with either an excess or deficiency of any of these factors. Examples of

temporal heterogeneity include regular patterns such as day-night, seasonal cycles and age dependent development (Cheplick, 1995), as well as irregular systems, such as pollen availability (Burd, 1994), treefall gaps in forests (Kanno & Seiwa, 2004), wildfire regimes, and flooding (Abrahamson, 1975). In such systems the change is limited in duration, and so the plants must act quickly to exploit the short lived opportunity (Kanno & Seiwa, 2004).

In either case, spatial or temporal, the plants response is mediated by allocation of limited resources to an appropriate growth pattern. The existing literature shows many examples of plants altering their resource allocation, growth and reproductive modes in response to spatial and temporal heterogeneity (Price & Marshall, 1999; Trewavas, 2005). Heterogeneity in the distribution of nutrients is one of the most common of these factors, having been observed in; *Glechoma hederacea* (Birch & Hutchings, 1994; Hutchings & Wijesinghe, 1997; D. K. Wijesinghe & Hutchings, 1997), *Trifolium repens* (Dekroon & Hutchings, 1995; J F Stuefer, De Kroon, & During, 1996), *Fragaria chiloensis* (P. Alpert, Holzapfel, & Slominski, 2003), and *Potentilla simplex* (D. Wijesinghe & Handel, 1994). Studies have also found this to be true for other resources, such as light (P. Alpert et al., 2003; Kanno & Seiwa, 2004) and water (Peterson & Chesson, 2002; Schenk, 1999). Long term trends such as population age (D.C.Hartnett and F.A.Bazzaz, 1985), land use history (Gonzales, Hamrick, & Smouse, 2008), and temporal heterogeneity (Abrahamson, 1975; Rusterholz, Kissling, & Baur, 2009) have also been observed to alter plant resource allocations. These individual examples are

also supported by several modeling studies which simulate the response of plants to different types of environmental heterogeneity (B. Oborny, Kun, Czárá, & Bokros, 2000; Beata Oborny, 1994; Beáta Oborny & Kun, 2002). Thus we see that, despite their sessile nature, plants are actively responding to environmental conditions in order to maximize their individual fitness.

Sexual vs. Asexual Allocation

One of the most important ways on which plants can respond to their environment is to alter their resource allocation toward two competing reproductive strategies; sexual reproduction, through seed, and asexual reproduction, through vegetative growth. Asexual reproduction, in the form of clonal growth, is of particular interest as it affects the fitness of individuals in two ways; 1) as a form of reproduction, and 2) as a physiological process for foraging and resource sharing (see division of labor below). The latter is particularly important for clonal plants, as they are more likely to encounter heterogeneous environments (Price & Marshall, 1999). Clonal growth can be beneficial in such environments, however, doing so means reducing the amount of resources being allocated to the structures and development needed for sexual reproduction (Bai, Sun, Wang, & Li, 2009; F. Liu et al., 2008; Metcalf, Stephens, & Rees, 2009; Thompson & Eckert, 2004). The trade-off between clonal spread and sexual reproduction has been observed in a wide range of plant species, including; *Sagittaria pygmaea* (F. Liu et al., 2008), *Calathea marantifolia* (Matlaga, 2008), *Geum reptans* (Weppler & Stöcklin, 2005; Weppler, Stoll, & Stocklin, 2006),

Butomus umbellatus (Thompson & Eckert, 2004), *Trillium cuneatum* (Gonzales et al., 2008), Helianthus tuberosus (Westley, 1993), *Eurya japonica* (Suzuki, 2001) and *Veratrum album* (Hesse, Rees, & Müller-Schärer, 2008). This trade off has also been the subject of several modeling studies as well (Bengtsson & Ceplitis, 2000; Metcalf et al., 2009). The sexual-asexual allocation trade-off means that responding to environmental heterogeneity by developing vegetative structures for resource foraging will, indirectly, affect the reproductive allocation of a plant. This is a result of the dual role of vegetative growth, such that it contributes both to resource foraging and asexual reproduction. For plants that maintain a mixed reproductive strategy, using both clonal and sexual reproduction, the maintenance of a clonal structure in response to resource heterogeneity will shift the relative balance toward asexual reproduction.

The shift toward greater investment in clonal structures as a result of environmental heterogeneity has been observed under many different systems. Existing studies have found that such links can be driven by overall nutrient levels (F. Liu et al., 2008; D. Wijesinghe & Handel, 1994), nitrogen availability (Bai et al., 2009), division of labor (Ikegami, van Hal, van Rheenen, Whigham, & Werger, 2008; Ikegami, Whigham, & Werger, 2008), land use history (Gonzales et al., 2008; Jesse, Nason, Obrycki, & Moloney, 2010), light heterogeneity (Nilsson & D'Hertefeldt, 2007; J F Stuefer, 1996; Josef F Stuefer et al., 1994), water heterogeneity (Peterson & Chesson, 2002), habitat harshness (Nilsson & D'Hertefeldt, 2007), urban vs. rural development (Gonzales et al., 2008), and

periodic disruptions in the form of temporal heterogeneity (Silvertown, 2008). Heterogeneity can also affect reproduction through biotic interactions; heterogeneity in the dispersal of arbuscular mycorrhizal fungi has been shown to alter clonal growth in *Potentilla reptans, Fragaria moschata*, and *Trifolium repens* (Du, Yu, Alpert, & Dong, 2009; Sudová & Vosátka, 2008). Conspecific competition was also observed to favor a shift toward sexual reproduction in *Potentilla anserina ssp. egedii* (Eged's Silverweed) (Rautiainen, Koivula, & Hyvärinen, 2004).

The largest number of field studies regarding the effect of environmental factors on mixed reproductive systems have been performed on the common reed, *Phragmites australis* (Alvarez, Tron, & Mauchamp, 2005; A. H. Baldwin, Kettenring, & Whigham, 2010; Engloner, Major, & Podani, 2010; Ishii & Kadono, 2002; Kettenring, McCormick, Baron, & Whigham, 2010; Paul, Kirk, & Freeland, 2011). *Phragmites australis* is a high impact invasive plant known for its aggressive spread throughout North American wetlands. The success rate of reproductive strategies, both clonal and sexual, for *Phragmites* has been shown to respond strongly in response to the hydrology of its site (Alvarez et al., 2005; A. H. Baldwin et al., 2010; Engloner et al., 2010; Ishii & Kadono, 2002). These studies were all in agreement that deeper water levels put sexual reproduction at a disadvantage, due to poor seed establishment success, resulting in populations largely dominated by the clonal growth so often associated with *Phragmites*. This consistent response to environmental conditions is even more notable due to *Phragmites* having such a wide variation in viable seed production (Kettenring et

al., 2010; Kettenring & Whigham, 2009). Even with viable seed production, environmental factors still limit the success of sexual reproduction. One study estimates that successful seed set chances for *Phragmites* in southwestern Japan ranged from 0.1% to as high as 59.6%, although with a mean of only 9.7% success (Ishii & Kadono, 2002). A similar study performed in Lake Biwa (located in central Japan) found seed set rates ranging between 0% and 45.9%, with a mean of 12.0% (Tachibana, 1984). These findings agree with other works, demonstrating a variable but overall low seedling set rate in *Phragmites* (Gervais, Moreno, Trahan, & Drolet, 1993; Gorenflot, 1976; Gustafsson & Simak, 1963; Ishii & Kadono, 2002). The genetic diversity of *Phragmites*, then, appears to be driven by occasional sexual reproductive events resulting in the establishment of seed to colonize a new area, which then grows clonally until the genet pushes up against a different clone (Alvarez et al., 2005; Engloner et al., 2010).

Various biotic factors have also been shown to affect reproductive strategies of *Phragmites australis*. Pollen limitation, exacerbated by the partially self-incompatible nature of Phragmites, played a large role in restricting the opportunity for sexual reproduction (Ishii & Kadono, 2002; Lambert & Casagrande, 2007). Pollen limitation was determined to be a major limiting factor in seed production of *Phragmites* (Kettenring et al., 2010; Kettenring & Whigham, 2009). This agrees with a study performed by Vallejo-Marin and O-Brien which examined 87 species of the family *Solanaceae*, the study linked pollen limitation to a higher rate of clonality (Vallejo-Marín & O'Brien, 2007). Additionally, damage

from a species of thrip, *Chirothrips manicatus Haliday*, also reduced survival of any *Phragmites* seeds that were produced (Ishii & Kadono, 2002). All of these examples can reduce the relative fitness of sexually reproducing individuals, altering the genetic trajectory of the community, and resulting in a highly clonal population.

Another environmental factor that alters reproductive balance, and a factor commonly associated with invasive species like *Phragmites*, is human disturbance. Anthropogenic eutrophication drove higher sexual reproduction levels in an invasive haplotype of *Phragmites* by increasing seedling growth (Chambers, Meyerson, & Saltonstall, 1999; Saltonstall & Court Stevenson, 2007). This change was likely mediated by elevated levels of nitrogen, which were also linked to elevated invasion success of *Phragmites* (King, Deluca, Whigham, & Marra, 2007). Previous work had concluded that populations were dominated by rhizome mediated clonality (Hudon, Gagnon, & Jean, 2005; Keller, 2000; Pellegrin & Hauber, 1999), indicating that human disturbance may be responsible for shifting this balance toward greater sexual reproduction. If true, human activity could have far reaching impacts on the genetic trajectory of invasive colonies of *Phragmites australis*.

Modeling studies have also been used to directly test how environmental heterogeneity alters resource allocation to sexual and vegetative reproduction (Sakai, 1995; Weeks, 1993). Sakai et al. modeled resource heterogeneity

through the quality of favorable patches and the total number of such patches. Under assumptions of a moderate probability of successful seed establishment Sakai's model concluded that higher quality patches resulted in enhanced clonal growth in the form of longer rhizomes (Sakai, 1995). More importantly, the model also predicted a corresponding reduction in seed production, a trade-off due to limited resources for building new structures (Sakai, 1995). Elevated heterogeneity was modeled through a higher number of patches, resulting in a more uneven distribution of resources. Under such conditions the model predicted the same result, higher clonal allocation and reduced investment in seed production (Sakai, 1995). When the model was altered to find conditions favoring seed production it resulted in the inversely corresponding predictions; seed production to the exclusion of rhizomes was found if the model contained low quality sites or very few patches (Sakai, 1995).

So we have seen that environmental heterogeneity tends to favor increased clonality, through physiological shifts and reproductive allocations of an individual plant. However, one modeling study also suggests that heterogeneity may be vital to maintaining mixed reproductive systems (Weeks, 1993). Weeks studied the long-term evolutionary effects when a population with mixed reproductive strategy, utilizing both sexual and asexual reproduction, compete under a set of temporally heterogeneous conditions. The default organism in his model was assumed to have a reproductive strategy of sexual outcrossing, while a variable mutation rate was used to convert these individuals to obligate asexuality. We

know that obligate asexuality is generally limited in scope in reality, due to long term fitness reduction (Muller, 1963), but under temporally homogeneous conditions Weeks' model actually predicted a complete loss of sexually outcrossing individuals (Weeks, 1993). The expected extinction of obligate asexual individuals was more than offset by the rate of mutation creating more such individuals, resulting in a situation that greatly favored asexuality as a reproductive mode. Situations which favored persistence of the sexual genotype were limited to models with a strong temporal fluctuation of resources (Weeks, 1993). This explains why we see such a large number of plant species demonstrating mixed reproductive modes, even as spatial heterogeneity drives them toward clonality. The overall balance between sexual and asexual reproductive modes may shift, but most plant species maintain at least some degree of both.

The understanding of how plants balance sexual and asexual reproductive strategies requires knowledge of how plants shift their non-sexual allocations in response to environmental heterogeneity. This is because vegetative structures serve a dual role, performing basic plant functions, such as resource foraging, while also allowing for clonal reproduction.

Resource Allocation and Heterogeneity

Many modeling studies have been used to simulate the response of plants to different types of heterogeneous environments. Oborny et al. (2000) used a

cellular automata model to examine the effects of spatial heterogeneity in resource distribution on resource allocation to foraging structures. The model simulated two strategies, clonal integration and splitting, and predicted that the maintenance of large integrated clonal networks was favored when the total number of nutrient rich sites was limited, resulting in a highly heterogeneous environment, and also when the total amount of resources was high (B. Oborny et al., 2000). These two strategies resulted in very different dispersal across the environment, showing the importance of responding to environmental heterogeneity. "Splitters" remaining close to areas of local resource abundance, while the integrated ramets were free to explore the full extent of their environment. This advantage was offset by the stronger performance of "splitters" in optimal patches (although "splitters" performed poorly in bad patches). Thus there is a trade-off between the two strategies, with the level of heterogeneity determining the balance.

Other models show that the number and size of resource patches in the environment strongly influence the effectiveness of a plant's foraging response (Michael L Cain, Dudle, & Evans, 1996). The position of heterogeneous patches was also found to be important, when resource patches were heavily clumped together it was found to reduce the probability of successful foraging. Cain et al. also demonstrated one of the major trade-offs that plants face, spatial heterogeneity of resources means that a plant will eventually suffer density

dependent crowding effects and reduced fitness unless it responds to environmental heterogeneity by expending resources for foraging.

Although these modeling studies are extremely useful in elucidating the underlying mechanisms behind how plants respond to heterogeneity, testing their predictions in a natural environment can be quite difficult. The abundance of environmental factors can make it difficult to ascertain if the model accurately reflects reality. As such, controlled greenhouse experiments serve as an important middle ground, allowing us to test mechanisms under conditions where any such signal is much more likely to be observed.

One of the most frequently used species for controlled greenhouse studies is *Glechoma hederacea*, a clonal herbaceous plant commonly known as ground ivy (Birch & Hutchings, 1994; Slade & Hutchings, 1987; D. K. Wijesinghe & Hutchings, 1997). The earliest of these studies, published by Slade and Hutchings in 1987, examined resource heterogeneity in its simplest form, two adjacent pots. Linked ramet pairs of *Glechoma* were tested under uniformly high and low nutrient loads (both treatments being homogeneous), as well as a an experimental treatment where one ramet of the pair had high nutrient availability and the other low (heterogeneous). The uniformly high nutrient treatment showed high levels of branching and an increased leaf area, indicating an environmental response by the plant to rapidly forage and make use of the abundant resources (Slade & Hutchings, 1987). The high nutrient load plants also had significantly

shorter stolon internodes, as the local abundance of resources negated any need for exploration of the landscape. The exact opposite effects were seen in the low nutrient treatments, where linked ramets significantly extended their internode distances to forage into wider areas (Slade & Hutchings, 1987).

However, response to local abundance of resources is not the same as responding to heterogeneity. Once a heterogeneous environment is built into the experiment we see that optimal foraging response can actually result in higher performance for heterogeneous plants than individuals from high resource homogeneous treatments (Birch & Hutchings, 1994). In their work Birch and Hutchings tested the response of *Glechoma hederacea* to resource heterogeneity with a single ramet planted with varying resource configuration. Treatments consisted of a planter of coarse sand with compost spread evenly across the plot (homogeneous/uniform) or concentrated in a region around the center (heterogeneous/patchy). The patchy treatment performed significantly better, showing a 2.5x higher biomass increase than the uniform treatment (Birch & Hutchings, 1994). Despite having access to the same total amount of resources, the spatial distribution of these resources caused a massive difference in growth of the plant. In addition to overall higher growth, the patchy treatment also had more stolons reaching the edge of the 80cm x 80cm planter, indicating a greater propensity for wide-ranging foraging response (Birch & Hutchings, 1994). This increased foraging spread in response to heterogeneity is in agreement with other findings existing in the literature (Birch & Hutchings,

1994; Michael L Cain et al., 1996; Rautiainen et al., 2004; J F Stuefer, 1996; W. J. Sutherland & Stillman, 1988). This also agrees with other work, which shows that the distribution of resources in the environment, even when the total amount present is held constant, can result in altered root:shoot ratios (Hutchings & Wijesinghe, 1997). Despite the stolons being physically distributed farther across the landscape, 80% of the root biomass was located in the resource-rich central circle (Birch & Hutchings, 1994). This is evidence that plants can react by altering their growth to specialize for exploiting environmentally abundant resources.

The response of plants to their environment is not just limited to nutrient heterogeneity, substrate composition and overall scale of heterogeneous patches are also important factors. Another study performed with *Glechoma hederacea* examined the effect of soil substrate heterogeneity, while holding then overall nutrient availability constant (D. K. Wijesinghe & Hutchings, 1997). In this study the substrate heterogeneity was tested by altering the size of patches of compost and sand, with the total amount of each substrate being held constant. The results showed that spatial heterogeneity is strongly dependent on scale. The overall increase in biomass was largest among plants grown within large patches, while the plants treated small scale heterogeneity as though it were of uniformly poor quality (D. K. Wijesinghe & Hutchings, 1997). Despite the difference in biomass accumulation the overall distribution of this biomass across new plant structures did not vary by treatment, indicating that the heterogeneity of the test environment was not sufficient to prompt a change in foraging

behavior. A difference was seen, however, in the placement of new root structures. Treatments within large patches had discernibly higher root foraging response, indicating a small scale response to local abundance despite the lack of a larger foraging response (D. K. Wijesinghe & Hutchings, 1997).

Division of Labor

The response to environmental heterogeneity is often strong enough that linked plants may allocate biomass to specialize in locally abundant resources, in a process analogous to economic division of labor (Hutchings & Wijesinghe, 1997; Ikegami, van Hal, et al., 2008; Ikegami, Whigham, et al., 2008; J F Stuefer et al., 1996). Division of labor is unusual in that it inverts the standard assumptions about how plants allocate resources in response to heterogeneity. An example of this inversion can be found in *Schoenoplectus americanus*, a common wetland sedge. When exposed to heterogeneous light-salinity treatments the isolated ramets of *Schoenoplectus americanus* had to dedicate biomass toward capturing the resources which were scarce, while linked ramets were shown to specialize in resources in high local abundance (Ikegami, van Hal, et al., 2008). This means that the optimal response to environmental heterogeneity is dependent upon the life history of the plant, and that plants capable of maintaining clonal growth are specifically well suited to exploiting such heterogeneous conditions.

This relationship has been shown to apply to other resources as well, including light availability. Working with *Potentilla reptans* (creeping cinquefoil) and

Potentilla anserina (common silverweed or silver cinquefoil), Stuefer et al. tested the effect of physiological integration of linked ramets under heterogeneous light conditions. Within the heterogeneous treatments, those ramets growing under shaded conditions gained more biomass because they benefited from photosynthetic products created by the well lit ramets (Josef F Stuefer et al., 1994). The reciprocal nature of water and light availability meant that the heterogeneous light conditions also drove an inverted heterogeneity in water availability. As a result it was observed that ramets growing under high light availability had more severe evaporative demands, which was compensated for by translocation of water from the shaded ramets (Josef F Stuefer et al., 1994). This demonstrates the ability of plants to respond to multiple heterogeneous conditions at once. A single heterogeneous condition would have driven a source-sink relationship between ramets (Slade & Hutchings, 1987), but the combination of light and water heterogeneity resulted in a mutual bi-directional exchange of water and carbohydrate, improving the performance of both ramets.

This light/water heterogeneity response was also observed in *Trifolium repens* with similar results, a trend toward local specialization and a shift in morphology (J F Stuefer et al., 1996). The response to local heterogeneity of resources was strong enough that integrated ramets with local shortages actually outperformed individual ramets with no shortage of resources. Once again, the environmental heterogeneity of water and light prompted a shift in plant development which resulted in a reciprocal water/carbohydrate exchange between the two ramets (J

F Stuefer et al., 1996). This is evidence that under heterogeneous conditions the correct plant response (e.g. clonal integration) does more than merely offset local resource shortages, maintaining a physiological connection actually promoted higher overall performance, resulting in increased fitness for the genet as a whole. Ecologically, this response has helped to contribute to *Trifolium repens'* success in colonizing disturbed areas, such as pastures (Burdon, 1983; Price & Marshall, 1999). The importance of heterogeneous response was also observed in *Potentilla simplex*, where a heterogeneous resource distribution once again drove a clonal integration response (D. Wijesinghe & Handel, 1994). The strength of the fitness benefit to integration was shown to vary in response to environmental heterogeneity, indicating that under this system clonal integration is maintained in response to environmental heterogeneity.

It is important to note that in all of these cases the benefits of division of labor are contingent upon both the existence of environmental heterogeneity and upon the maintenance of physiological integration across a genet. By necessity this means that the benefits of division of labor are only available to plants which are, at least in part, reproducing clonally. However, it has also been demonstrated that the degree of clonality is itself influenced by environmental heterogeneity. Thus we return to our initial question; what environmental factors favor allocation towards clonal reproduction?

Introduction to Our Study

In order to test what causes plants to shift reproductive strategies in nature we first directed our efforts toward small scale testing of the underlying concepts. The species we chose for field work (see chapters 2 and 3) have very broad global impact, but are not well suited to short-term preliminary studies due to the length of their maturation times and the large size of the mature individuals. For this reason, our initial pilot work needed to be conducted on a small herbaceous species which could be grown rapidly in a greenhouse, and still exhibit the dual forms of clonal and seed based reproduction. We have chosen to utilize *Trifolium repens* (common clover) due to its ubiquity, hardiness, ease of propagation through cuttings, and its use in existing studies (J F Stuefer et al., 1996). Other species, such as *Microstegium vimineum* (Japanese stiltgrass) and *Oxalis* (wood sorrel), were used in initial trials but rejected due to difficulty cultivating them under experimental conditions. The scale of growth of these species is on the scale of centimeters to decimeters and the maturation time is measurable in weeks, which enabled us to test our concepts regarding the sexual-asexual dichotomy under a controlled environment in a reasonable time frame.

Existing literature reports that both abundance of resources and the heterogeneity of those resources in the environment are important in determining the reproductive allocation of plants between seed production and clonal growth (Bai et al., 2009; Du et al., 2009; Gonzales et al., 2008; Ikegami, van Hal, et al., 2008; Ikegami, Whigham, et al., 2008; Jesse et al., 2010; F. Liu et al., 2008; Z.

Liu et al., 2008; Nilsson & D'Hertefeldt, 2007; Peterson & Chesson, 2002;

Silvertown, 2008; Sudová & Vosátka, 2008; D. Wijesinghe & Handel, 1994).

From this evidence we decided to measure the degree of clonality through two

parameters; proportion of biomass allocated to horizontal stem growth,

representing clonal spread, and inflorescence production per unit of biomass,

representing allocation to sexual development. Based on this we derived the

following hypotheses:

Hypothesis 1.1: Plants grown in a more heterogeneous

environment exhibit a greater allocation of biomass toward

horizontal stem development than those in a homogenous

environment.

Hypothesis 1.2: Plants grown in a more heterogeneous

environment exhibit a lesser allocation of biomass toward

inflorescence development than those in a homogenous

environment.

Previous work has also shown a link between resource level and sexual-asexual

trade-offs (Piquot et al., 1998; S. Sutherland & Vickery, 1988). Clonal structure

development is known to be linked to high nutrient abundance (Caraco & Kelly,

1991; B. Oborny et al., 2000), and specifically to higher nitrogen availability (Bai

et al., 2009). Modeling studies have also predicted that favorable conditions

promote increased clonal growth (Gardner & Mangel, 1999; Sakai, 1995). Based on this background we predicted that:

Hypothesis 1.3: Plants grown in a more nutrient rich environment exhibit a greater allocation of biomass toward horizontal stem development than those in a nutrient poor environment.

Hypothesis 1.4: Plants grown in a more nutrient rich environment exhibit a lesser allocation of biomass toward inflorescence development than those in a nutrient poor environment.

Methods: Experiment 1

In an exploratory initial experiment environmental heterogeneity was created through use of plastic planting trays with different levels of soil fertility heterogeneity; each treatment consisted of 10 trays divided into regions in a 3x3 array of varying soil nutrient levels. Cuttings of sexually reproductive *Trifolium repens* were introduced into the center of the plot, with all eight of the surrounding regions empty to provide locations for expansion. The regions were defined by treatment application only; no artificial barriers were used to avoid any possible interference with vegetative growth. Nutrient availability was controlled by addition of a Miracle-Gro® continuous release NPK fertilizer (10-10-10 composition with 20% sulfur content) to the plastic trays according to the proscribed heterogeneity regime. Trays were 40cmx30cmx8cm, with 3cm deep

of unfertilized topsoil. Although some degree of leaching across treatment
regions may have occurred, but the use of slow release fertilizer pellets ensured
that the highest concentration of nutrients remained centered around the
prescribed treatment location.
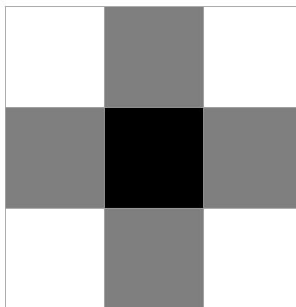
Figure 1.1 A 3x3 array of resource patches. White boxes are farther away from the center than
grey boxes. To account for this, 4 of the 8 boxes were fertilized in a random fashion. Black
indicates the planting location of the initial plant (no fertilizer).

Minimal heterogeneity was expressed through equal levels of nutrients across all
nine of the regions. In establishing maximum heterogeneity treatments the
regions of the trays were separated into two groups; those directly adjacent to
the central well and those diagonal (Figure 1.1). Randomization was applied
independently for both groups. This separation was necessary due to the
trigonometric relationship intrinsic to squares; meaning that there exists a greater
distance between the central and diagonal wells as compared to those
immediately adjacent. The maximum heterogeneity in our study is represented
by the treatment where half of the peripheral wells had additional nutrients added
through the NPK fertilizer and the other half received none. Taking into account

the trigonometric design, two of the four adjacent regions have added nutrient load. The same is true of the four diagonal regions. The remaining region, the central area that contains the initial individual, also received no additional nutrient load. The lack of excess nutrients in the initial well was chosen to avoid clustering of offspring in the initial well and to encourage expansion into the "environment" of the other wells.

Three treatments were run for the initial trial; fertilizer applied with minimum heterogeneity, fertilizer applied with high heterogeneity, and a control. All fertilizer treatments contained a total of 21 grams of Miracle-Gro® slow release fertilizer pellets per tray. The minimum heterogeneity treatment has that amount distributed equally across 8 areas of the tray, resulting in approximately 2.62 grams of fertilizer per region. The high heterogeneity treatment condenses the available resources into half the space, with 5.25 grams applied per region. The choice of which regions will receive fertilizer was determined by random generation using a custom script written in *Mathematica* 8.0. Control trays received no additional nutrients beyond what is present in top soil used to construct the trays. Multiple trays were set up to provide a total of 10 replicates. The experiment was run for a period of 3 months, between September and December of 2011.

Behavioral shifts between seed-based and clonal reproduction were observed through counts of produced inflorescences. At the end of the study counted

runners to directly account for asexual behavior; measurements taken at this time included above ground biomass and the number of adventitious roots developed. All of these measurements are dependent upon the overall size of an individual, thus we normalized the data to represent allocation per unit of total biomass. We chose to represent sexual reproduction through flower production rather than seed count because of growing conditions and isolation from pollinators due to being grown in the greenhouse. Additionally, we are most interested in the plant's allocation of resources to reproductive strategies, and not the effectiveness of pollination syndromes. Flower formation represents the initial energy investment towards sexual reproduction, and thus it is a useful proxy for resource distribution.

Data was analyzed using *Mathematica* 10.0.4 to test for normalcy, perform mean difference testing, and ANOVA.

Power Analysis

Due to the inconclusive results of the preliminary experiment, we refined the experiment for a second run. We used the parameters from out first experiment to perform a power analysis using a custom script in *Mathematica,* which allowed us to determine the appropriate number of replicates to run (figures 1.2 and 1.3 below).
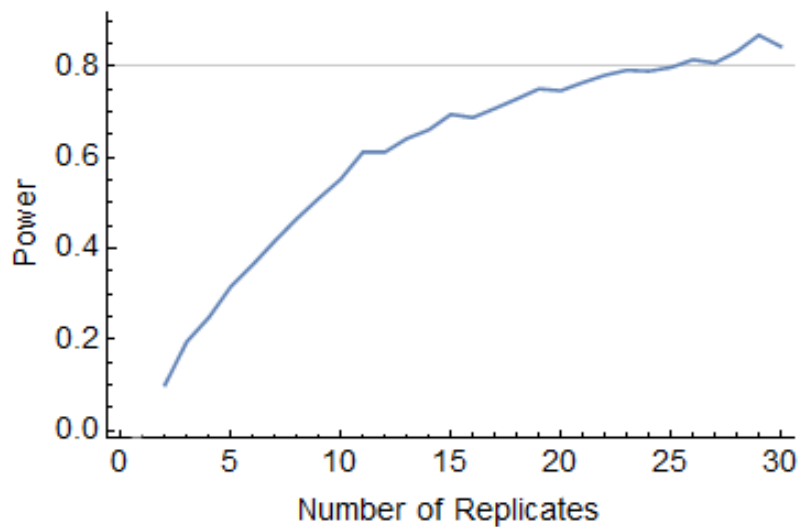
Figure 1.2 Power analysis for the number of replicates needed to detect 1g difference of horizontal stem biomass between treatments. 80% power occurs at 21 replicates, while 15 replicates results in 70% power.
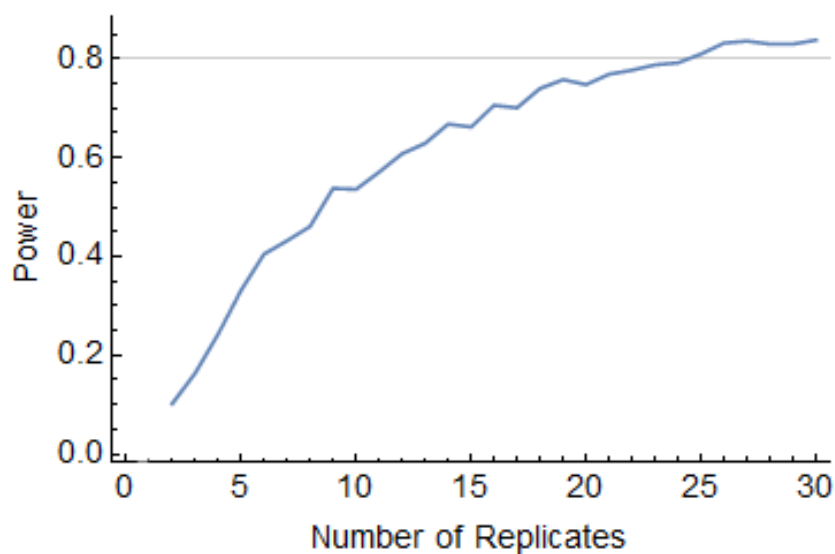
Figure 1.3 Power analysis for the number of replicates needed to detect 4g difference of total plant biomass between treatments. 80% power occurs at 24 replicates, while 15 replicates results in 70% power.

Experiment 2

Our second experiment was designed to test our hypotheses based on the following diagram.
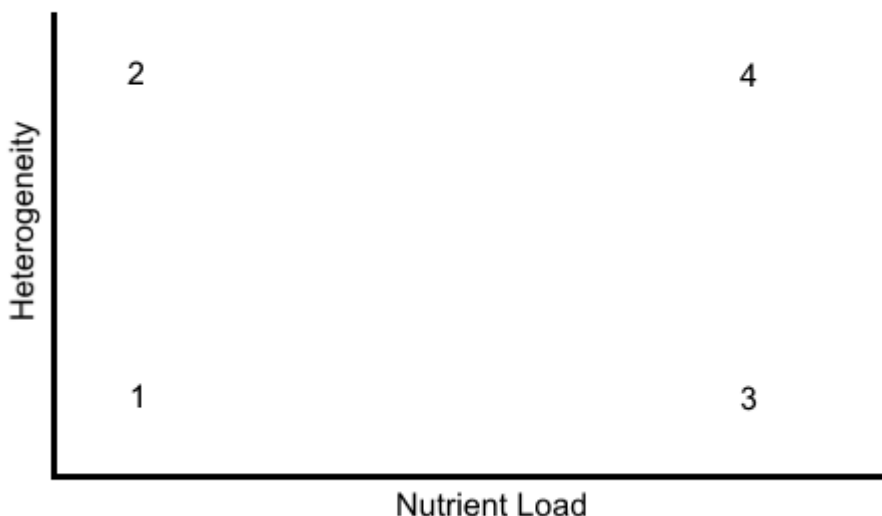
Figure 1.4 Showing 4 experimental treatments to be tested.

Figure 4 illustrates our 4 treatment model, comparing both heterogeneity and total nutrient load in combination. Treatments 1 and 3 have a uniform distribution of resources, while treatments 2 and 4 have their resources concentrated into two out of the four quadrants. Treatments 3 and 4 both received limited amounts of fertilizer, 2g each, while treatments 3 and 4 received 12g each.

Unfortunately, the limited amount of space in the greenhouse prevented us from using the number of replicates needed for 80% power. We instead were forced to settle for 70% power, which corresponded to 15 replicates per treatment. We achieved this higher number of replicates by utilizing smaller trays. The new trays

were constructed from aluminum catering trays measuring 30cm x 24cm x 6.6

cm, modified to allow for drainage. Soil was 3cm deep. We ran four treatments,

as described above, allowing us to test both heterogeneity treatments under high

and low nutrient loads, with 15 replicates for each treatment. Due to limited

space in the smaller trays we redesigned the plots from 3x3 grids down to 2x2

grids, as depicted below in figure 1.5. All other conditions were held the same,

including fertilizer levels. A schematic explanation of our treatments can be found

below.
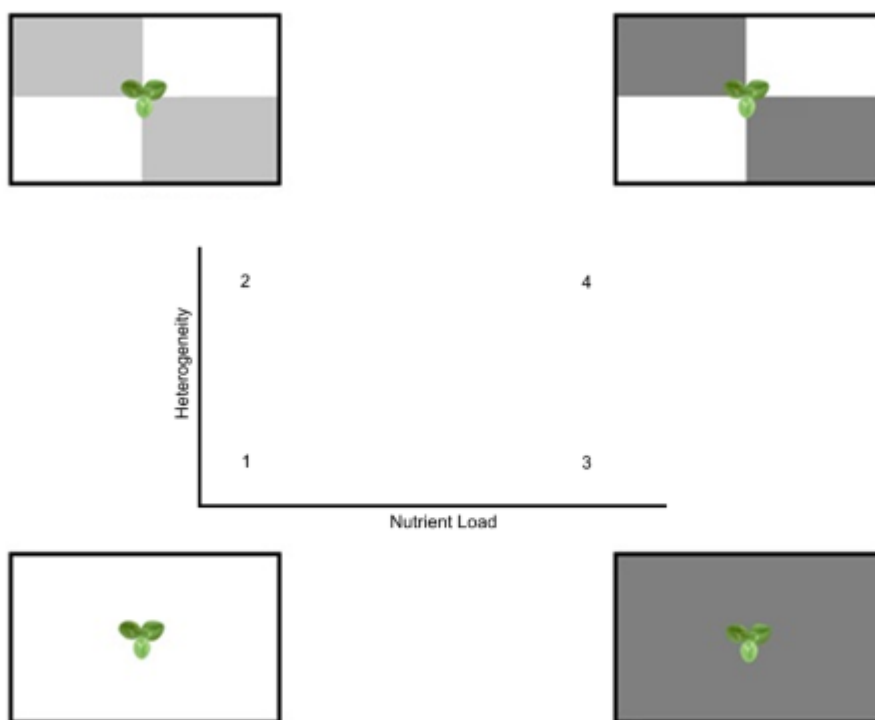
Figure 1.5 Treatments for greenhouse study #2. Shading indicates fertilizer application.

Experiment 2 was run for 2 months, from 2/24/2013 to 4/20/2013. At the end we recorded the mass of leaf, horizontal stem, and adventitious root tissue biomass. We also performed direct measurements of the number of flowers and total dry biomass.

Our 4 way comparison of nutrient load and heterogeneity was largely inconclusive, as 15 replicates of each treatment provided insufficient power to detect a signal at the scale we considered (see power analysis section above). We were, however, able to pool treatments together by heterogeneity, bringing the number of replicates up to 30. This was sufficient power to detect a significant difference between the heterogeneous and homogeneous treatments, as demonstrated in the Results of Experiment 2 section below.

Results of Experiment 1

Untransformed data from experiment #1provided little information; a comparison of the aboveground biomass, runner biomass, total root count, and adventitious root count for experiment #1 can be seen below (figures 1.6-1.9). All of these parameters were non-significant, due to variation and low number of replicates (aboveground biomass p=0.142; runner biomass p=0.274; total root count p=0.145; adventitious root count p=0.166).
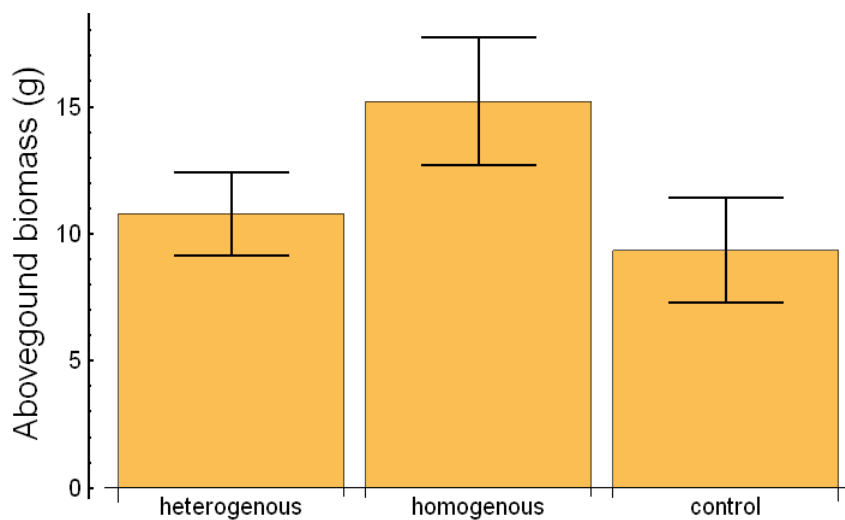
Figure 1.6. Aboveground biomass across 3 treatments (ANOVA p=0.142). Error +/- 1 SE.
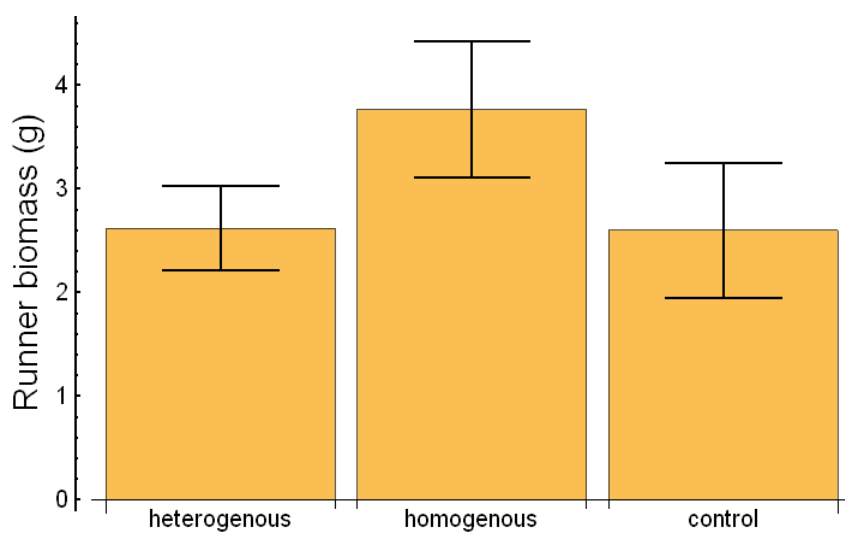
Figure 1.7. Runner biomass across 3 treatments (ANOVA p=0.274). Error +/- 1 SE.
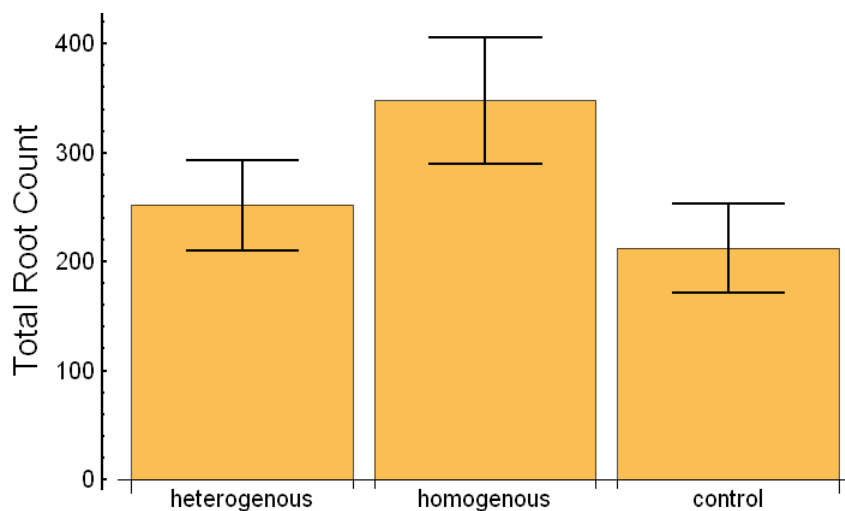
Figure 1.8. Adventitious root counts across 3 treatments (ANOVA p=0.145). Error +/- 1 SE.
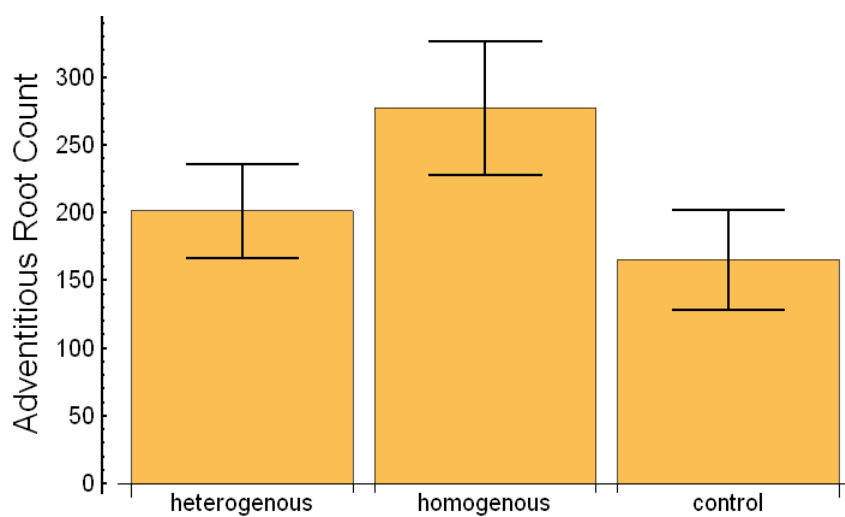
Figure 1.9. Adventitious root counts (not including the central location) across 3 treatments

(ANOVA p=0.166). Error +/- 1 SE.

Normalizing the parameters to account for wide variations in each individuals'

biomass was more informative. A one-way ANOVA analysis of inflorescence

count normalized by total biomass was found to be significant (p=0.0339). The

control treatment was uniformly the lowest treatment in terms of flowers per unit biomass, but the heterogeneous and homogeneous treatments were non-significant (Figure 1.10).
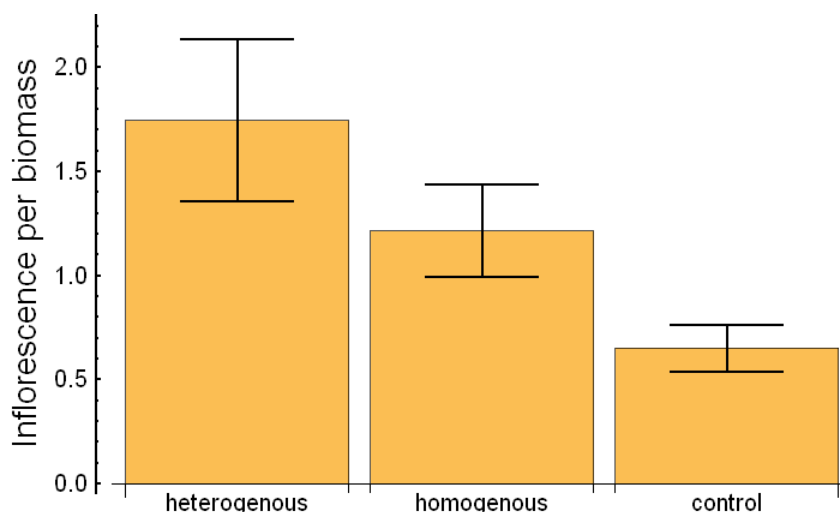
Figure 1.10 Inflorescence counts across 3 treatments, after being normalized by for total above ground biomass (ANOVA p=0.0339). Error +/- 1 SE.

A Bonferroni post-hoc test showed that the heterogeneous treatment resulted in a higher number of flowering structures per total biomass (p= 0.022) compared to control, indicating a greater focus on sexual reproduction regardless of the size of the plant. When compared against the homogenous treatment the post-hoc test showed that the effects were not significant (p= 0.253), indicating that significance was driven by nutrient levels rather than distribution. We verified this by comparing the homogenous treatment to the control, which also showed a significant increase in relative investment in flowering structures as compared to

the control (p= 0.046). These results were closely mirrored by the observation of raw inflorescence counts, as seen in figure 1.11.
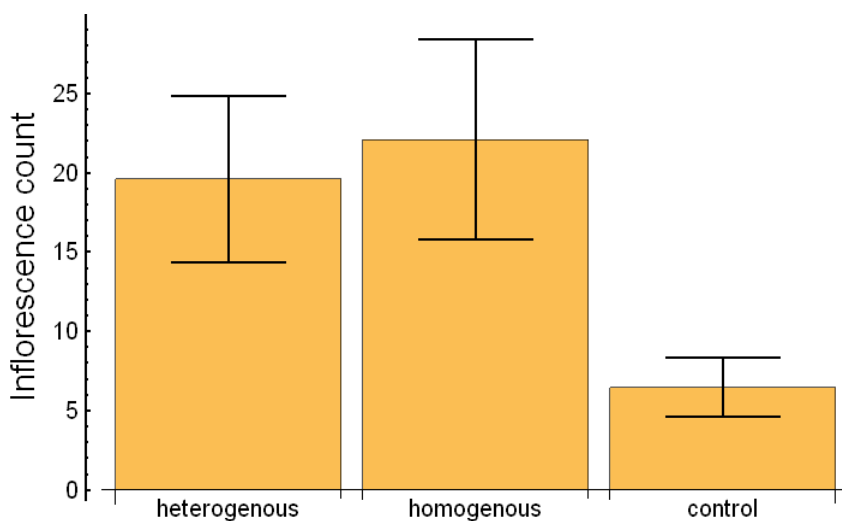
Figure 1.11 Raw inflorescence counts across all three treatments (ANOVA p=0.084). Error +/- 1 SE.

Wide variation prevented us from finding any significant difference in raw flower count though ANOVA (p= 0.084). Raw flower counts also did not vary between heterogeneous and homogenous treatments (p=0.764). Both fertilizer treatments featured significantly higher maximum flower counts than control (homogeneous p=0.0375, heterogeneous p=0.0370).
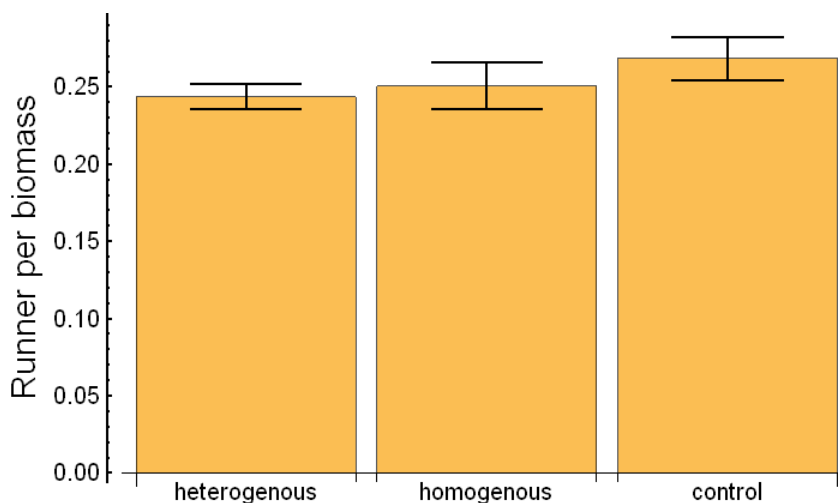
Figure 1.12 Proportion of biomass allocated to horizontal stem growth. Lower values indicate a higher investment in other tissues, such as leaves or flowers (ANOVA p=0.393). Error +/- 1 SE.

In addition to the number of flowers per unit of biomass, we also compared the ratios of horizontal stem biomass to total aboveground biomass. Figure 1.12 shows this comparison of horizontal stem biomass as a proportion of total biomass. Despite showing a slight trend, the overall effect was not significant (p= 0.393). The heterogeneous treatment had a lower proportion of plant biomass directed toward horizontal growth, possibly indicating a greater emphasis on leaf and flower development; however significance is so low that we cannot consider it to be supporting evidence.

Results: Experiment 2

Similar to experiment #1, the untransformed data from experiment #2 provided very little information. The results of experiment number 2 can be seen below (Figures 1.13-1.20). Due to low statistical power, none of our parameters showed any significant difference between the 4 treatments when compared through

ANOVA (horizontal stem allocation, p=0.119; inflorescence per biomass p=0.561;

adventitious root allocation, p=0.239).
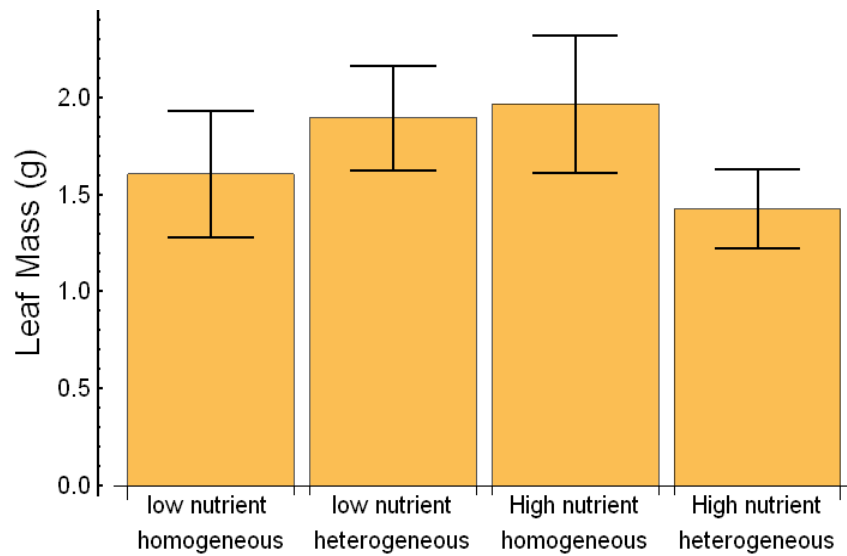
Figure 1.13 Leaf mass across four treatments of experiment 2. ANOVA found no significance

(p=0.534). Error +/- 1 SE.
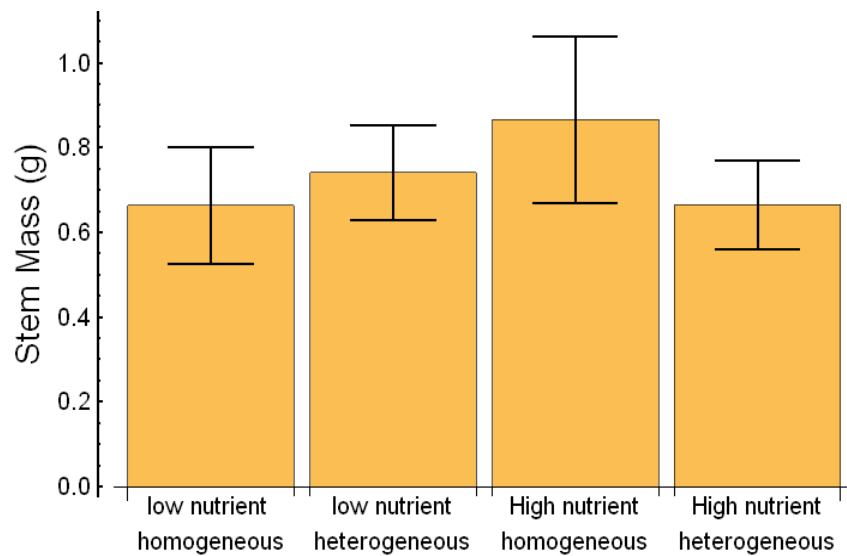
Figure 1.14 Stem mass across four treatments of experiment 2. ANOVA found no significance

(p=0.720). Error +/- 1 SE.
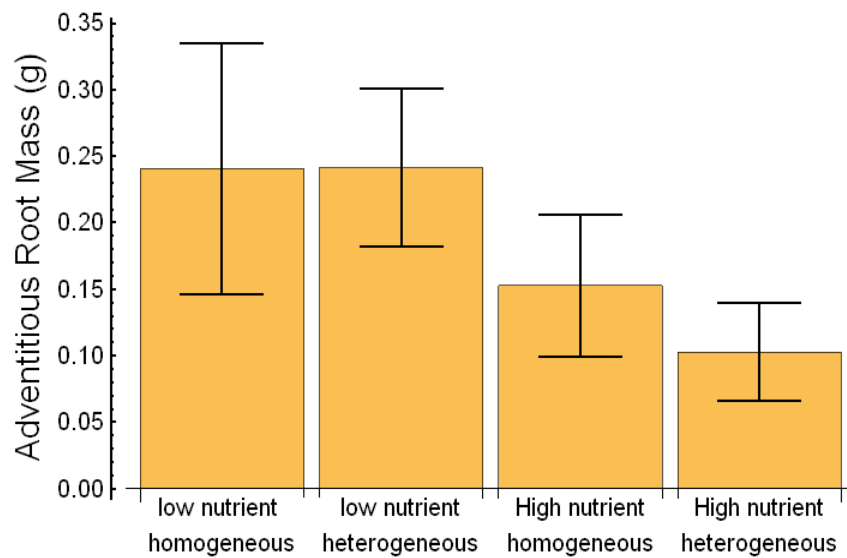
Figure 1.15 Adventitious root mass across four treatments of experiment 2. ANOVA found no significance (p=0.343). Error +/- 1 SE.
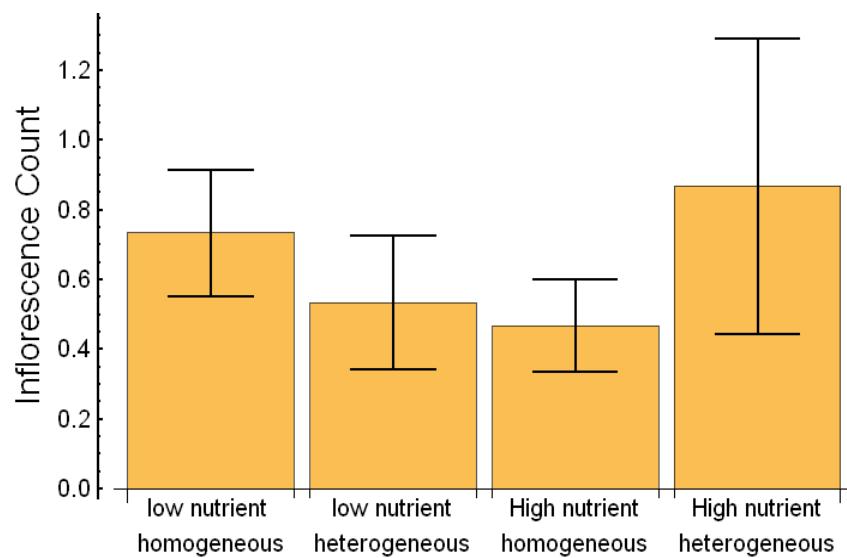
Figure 1.16 Inflorescence counts across four treatments of experiment 2. ANOVA found no significance (p=0.681). Error +/- 1 SE.
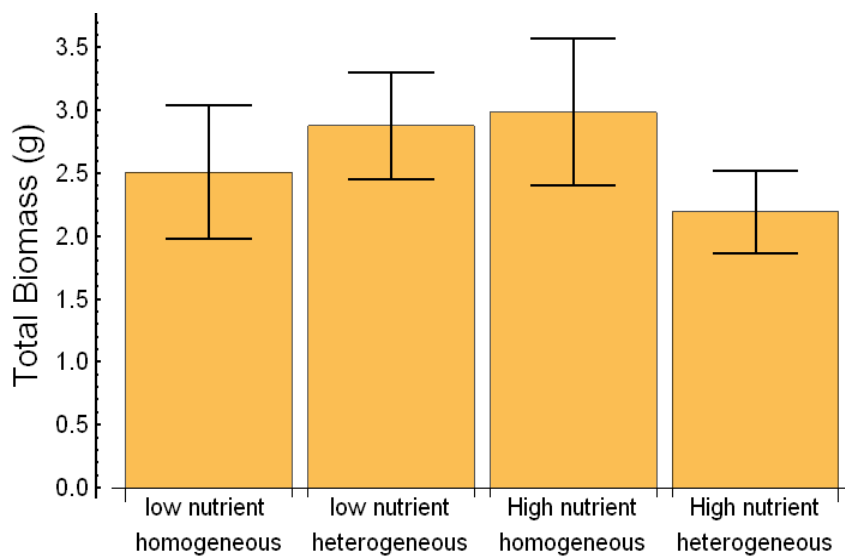
Figure 1.17 Total biomass across four treatments of experiment 2. ANOVA found no significance

(p=0.637). Error +/- 1 SE.
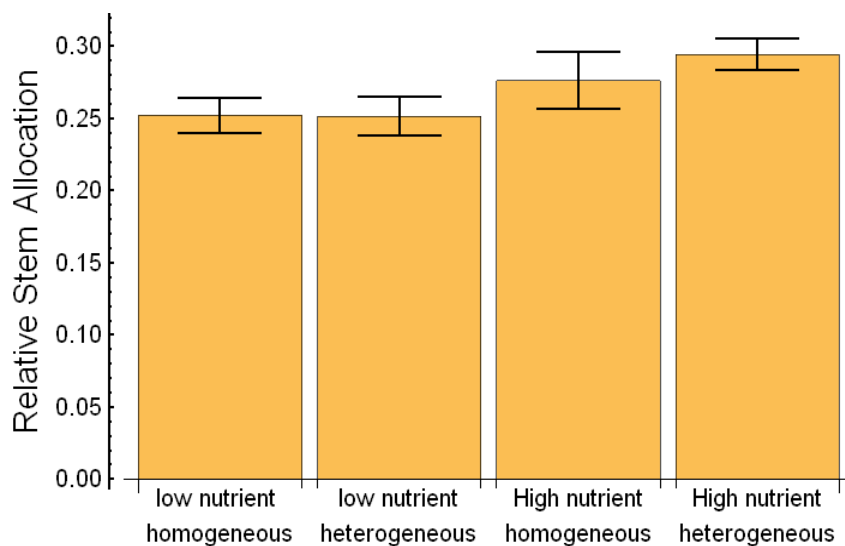
Figure 1.18 Allocation to horizontal stem as a proportion of total biomass across four treatments

of experiment 2. ANOVA found no significance (p=0.119). Error +/- 1 SE.
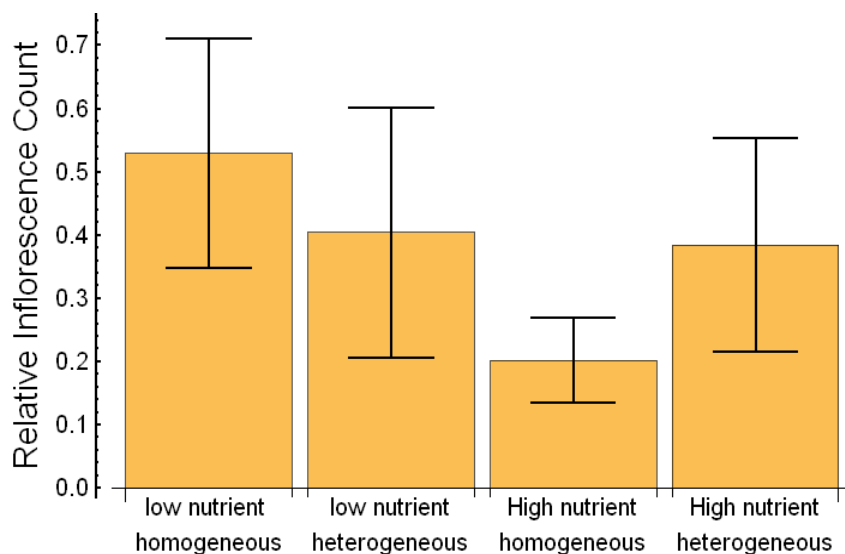
Figure 1.19 Inflorescence counts normalized for biomass across four treatments of experiment 2. ANOVA found no significance (p=0.561). Error +/- 1 SE.
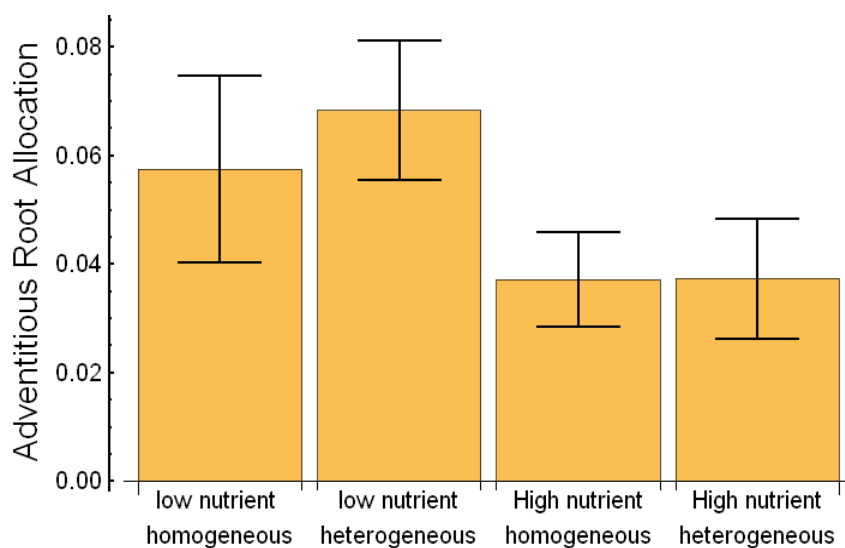
Figure 1.20 Adventitious root allocation normalized for biomass across four treatments of experiment 2. ANOVA found no significance (p=0.239). Error +/- 1 SE.

A two-way ANOVA of our data showed that heterogeneity treatments demonstrated no significant difference in any of our comparisons; leaf mass (p= 0.669), stem mass (p=0.664), adventitious root mass (p=0.706), inflorescence

count (p=0.699), and total biomass (p=0.657). Two-way ANOVA of the

normalized parameters also showed that heterogeneity treatments lacked any

significant differences (Figure 1.21-1. 23) in relative stem allocation (p=0.3558),

relative inflorescence count (p=0.860) and relative allocation to adventitious roots

(p=0.676).
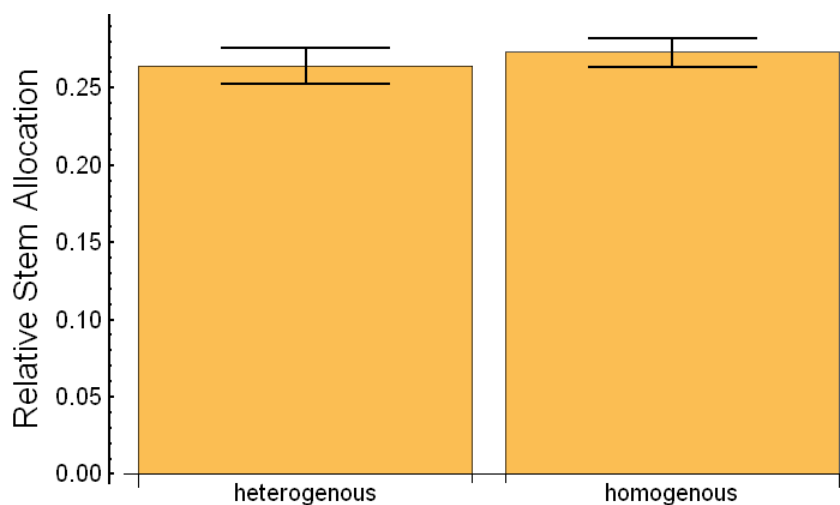
Figure 1.21 Relative stem allocation showed no difference when compared across pooled
heterogeneity treatments (p=0.558). Error +/- 1 SE.
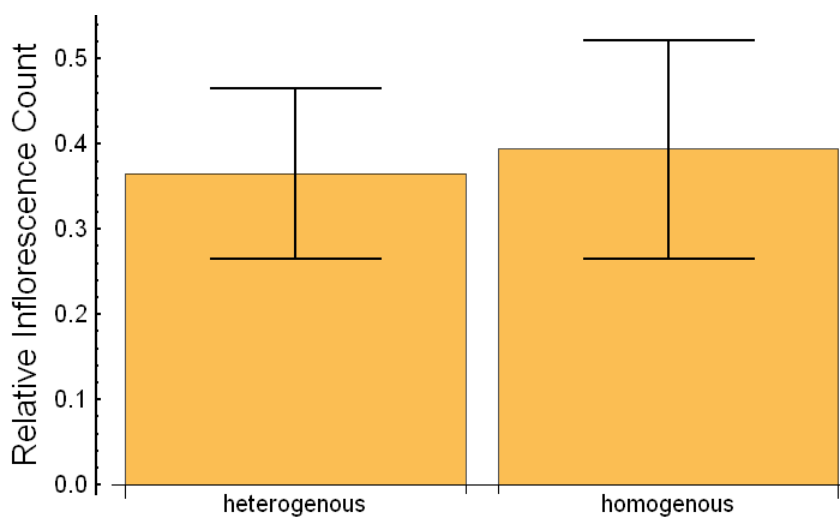
Figure 1.22 Inflorescences per total biomass showed no difference when compared across
pooled heterogeneity treatments (p=0.860). Error +/- 1 SE.
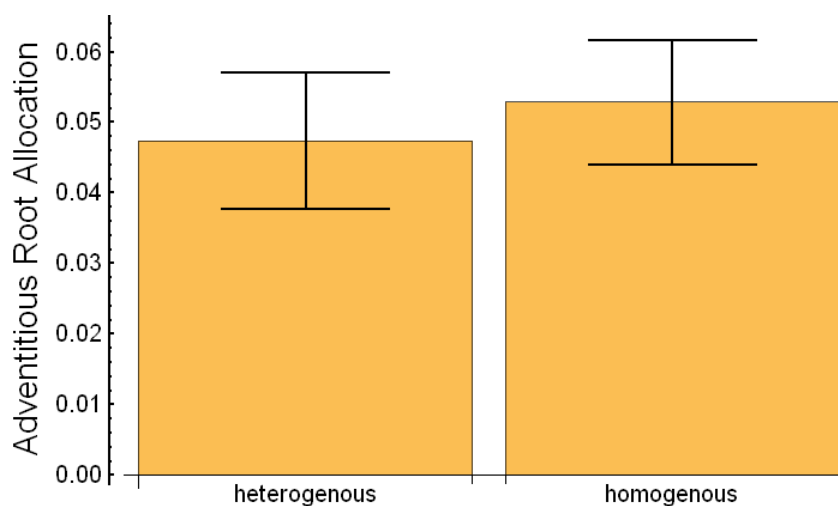
Figure 1.23 Relative allocation to adventitious roots showed no difference when compared across

pooled heterogeneity treatments (p=0.676). Error +/- 1 SE.

The two-way ANOVA did, however, show significance driven by nutrient levels.
We observed a significant increase in the proportion of total biomass allocated to
horizontal stem growth under the high nutrient treatments (figure 1.24,
p=0.0232). Individuals growing in a more nutrient rich environment demonstrated
a growth form that favored horizontal spread, as demonstrated by the higher
amount of biomass dedicated to producing new horizontal stem tissue. Plants in
the low nutrient treatments tended to exhibit less tendency to spread, and made
use of more localized resources.

Relative allocation of biomass to adventitious root development, adventitious root
tissue that was not a part of the original cutting mass, also showed significance
(figure 1.25, p= 0.0477), with a higher amount of root growth found in low nutrient
treatments. This indicates a greater level of resource scavenging from a

generalized area, while high nutrient treatment plants deployed adventitious roots in a more limited area.

Contrary to experiment 1, the nutrient levels in experiment 2 caused no significant difference in inflorescence development between treatments (figure 1.26, p=0.289).
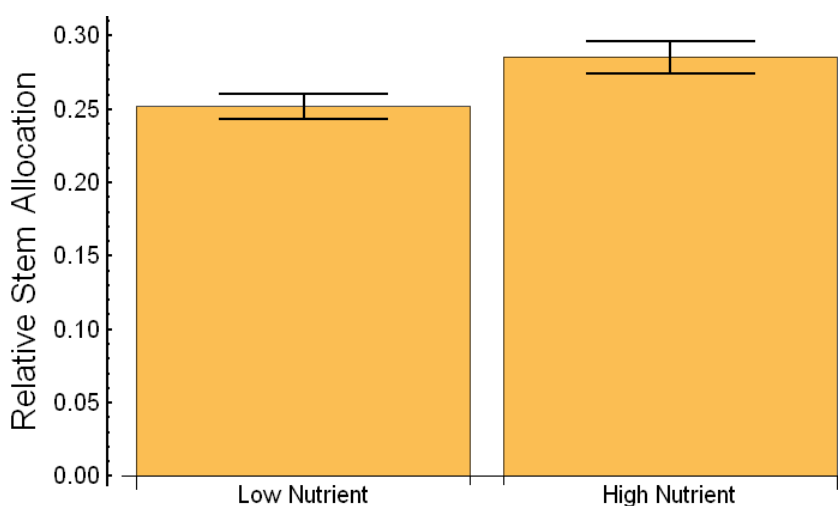
Figure 1.24 Allocation of resources toward horizontal stem growth differs in response to environmental nutrient load (p=0.0234). Error +/- 1 SE.
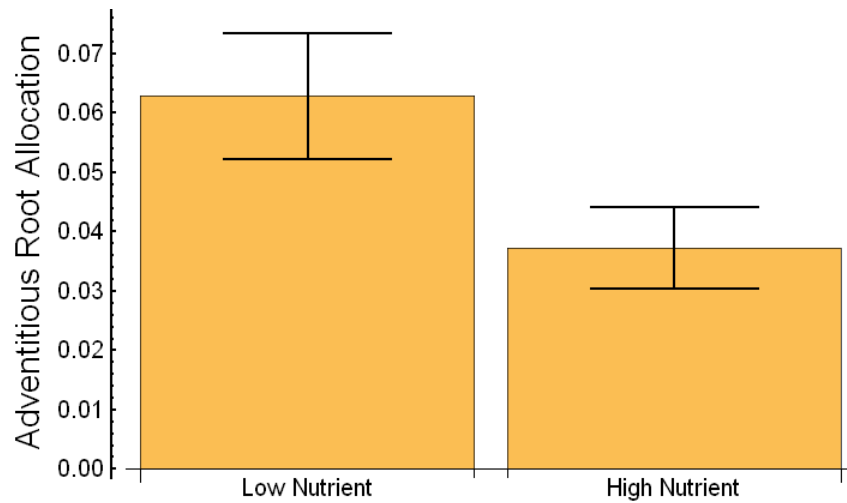
Figure 1.25 Allocation of resources toward root allocation differs in response to environmental

nutrient load (p=0.0477). Error +/- 1 SE.
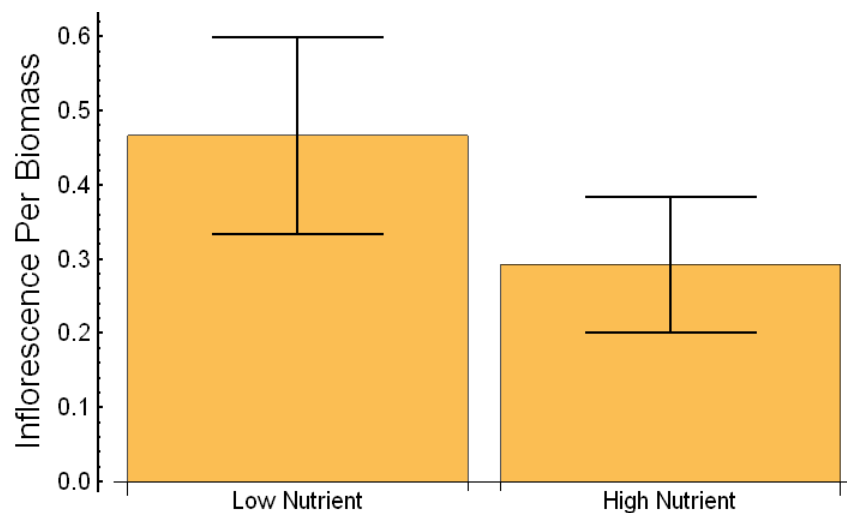
Figure 1.26 Number of inflorescences per unit biomass did not differ in response to environmental

nutrient load (p=0.289). Error +/- 1 SE.

Discussion

Ultimately we found that the level of resource abundance played a far larger role

than how those resources were distributed. Allocation to clonal growth was

enhanced under high nutrient treatments, but we found no link between resource heterogeneity and allocation toward sexual vs. clonal reproduction.

Experiment 1 did not have sufficient statistical power due to a low number of replicates (N=10), thus above ground biomass and adventitious root counts showed no significantly different behavior responses to treatments. Counts of inflorescences also did not differ significantly among treatments. The inflorescence count results, however, were confounded by the highly variable biomass measurements of our plots. Since total biomass and flower count are not independent of each other, we normalized the data by total biomass to account for the wide variation in individual performance. The normalized inflorescence data, a count of the number of inflorescences per unit of biomass, was significantly different between treatments.

In experiment 1 our comparison of flowers per unit of biomass (figure 1.10) showed that both fertilizer treatments resulted in higher levels of inflorescence production than the control (p=0.0339). However, post-hoc testing found no significant difference between the heterogeneous and homogeneous treatments, both of which had elevated nutrient load over the control treatment. Taking both into account, we concluded that the significance in flowers per biomass unit was driven by fertilizer treatment, and not by the spatial distribution of those resources. A comparison of the heterogeneity treatments was found to be non-significant (p=0.253). Further, these results indicate two things; 1) that higher

nutrient availability in clover increases flower production (flower production is nutrient limited) and 2) that a non-significant trend suggests that heterogeneity may favor sexual reproduction more than evenly distributed resources. The latter is conjecture based on the non-significant patterns of figure 1.10. Unfortunately, due to limited sample size (N=10) we were unable to draw firm conclusions between the heterogeneous and homogeneous treatments; the only significance we found showed that inflorescence production was linked to nutrient availability. Previous work has shown that plants in a poor habitat (e.g. low nutrient levels) allocate more resources to foraging (Slade & Hutchings, 1987). This explains the lower occurrence of flowering in our control, as low nutrient conditions promoted allocation of resources to vegetative tissue instead of developing inflorescences. However, once nutrients were no longer limiting we were unable to discern any difference in reproductive allocation based on heterogeneity.

We also attempted to characterize the growth patterns of the *Trifolium repens* individuals by examining the ratio of biomass allocation between horizontal stem tissue and other above ground biomass. Above ground biomass includes both flowers and leaf tissue, thus the horizontal stem ratio gives us a sense of how the individual plants utilize the surrounding space. A higher horizontal stem ratio indicates a plant that is more spread out in its environment, while a lower value indicates a more compact individual. Resource availability and distribution are well known to affect the development of stolons and rhizomes (Birch & Hutchings, 1994; Hutchings & Wijesinghe, 1997; Slade & Hutchings, 1987; Josef

F Stuefer, During, & Schieving, 1998; D. K. Wijesinghe & Hutchings, 1997; D. K. Wijesinghe & Whigham, 1997). Our results, however, were inconclusive. Given that we failed to replicate a phenomenon so widely documented, we concluded that our sample size was insufficient.

Our second experiment with *Trifolium repens* was more informative, although still dominated by fertilizer effect. We found significant results by pooling treatments together by nutrient level. This resulted in a significant difference in relative stem allocation (p=0.0234; figure 1.24), which provides insight regarding the foraging strategies of these plants. A higher relative stem allocation indicates that more biomass was being dedicated to allowing the individual to spread across the landscape, making it better suited to exploit local abundances of resources. Similar strategies have been seen in other clonal plant species as well (Birch & Hutchings, 1994; Dekroon & Hutchings, 1995; W. J. Sutherland & Stillman, 1988; D. K. Wijesinghe & Hutchings, 1997). Individuals growing in lower nutrient treatments still spread out horizontally, but this expansion was not as pronounced. The difference in clonal spread away from the original planting can be explained as a dispersal mechanism to escape density dependent fitness reduction, which is common to clonal species (Abrahamson, 1975; Michael L Cain et al., 1996; Nishitani, Takada, & Kachi, 1999; Price & Marshall, 1999).

The difference in allocation to adventitious root tissue between treatments shows that resource availability can have a significant impact on foraging strategy.

Individuals growing in a low nutrient treatment had significantly higher levels of biomass allocation to adventitious root growth than individuals in high nutrient treatments (p=0.0476). This response to the environment may be seen as a foraging strategy; in an environment with a scarcity of resources plants will allocate biomass to build foraging structures for the limiting resource. Spatial heterogeneity of resources can reverse this strategy and allow plants to allocate growth to exploit locally abundant resources, a strategy which has been widely observed in other species (Birch & Hutchings, 1994; Hutchings & Wijesinghe, 1997; J F Stuefer et al., 1996; Josef F. Stuefer, 1998; Josef F. Stuefer, Gómez, & Mölken, 2005). The more strongly heterogeneous an environment is the better the fitness return for this foraging strategy, even surpassing the performance under high quality homogeneous conditions (Hutchings & Wijesinghe, 1997; J F Stuefer et al., 1996; D. Wijesinghe & Handel, 1994). Unfortunately, we did not observe such an effect in our study.

This results was unexpected due to the clonal nature of *Trifolium repens,* which was anticipated to play a role in shifting such resource allocations. By maintaining connections between different ramets, clonal plants are able to specialize in the locally abundant resource rather than allocating growth toward accumulating scarce resources (P. Alpert et al., 2003; Peter Alpert, 1999; Hutchings & Wijesinghe, 1997; Ikegami, van Hal, et al., 2008; Josef F. Stuefer, 1998; Josef F Stuefer et al., 1994). Under this model clonal propagation is important both for reproduction and for allowing the plant to take advantage of

spatial resource heterogeneity. Instead we observed that *Trifolium repens* allocated more biomass to root development when resources were scarce and increased horizontal stem growth when resources were abundant, without regard to the spatial distribution of these resources. This indicates that *Trifolium repens* was not participating in the "division of labor" strategy commonly seen in many clonal plants labor (Hutchings & Wijesinghe, 1997; Ikegami, van Hal, et al., 2008; Ikegami, Whigham, et al., 2008; J F Stuefer et al., 1996).

We did not directly observe any impact of heterogeneity or resource abundance on reproductive allocation in our second experiment, as flower per unit of total biomass showed no significant difference between treatments. This may be due to the fact that our pooled data sets consisted of both high and low resource groups, which itself drives flower production (see experiment #1, figure1.10). However, when pooled by resource abundance we also saw no significant increase in inflorescence development between treatments (figure 1.26). This lack of significance can be taken as evidence of indirect effect of resource abundance on reproductive allocation. If the elevated nutrient load had no effect on reproductive strategy then we would expect that both inflorescence production and horizontal stem production would increase by similar amounts. Instead we observed that the high nutrient treatment drove an increase in clonal stem growth, but no corresponding increase in sexual structures was observed. The difference in biomass increase is important because plants have limited resources, and allocation of these resources toward clonal growth means a

necessary diversion away from investing in the structures needed for sexual reproduction (Bai et al., 2009; Bazzaz, Chiariello, Coley, & Pitelka, 1987; F. Liu et al., 2008; Metcalf et al., 2009; Thompson & Eckert, 2004). This means that the significant increase we observed in biomass allocation toward horizontal stem development in experiment #2 also represents proportionally less allocation for flower development. Thus, although horizontal stem development may be primarily a foraging response, it also represents a shift in relative reproductive investment.

Based on this interpretation, our study shows that resource abundance acted as a driving force in favor of greater clonality (e.g., investment in clonal spread) in *Trifolium repens*. This conclusion is also supported by existing literature, which shows a link between resource level and sexual-asexual trade-offs (Piquot et al., 1998; S. Sutherland & Vickery, 1988). High nutrient abundance has been shown to favor clonal structure (Caraco & Kelly, 1991; B. Oborny et al., 2000), and higher nitrogen availability has been directly shown to increase clonality (Bai et al., 2009), both of which are in agreement with our findings. We offer our findings as further support for the predictions made by (Gardner & Mangel, 1999; Sakai, 1995) that favorable conditions act to promote increased clonal growth. Sexual reproduction, in contrast, is largely promoted by density dependent mechanisms (Hamilton & May, 1977; Kleunen, Fischer, & Schmid, 2001; Nishitani et al., 1999; Olejniczak, 2003; Reekie, 1991). The significant effects observed during experiment #1 were likely driven by these density dependent mechanisms. The

longer run time for the first experiment allowed both fertilizer treatments of *Trifolium* enough time to grow to a higher density, which would have promoted the development of inflorescences.

We propose a follow-up experiment run over a longer period of time, tracking the temporal changes in resource allocation of our *Trifolium repens* system. A longer study would allow enough time for these density dependent mechanisms to come into play. Allowing individuals more time to grow may also help to investigate reproductive allocations more fully, as the trade-off between sexual and asexual reproduction has been linked to the size of individuals (M L Cain & Damman, 1997; Hartnett, 1990). Such an experiment would also allow individuals a chance to fully explore the extent of their environment, and eliminate situations where an individual under heterogeneous conditions immediately stumbled into a high resource patch. Based on this, we propose that a temporal study would show an immediate increase in clonal growth, followed by a gradual trade-off as clonality declines in favor of more allocation to sexual reproduction.

Having investigated the role of environmental conditions on allocation to sexual and asexual reproduction, in the next chapter we investigate environmental factors which affect the fitness of sexual propagules and the potential role this has on the return on investment in sexual structures.

Chapter 2: Land-use Legacy and Germination in *Ailanthus altissima*

Introduction

In the previous chapter I examined the role of environmental signals in determining the reproductive strategies of plants, and the allocation between sexual and asexual reproduction. Existing literature showed many different factors that affect this, including soil composition (D. K. Wijesinghe & Hutchings, 1997), habitat quality (Sakai, 1995), environmental heterogeneity (Birch & Hutchings, 1994; Michael L Cain et al., 1996; Rautiainen et al., 2004; J F Stuefer, 1996; W. J. Sutherland & Stillman, 1988), and capability for resource translocation between ramets (J F Stuefer et al., 1996; D. Wijesinghe & Handel, 1994). Expanding on this knowledge, we demonstrated a proof of concept showing that under controlled greenhouse conditions we can experimentally manipulate reproductive allocation in *Trifolium repens*.

Building on this foundation, we recognized that allocation to the development of flowers and seeds does not immediately confer an increase in fitness. Flowers, fruits and seeds are complex structures which require a large investment of resources to develop. All of these structures require complex events to meet their function; tissue development, pollination, germination, etc. are examples for this. If any of these crucial steps fail then no offspring is created and all of the resources invested thus far are wasted. Despite this risk, the adaptive benefits,

complex dynamics of population structure and genetic drift act to maintain sexual reproductive strategies (Otto, 2009).

Our next step was to move out of the controlled greenhouse environment and to test environmental effects on plants which had grown in their natural environment. Recognizing that controlling experimental variables in an outdoor setting is not viable, we chose to work in the greenhouse with two extreme conditions and to directly observe relative differences in reproductive output between these groups. The field conditions that we examined were pollution caused by prior industrial development, hereafter referred to as brownfield and non-brownfield sites. These categories are supported by existing literature shows evidence of human development impacting plant reproductive strategies (Kettenring et al., 2010; McCormick, Kettenring, Baron, & Whigham, 2010; Rusterholz et al., 2009; Warburton, James, Fripp, Trueman, & Wallace, 2000). Brownfield sites were chosen to have high levels of industrial development, either historically or currently, while non-brownfield sites were chosen by the lack of the former site characteristics We chose this brownfield/non-brownfield comparison because our industrial sites were known to have substantial levels of heavy metal contamination (Gallagher, Pechmann, Bogden, Grabosky, & Weis, 2008b; United States Environmental Protection Agency, 1994; US Army Corps of Engineers, 2005). Although heavy metal contaminants are detrimental, many invasive species have an elevated tolerance to heavy metals. By determining if heavy metal contamination in the environment has a detrimental effect on a species

seed viability we can get a better sense of what selective pressures are acting to alter the plant's reproductive strategies.

With field sites chosen we than had to decide which dependent variable we would use to test for an effect. Considering the various processes required for successful sexual reproduction, we had many potential variables with which to continue our investigation. We chose to examine the environmental effects on seed germination, as germination success is quite simple to test but would yield useful information regarding individual fitness through offspring survival. Although there are many different fitness benefits involved in seed germination (e.g. dispersal, seed bank accumulation, recombination, etc.) these benefits are irrelevant if the seed fails to germinate (Harper, 1977). A non-germinated seed has a fitness of zero, and thereby confers a fitness loss to the parent plant as well. Therefore, we instigate the role of germination, a process which must be successful before other factors come into play.

The species of choice for this experiment, and the following chapter, is *Ailanthus altissima*, commonly known as the "Tree of Heaven" and one of the most widely spread invasive trees in both the United States and across Europe (Invasive Species Specialist Group, 2015; United States Department of Agriculture, 2016). Originally native to NE Asia (China, etc., *Ailanthus* is an many early successional tree that thrives in disturbed habitats and tolerant to both soil and air pollution, leading it to be a common sight among urban settings (Kostel-Hughes, Young, &

Wehr, 2014; Pannill, 2000; Virginia Department of Forestry, 2009). *Ailanthus altissima* produces both seeds and root suckers in high volume, allowing it to rapidly spread into open areas (Invasive Species Specialist Group, 2015; Kowarik & Säumel, 2007; Martin & Canham, 2010; United States Department of Agriculture, 2016). When combined with the possibility of creating multiple crops of seeds under optimal conditions, estimates of seed production from a single tree can be as high as 300,000 seeds in a single year (Pannill, 2000; Virginia Department of Forestry, 2009). Additionally, despite the high volume of low cost seeds produced, most of these seeds are actually highly viable (González-Muñoz, Castro-Díez, & Fierro-Brunnenmeister, 2011; Virginia Department of Forestry, 2009). This high seed production is the result of large number of tiny flowers per inflorescence and the fact that *Ailanthus* pollination is highly generalist in nature, utilizing whichever species are in local abundance (Aldrich, Brusa, Heinz, Greer, & Huebner, 2008). The seeds have an elongated samara shape which aids in wind dispersal, allowing for more rapid spread into new areas (Invasive Species Specialist Group, 2015; Virginia Department of Forestry, 2009). Studies have also shown that *Ailanthus* samara are able to retain viability and be transported long distances via waterways, such seeds even demonstrated higher rates of generation compared to controls (Kaproth & Mcgraw, 2008; Kowarik & Säumel, 2008). Ailanthus seeds were capable of remaining buoyant for 24 hours in riverine conditions, giving the seeds a much farther distribution than expected under wind dispersal. In addition, these seeds often performed better at germinating than terrestrial seeds, indicating an

unexpected increase in propagule pressure to downstream habitats. Additionally, *Ailanthus altissima* demonstrates a strong propensity for asexual reproduction, a trait often seen in many invasive plants (Pysek, 1997; Williamson & Fitter, 1996a). Vegetative fragments of *Ailanthus* have been shown to be capable of propagation after extended immersion in flowing water, indicating that trees can utilize waterways to extend the range of both sexual and asexual reproduction (Kowarik & Säumel, 2008). Such secondary propagation can enhance the range of dispersal by as much as two orders of magnitude as compared to wind-based dispersal (Kaproth & Mcgraw, 2008). Root suckers proliferate rapidly, even with the removal of the parent tree, requiring removal treatments to be continued for several seasons. (Espenschied-Reilly & Runkle, 2008; Invasive Species Specialist Group, 2015). The growth of these root suckers has actually been hypothesized to increased in response to such control efforts, making efforts to contain *Ailanthus altissima* populations more difficult (Pannill, 2000).

Ailanthus altissima is well known as a particularly nasty invader of disturbed areas, and possesses many of the characteristics associated with early successional species. However, despite these early successional traits, encroachment of *Ailanthus altissima* into established communities has become a concern in recent years (Espenschied-Reilly & Runkle, 2008; Knapp & Canham, 2000; Landenberger, Kota, & McGraw, 2007; Martin & Canham, 2010). If these observations hold true and an aggressive invasive species is able to expand its range into areas which had previously been thought safe, then understanding the

reproductive mechanisms of this species is of critical importance. In light of these

discoveries, the relative viability of *Ailanthus* seeds could be of great ecological

importance, as a reduction in seed viability could offset some of the propagule

pressure on the surrounding ecosystems.

Reproductive Balance in *Ailanthus altissima*

The spread of invasive species is linked to many factors, including hydrology,

human traffic and land development activity (Peter Alpert, Bone, & Holzapfel,

2000; Alvarez et al., 2005; A. H. Baldwin et al., 2010; Barney, 2006; Engloner et

al., 2010; Ishii & Kadono, 2002; Silliman & Bertness, 2004; Williamson & Fitter,

1996a, 1996b). *Ailanthus altissima* is rarely found in wetlands, so impacts from

hydrology would be mostly limited to water retention in the soil. (Although

Ailanthus altissima has been shown to use waterways for both seed and

vegetative fragment dispersal, see: (Kowarik & Säumel, 2008; Säumel &

Kowarik, 2010)). Human development, however, is a factor which is quite

prevalent in many *Ailanthus* habitats. Human development has been shown to be

positively correlated with seed development for *Phragmites* (Chambers et al.,

1999; Saltonstall & Court Stevenson, 2007), and we investigated to see if a

similar pattern could be found with *Ailanthus altissima*. Our prediction, however,

ran in the opposite direction. The reproductive shift in *Phragmites* was driven by

a fertilizer effect from nitrogen run-off into waterways, which Ailanthus would not

be exposed to. Instead, the influence of human activity on *Ailanthus* would likely

be detrimental as our urban sites were post-industrial brownfield sites with a

combination of poor soil and elevated metal loads (Gallagher et al., 2008b; United States Environmental Protection Agency, 1994; US Army Corps of Engineers, 2005). Poor growing conditions for parents should also be reflected in their offspring; based on this we developed the following hypothesis for *Ailanthus altissima* in brownfield and non-brownfield sites.

Hypothesis 2.1: Germination rates of seeds from brownfield sites will be lower than the rate for seeds from non-brownfield sites.

We also tested a secondary hypothesis, based on the premise that urban land use and green-space policy can help to mitigate some of these detrimental effects. To test this we expanded our sampling to include individuals from Illinois, looking at brownfield sites with suburban land use policies (United States Environmental Protection Agency, 1994).

Hypothesis 2.2: Germination rates of seeds from sites in Illinois will be higher than the rate for seeds from sites in New Jersey.

Brownfield sites, particularly places like Liberty State Park with its old railroad yards, have a much higher concentration of harmful compounds like heavy metals (Gallagher, Pechmann, Bogden, Grabosky, & Weis, 2008a; Gallagher et al., 2008b; US Army Corps of Engineers, 2005). One of the invasive characteristics of *Ailanthus altissima* that helps it to succeed is a very high

tolerance of such contamination, however these toxic materials may still impose a physiological stress upon the plant. We investigate to see if the deleterious effects of heavy metals extend to the offspring by comparing the successful germination rates for seed collected from both brownfield and non-brownfield sites. Lower germination rates would reduce the fitness benefits of seed investment, giving a relative advantage to the alternate (clonal) strategy.

This proposed exchange between sexual and asexual strategies is supported by existing literature, if the form of both modeling and field studies. Dorken and Van Drunen looked at reproductive systems from an evolutionarily stable strategy (ESS) approach, and found that clonal reproduction had a negative impact on the fitness of female functions (Dorken & Van Drunen, 2010). Although the causal relationship runs the other direction, the Dorken and Van Drunen model predicts that clonality selects against female reproductive behavior (e.g. seed production) while our hypothesis predicts that lower female fitness will drive clonality, both approaches agree that clonality and female function are inversely related. This is also supported by other work, which shows a tradeoff between sexual and asexual allocation (Groenendael, Klimes, Klimesova, & Hendriks, 1996; Harada & Iwasa, 1994; Huber & During, 2000; Stearns, 1989, 1992).

Methods

We tested these hypotheses by performing a greenhouse study using seed germination techniques as put forth by (Marshall & Furnier, 1981). Seed samples

were collected from sites in northern and central New Jersey and west-suburban Illinois. Both regions had already been well characterized in previous works (Aldrich et al., 2008; Gallagher et al., 2008b). Both regions had brownfield sites, although the industrial parks of suburban Illinois had much higher levels of green space than those of northern New Jersey. The number of sites and individuals sampled for our study can be seen in table 2.1 below.

Table 2.1. Samples used in germination study taken from both Illinois and New Jersey sites.

Site	State	Trees sampled	Seeds per tray	Land use History	Latitude	Longitude
Veteran's Park	IL	7	50	Non-brownfield	41°45'32.13"N	88° 8'25.04"W
Batavia	IL	2	50	Brownfield	41°51'23.08"N	88°17'22.61"W
Riverwalk	IL	6	50	Non-brownfield	41°46'4.64"N	88° 8'49.26"W
Liberty State Park	NJ	8	50	Brownfield	40°42'14.78"N	74° 3'1.13"W
Hutchinson Memorial Forest	NJ	5	50	Non-Brownfield	40°29'57.98"N	74°33'50.83"W
Newark	NJ	2	50	Brownfield	40°44'23.29"N	74°10'37.58"W
Watchung Reservation	NJ	5	50	Non-Brownfield	40°40'50.00"N	74°23'19.50"W

Germination testing was conducted using trays of moist vermiculite placed on greenhouse benches. The seeds of *Ailanthus altissima* are known to have

extremely high viability, a great asset to an invasive plant, thus specialized

conditions are not necessary (Gonzalez-Munoz *et al*., 2011; Marshall and

Furnier, 1981; Virginia DoF, 2009). Simulation of winter conditions through

stratification has also been shown to be unnecessary (Marshall & Furnier, 1981).

Light availability is known to be an important factor in germination of *Ailanthus*

altissima, with high light environments resulting in better rates of germination

(González-Muñoz et al., 2011; Kota, Landenberger, & McGraw, 2007; Pannill,

2000) and low light significantly inhibiting germination (Kota et al., 2007). In

response to this finding we took precaution to ensure that the germination

treatments were not shaded out by nearby greenhouse plants. The germination

experiments were also delayed until late spring, so that we could match the

photoperiod found in nature during germination period. Existing leaf litter has

been shown to inhibit the germination of *Ailanthus* seeds, so there is no need to

simulate such conditions (Kostel-Hughes et al., 2014). The germination trials

were run using 50 seeds per replicate in aluminum catering trays measuring 11.8

x 9.4 x 2.6 inches, which had modified to allow for drainage. Based on existing

literature the temperature was maintained at 20 degrees centigrade (Kowarik &

Säumel, 2008) and moist vermiculite was used in place of soil (Marshall &

Furnier, 1981). Seeds were treated using a dilute bleach solution to clean the

surface, as a preventative measure to reduce the risk fungal infection. Following

the bleaching step, the seeds were immersed in water for a period of 3 days.

This immersion step was included in the protocol because it has been observed

to increase both germination success and speed of seedling maturation (Kowarik

& Säumel, 2008; Säumel & Kowarik, 2010). Measurements of initial dry weight and number of seeds were conducted before bleaching. The germination experiment ran for 2 months, from July 19th through September 23rd. Observations were taken every two days, and germinated seeds were removed following each observation. All seeds germination trials were run simultaneously. Data collection was a simple count of successfully germinated seeds, as well as records of the total number of seeds taken from each site, from which percent germination could be calculated. Germination data failed a test for normalcy, so we performed a logit transform, as recommended by (Warton & Hui, 2011), before conducting a Mann-Whitney U test. Comparisons were also conducted for seed mass to determine if offspring investment varied by either land use or geographic region.

Results

The majority of viable seeds germinated during a 2-3 week period, which matches information found in existing literature for *Ailanthus altissima* (Marshall & Furnier, 1981).

We found significant evidence to support our hypothesis that brownfield sites showed lower seed germination rates than non-brownfield sites. The results of all brownfield sites pooled and all non-brownfield sites pooled can be found below in figure 2.1.
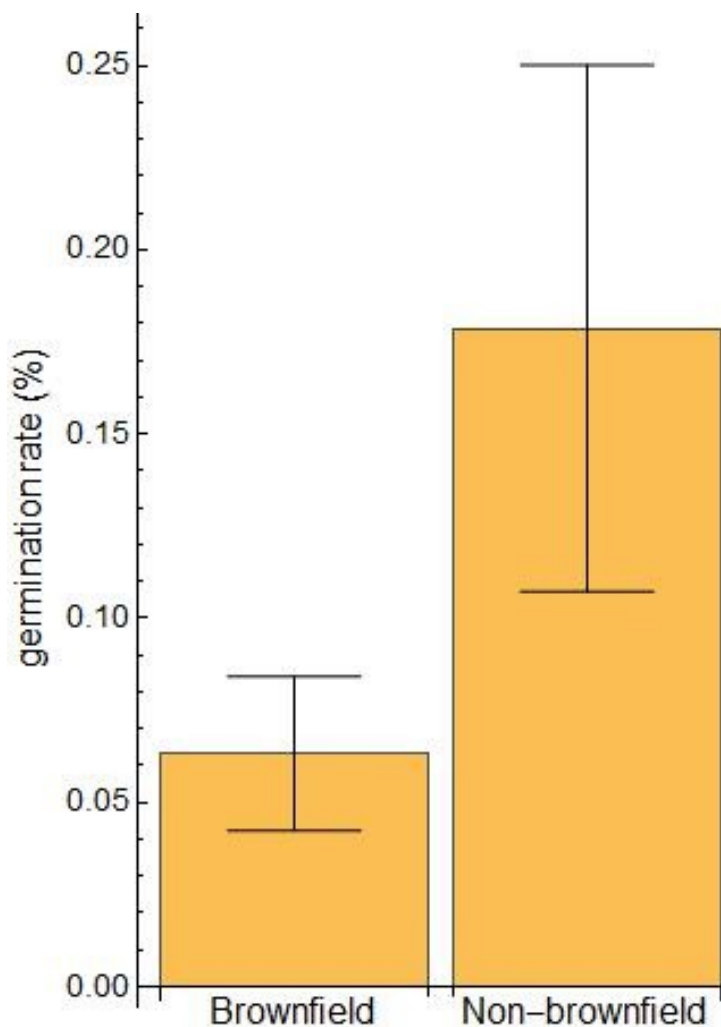
Figure 2.1 A comparison of *Ailanthus altissima* germination rate with sites pooled by brownfield/non-brownfield treatment. Brownfield sites show a significantly lower proportion of successful seed germination as compared to seeds from non-brownfield sites. Shown are means with 1 standard error (p=0.00732).

Our brownfield seed samples, primarily collected from locations with current or previous industrial land use, showed a lower overall rate of seed germination. Non-parametric tests showed that this difference was highly significant (p=0.00732). This result is direct verification of hypothesis 2.1, showing that brownfield sites display inhibited germination of *Ailanthus altissima* seeds.

We also compared germination data based on geographic region. Illinois samples came from a region with a much shorter industrial history, with lower population density allowing for higher amounts of greenspace. Figure 2.2 shows that this resulted in a significant difference in germination rates. A likely explanation for this is that the Illinois sites, despite having varying degrees of industrial development, were all still suburban by the standards of New Jersey. Suburban Illinois sites are also known to frequently use surface fill, replacing the upper soil layers, which would eliminate previous buildup of heavy metals. Without this build up, Illinois seeds performed the same regardless of human development levels ($p=0.0025$).
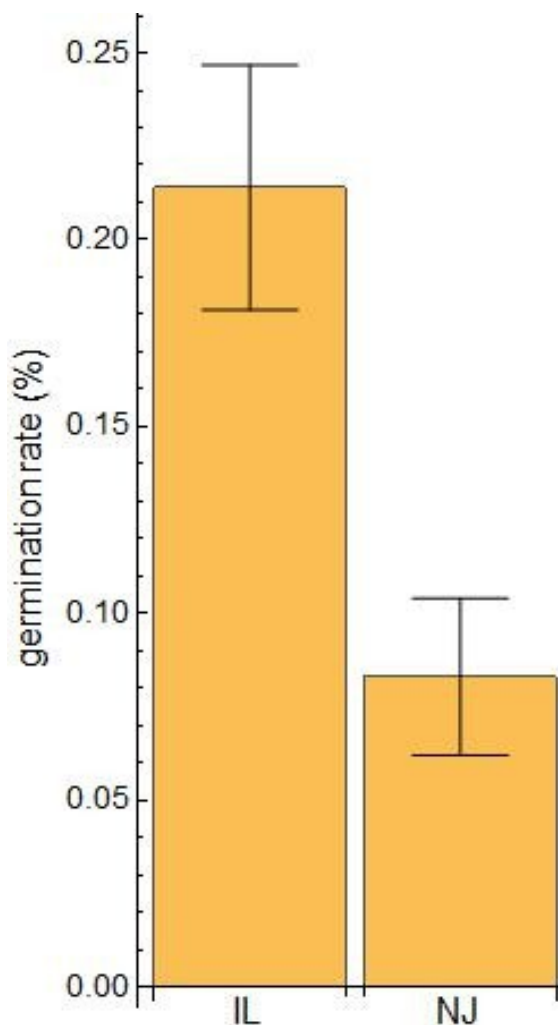
Figure 2.2 A comparison of germination rates for *Ailanthus altissima* seeds between Illinois and New Jersey collections. Illinois showed a significantly higher successful germination rate as compared to New Jersey. Error bars represent standard errors. (p=0.0025)

We also measured the initial seed mass before germination. We used this data to test for a correlation between seed mass and germination success. As demonstrated in figure 2.3, we found a slight positive and significant relationship between these two factors (p=0.00195), however the correlation coefficient was rather low (R^2=0.244).
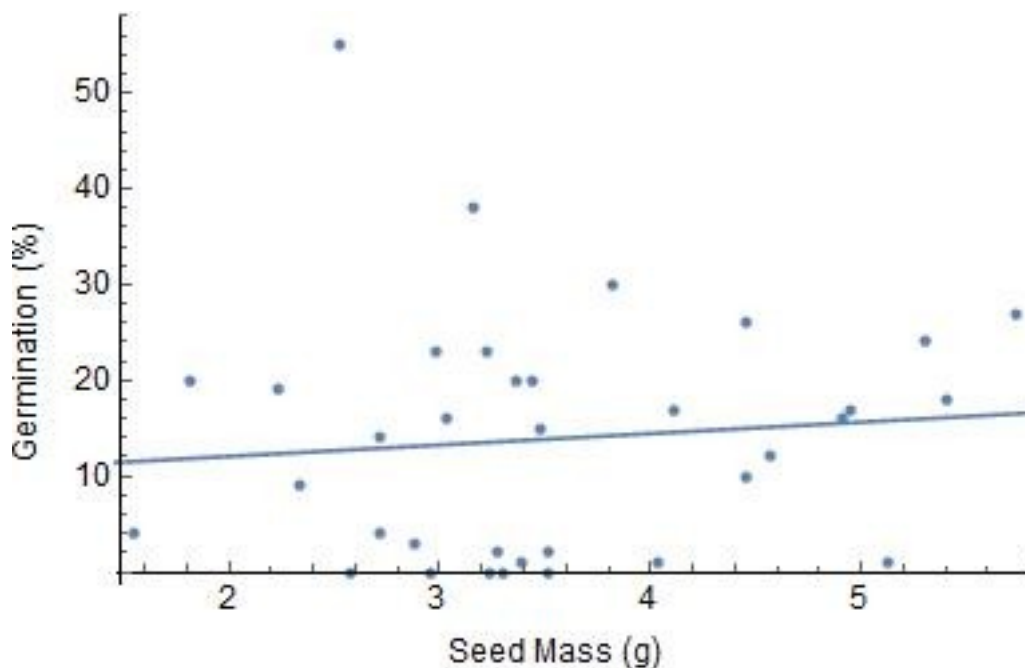
Figure 2.3 Initial seed mass positively correlates with germination success. (p=0.00195, R^2=0.224)

We then examined the effect of brownfield sites on seedmass, to determine if elevated pollution levels impacted resource allocation to offspring. Seed mass showed no significant differences between brownfield and non-brownfield sites, indicating that parental investment in seed quality did not differ based on historic land use (p=0.614). Similarly, no difference was found between seed mass between Illinois and New Jersey sites (p=0.264), indicating that *Ailanthus altissima* investment per seed was consistent across all of our samples (see figure 2.4 below).
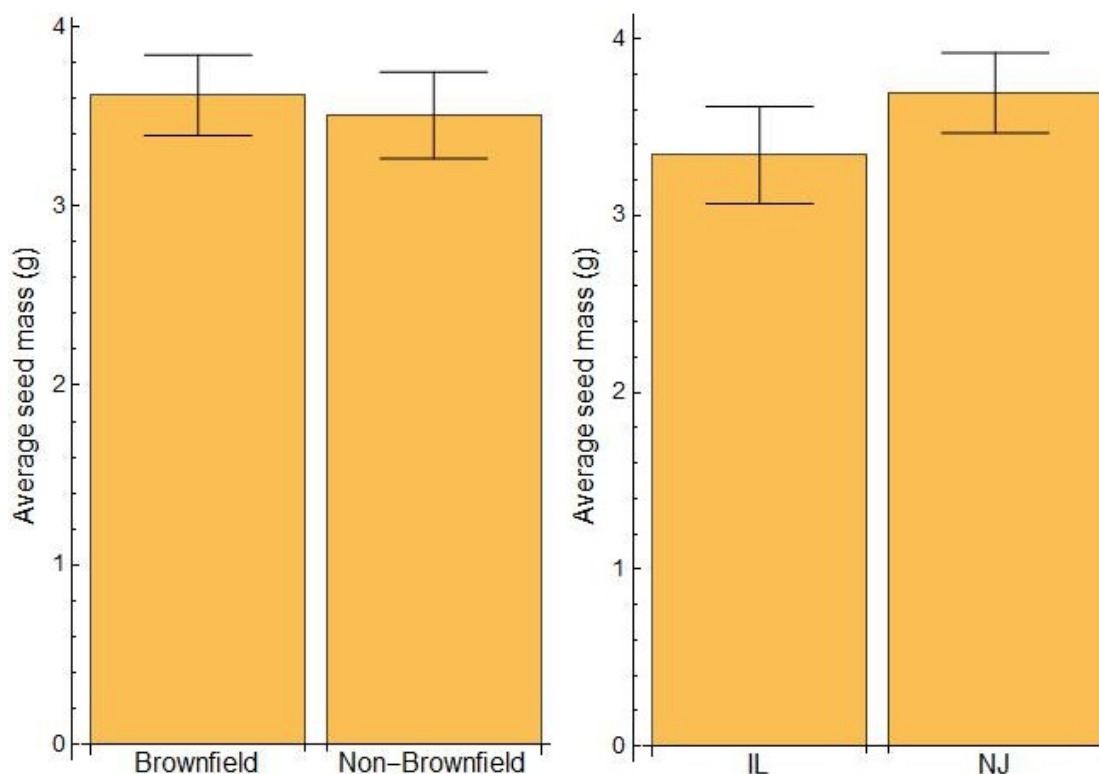
Figure 2.4. The average mass of *Ailanthus altissima* seeds was consistent, and did not differ between brownfield and non-brownfield sites (p=0.614) or by state origin (p=0.264). Shown are means with 1 standard error.

Discussion

Germination rates in this study were lower than initially expected, some previous studies had reported germination rates as high as 86.8% (Kowarik & Säumel, 2008). The lower overall germination of the study samples might be attributed to environmental conditions of the greenhouse. *Ailanthus altissima* is a high impact invasive species, known for rapid colonization of patches of open and disturbed land and extremely prolific germination in these sites. However, *Ailanthus altissima* is also known to have much lower (although non-zero) success at invading established forest canopies (Knapp & Canham, 2000; Kowarik &

Säumel, 2007, 2008). Our greenhouse was unable to mimic elevated light levels of disturbed habitat due it its location between buildings. Our germination rates were consistent with literature demonstrating a severe decline in germination rate when *Ailanthus altissima* seeds are shaded (Kota et al., 2007).

Although seed mass overall was observed to be positively correlated with germination success (figure 2.3), the correlation was weak (R^2=0.224). These results conflict with previous work, which found no relationship between samara weight and germination (Delgado, Jimenez, & Gomez, 2009). The lack of significance in Delgado's work may be driven by the low correlation which we observed in our own study. Ultimately we found that seed mass was not different across both region and habitat (figure 2.4). The lack of significant difference in seed mass indicated that parental investment in offspring was the same for all sampled sites, and should not influence the results of the germination study. Removing seed mass as a confounding variable allowed us to focus more specifically on land use as the determining factor for germination success.

Our germination study showed that, under controlled greenhouse conditions, *Ailanthus altissima* seeds taken from sites with heavy industrial development have significantly lower germination success than seeds taken from the non-brownfield sites. Our comparison included sites that were located in distinct two geographic regions, New Jersey and Illinois, to ensure a wider genetic diversity among samples. The Illinois sites showed little relationship between human

development and germination success, while the New Jersey sites clearly differed in germination rates between brown and non-brownfield sites.

The difference in performance between New Jersey and Illinois seeds was quite substantial (NJ=8.3, IL=21.4; p=0.0025). Although geographic isolation and genetic divergence could explain the difference in seed germination (and therefore individual fitness of the parent trees), this seems unlikely. Work by Aldrich *et al*. Showed a large amount of geneflow between populations of *Ailanthus altissima* across the entire continental United States, which should eliminate any major fitness differences (Aldrich et al., 2010). We also found that the mass of seeds did not vary, indicating a consistent level of parental investment across all groups. A more likely explanation for the difference in these two locations is human impact and land-use history. Locations in New Jersey have a much longer history of industrial presence compared to Illinois. Sites like Liberty State Park and the city of Newark are well known to have contaminated soils. Liberty State Park in particular has heavy metal contamination due to its use as a railroad yard.

The sites examined in Illinois, by contrast, had a history of much more moderate industrial development. Our most industrial site in Illinois was Batavia, which was located in a region known to have been contaminated with radioactive thorium from previous industrial activity (United States Environmental Protection Agency, 1994). Due to close proximity to residential zones, however, the site had been

remediated and the contaminated soil removed. The replacement of soil would have removed any other industrial contaminants which had built up, explaining why the Illinois industrial site performed better than anticipated. The land use history of Illinois suburban sites was primarily old agricultural fields. Additionally, developers of subdivision housing often replace the top layer of soil, which would create an effect similar to the hazardous materials remediation seen in Batavia. Taking these records into account, it is not surprising to see than suburban Illinois sites show such uniformly high levels of seed germination.

Knowing what we do about the land use history of Illinois sites, we can see that their higher performance relative to New Jersey industrial is expected. Without sustained accumulation even the industrial suburban sites showed high seed germination. It is only the sites with prolonged and continued contamination (e.g. Liberty State Park, Newark) that showed fitness impacts. The suburban nature of the Illinois Batavia site also likely played a role, as the periphery of the industrial building was a heavily maintained lawn surrounded by other green spaces. Frequent watering, and possible fertilizer treatments, would alleviate some of the physiological stresses often associated with industrial sites. Water stress due to impermeable surfaces and limited nutrient availability due to poor quality soil would be found in Newark and Liberty State Park sites, but not in Batavia. All told, the elevated seed germination observed in the Batavia samples as compared to Newark and Liberty State Park is not unusual, and can be explained

by human activity. Indeed, the higher germination may been seen as a success of the policies promoting green spaces in suburban industrial parks.

With the Illinois sites being so consistent, the New Jersey sites acted as the drivers of our observed brownfield/non-brownfield differences. The lower seed germination rate of *Ailanthus altissima* in New Jersey brownfield sites should directly impact the reproductive fitness of the individual trees. In addition to the expense of developing flowers, the final development of seeds represent an investment of resources which could have been directed to other growth instead (Cheplick, 1995; Groenendael et al., 1996; Price & Marshall, 1999; Suzuki, 2001; Watkinson & White, 1986). Each non-viable seed wastes resources, and that waste reduces the relative fitness of the tree. Despite this reduced fitness, we still see large stands of Ailanthus altissima in these contaminated sites. In order to resolve this conflict it is important to note that, as an invasive tree, *Ailanthus altissima* is well suited to disturbed environments. This means that it often grows in areas with far less competition from established trees. While contaminated soils may reduce the fitness of *Ailanthus altissima*, the contaminants also reduce the fitness of the rest of the plant community as well. If the rest of the plants are similarly impacted, or possibly worse, then the high prevalence of *Ailanthus altissima* can be explained. Indeed, *Ailanthus altissima* is known for tolerance to poor soil conditions, including heavy metal contamination (Feret, 1985; Kowarik & Säumel, 2007; Landenberger et al., 2007; Shah, 1997).

The effect of human development on reducing seed viability has implications at the broader landscape and geographic scales. Although *Ailanthus altissima* is known to propagate through vegetative fragmentation, particularly along waterways (Kowarik & Säumel, 2008; Säumel & Kowarik, 2010), the overwhelming majority of its invasion potential is undeniably the result of its extremely prolific production of seeds. Given this heavy reliance on seed dispersal for expansion, a reduction in seed viability caused by heavy metals would be expected to impair the invasive spread of *Ailanthus*. Why, then, do we see such prolific amounts of *Ailanthus altissima* in areas of heavy human development, including industrial sites? There are several factors which can explain this apparent paradox, and serve as direction for future investigation.

One important factor to consider is that our experiment only examined the effect of land use legacy on germination *Ailanthus altissima*, and not the other members of the local plant community. While sampling of these other species was beyond the scope of our project (and beyond the scope of our facilities), it is important to remember that no species exists in a vacuum. Heavy metals are known to be generally harmful to most organisms, unless they have specific mechanisms to sequester the metals safely. However, the degree of harm is not the same for all species. We know that under heavy metal load seed germination is reduced in *Ailanthus altissima*, but we do not know how its competitors respond to the same environmental stress. If metals more severely impact the

competing plants then Ailanthus will actually benefit from the presence of heavy metals in the soil, as its relative fitness compared to competitors will increase.

Along the same lines, despite the reduction in seed germination, *Ailanthus altissima* adults are known to be tolerant to heavy metals. Early stages of life history are often associated with high mortality, a fact which is particularly notable in the establishment phase of biological invasions (Niinements & Valladares, 2006). The reduction in new seed recruitment caused by heavy metals may be of lesser importance when the mature individuals are pollution resistant and also capable of such massive levels of seed production. Under this model the different life-history stages of *Ailanthus altissima* should react differently to heavy metal stress, and we would expect to see a decline in metal sensitivity as age classification increases.

Finally, we should consider the role that human impact plays in propagule dispersal. Invasive species are well known to often spread along routes of major human traffic; highways, railroad lines, harbors, etc. Given the prolific seed production of *Ailanthus altissima*, the effect of seed recruitment from immigrants could potentially be high enough to offset the loss of seed viability. Under this model the detrimental effect of heavy metals on *Ailanthus altissima* still exists, but the effect of metals is swamped by a much larger immigration effect. The advantage of this model is that heavy metal levels and propagule influx are both positively correlated to human disturbance. This means that sites with a high

heavy metal load tend to be highly developed, which means we should also expect to see a corresponding increase in immigrant seed recruitment. Any effect on population size from increased metal levels should then be offset by this increased recruitment.

All of these explanations are worth pursuing in further studies, but perhaps a simpler explanation may suffice here. The prolific seed production of *Ailanthus altissima* is an example of one of Darwin's original principles; more individuals are born into a population than can possibly survive. Heavy metal contamination may simply act as a selective pressure, allowing only more resistant seedlings to emerge and in addition reducing competition by reducing the number of viable seeds. Even with this reduction we still estimate tens of thousands of viable seeds per individual, more than enough to sustain a brownfield population. Thus the high fecundity of *Ailanthus altissima* overcomes environmental pressures, leading to rising populations in the face of detrimental conditions. As long as the tolerance to abiotic stress is high enough for surviving seeds to replace aging adults, there is no conflict between metals and stable populations.

So we have multiple, non-exclusive, explanations that may account for the high prevalence of *Ailanthus altissima* in a system where it suffers from a consistent source of fitness reduction. The persistence of the population may be driven by 1) less reduction in relative fitness than competitors, 2) reduced severity of effects based on age category, 3) immigrant establishment supporting the

population, 4) the sheer number of propagules offsets the fitness loss of heavy metals.

Ultimately we realize that fitness must be judged in context of competition. A situation that results in reduced fitness can still be beneficial, if all of the competing organisms have their fitness reduced by even higher amounts. This principle underlies the strategies of many invasive plants as well as pioneer species. Establishing a population under strong abiotic stresses allows organisms with poor competitive ability to avoid biotic pressures, effectively trading a small decrease in fitness (harsh environmental conditions) in exchange for avoiding the much larger fitness penalty of competing against better adapted species.

Considering these findings, we hypothesize that the reduction of germination success for *Ailanthus altissima* could serve as a selective pressure in favor of alternate reproductive strategies. Our initial line of inquiry suggested that lower germination success could be tied to elevated levels of clonality and/or fragmentation. The rationale for this is straightforward, non-viable seeds represent a large investment of both time and resources for the organism while asexual reproduction is rapid and relatively cheap (Agrawal, 2006; Otto, 2009; Otto & Lenormand, 2002).

This trade-off between allocation to sexual and asexual investment is supported by several modeling studies, and even relatively small differences in parameters such as seed set rates, sex ratios or population density can dramatically affect returns on reproductive investments (Charlesworth & Charlesworth, 1987; Dorken & Van Drunen, 2010; Nishitani et al., 1999; Sakai, 1995). These variations are large enough that many models predict a mixed reproductive system only under a narrow range of conditions.

Models running under a lattice structure habitat show that the coexistence of both sexual and clonal reproductive modes is precariously balanced based on fitness returns of both approaches (Harada & Iwasa, 1994). Other models show that maintenance of mixed reproduction requires that the propagule fitness of a population varies across multiple years (Bengtsson & Ceplitis, 2000). Our results demonstrated that propagule fitness, measured as germination success, was significantly lower in brownfield populations, while the Bengtsson's and Harada's models both demonstrate that propagule fitness can have a direct effect on a population's reproductive strategy. Taken together this lends support to the idea that human development of sites can, potentially, affect the reproductive balance of a population.

Other modeling systems show similar results. A model based on *Syneilesis palmate* (Umbrella Plant) makes similar assumptions to what we would expect from *Ailanthus altissima*; propagule fitness was assumed to be density

independent, based on its ability to disperse away from competition, while fitness effects of density on clonal growth was modeled as a negative relationship to simulate crowding (Nishitani et al., 1999). This model showed similar findings to another model, where continuation of the mixed reproductive system required a narrow range of relative fitness differences (Bengtsson & Ceplitis, 2000). The density independence of dispersed seeds lost out to rapid clonal growth during most conditions, and only became dominant once severe crowding effects reduced the relative fitness of the asexual approach (Nishitani et al., 1999). This demonstrates that selection acting in aggregate at the population level can be strong enough to actually change the reproductive balance of the group as a whole.

These models assume that the propagule successfully manage to establish once it has dispersed, and our work shows that this is not always the case. The success rate of seed establishment was directly tested by Satoki Sakai in a 1995 modeling study, where he demonstrated that the optimal resource allocation between reproductive systems directly changed based on the favorability of habitat, specifically resource availability and degree of patchiness (Sakai, 1995). The rate of successful seed establishment can be affected by other factors as well; in our work we demonstrated a reduction in seed viability caused by parental exposure to brownfield habitats. Building off of Sakai's findings, this should reduce individual fitness and shift the optimal allocation away from seed production. Such a situation would not necessarily eliminate seed production,

however. Asexual species had a strong advantage in environments which showed very little variation in conditions over time, but frequent perturbation of the environment shifted the fitness in favor of sexual reproduction (Weeks, 1993). Application of this model under real world conditions means that the heterogeneity of urban brownfield sites could act to preserve sexual reproduction, even as it also reduces the fitness of individual seeds. The overwhelming volume of seeds produced by *Ailanthus altissima* may enable the populations to tolerate some fitness loss in reduced seed viability in exchange for fitness benefits when facing temporal heterogeneity.

Ultimately these models agree we can assume that sexual and asexual reproduction are in some level of balance relative to each other, as determined by selection pressure, we can view this relationship as a sort of equilibrium. If we perturb this balance, e.g. by reducing seed viability, then we would anticipate the relative allocation to shift away from sexual reproduction and closer to asexual. Under moderate conditions both strategies would still be present in the system, but the relative allocation of resources between the two strategies would have shifted. Harsher conditions could drive the system toward only one reproductive mode, but such a loss is unlikely as *Ailanthus altissima* is known to be prolific in both reproductive strategies. We conclude that individuals of *Ailanthus altissima* growing in more polluted environments produced seeds with a lower germination success, and that this should result in selective pressure in favor of higher levels

of clonality than we would otherwise see. We further investigate this prediction by

examining the underlying genetics of *Ailanthus altissima* populations in chapter 3.

Chapter 3: Population Structure of *Ailanthus altissima* in Northern New Jersey: the Role of Land-use History and Management

An Introduction to Population Index Values

One of the most commonly reported parameter in a population genetics study is a single population index value which is an indicator for the degree of structuring, internal barriers to geneflow, within the populations. Often this takes the form of some type of fixation index, similar to Wright's original F_{ST} index (Wright, 1949). Due to the fact that Wright's F_{ST} was originally formulated as an estimate of probability of identity by descent, all of these index parameters have values that theoretically range from 0 to 1, although we will see that this maximum value may change in some circumstances. Many different population indices and F_{ST} analogs have been developed in the decades since Wright's original 1949 work, including G_{ST} (Nei, 1973), two different formulations of G'_{ST} (Hedrick, 2005; Nei, 1987), G''_{ST} (Meirmans & Hedrick, 2011), R_{ST} (Slatkin, 1995), Phi_{PT} (L. Excoffier, Smouse, & Quattro, 1992), and D_{est} (Jost, 2008). Of note, D_{est} is not actually an F_{ST} analog, but is an unrelated population index that also ranges from 0 to 1 (Jost, 2008; Meirmans & Hedrick, 2011). Each of these population indices is based upon different assumptions, and we will examine each of them in turn. Before we can do so, we must the underlying principles behind the various population index types.

Ultimately, most aspects of population genetics are connected, directly or indirectly, with the well known Hardy-Weinberg Equilibrium model. Under a given set of assumptions, the distribution of genotypes in a populations will rapidly reach a stable equilibrium state with proportions predicted by the binomial expansion (eg. $p^2+2pq+q^2$ for two allele models). The assumptions of the Hardy-Weinberg equilibrium model and their opposing mechanics are reviewed in Table 3.1.

Table 3.1. The assumptions of the Hardy-Weinberg equilibrium model, and the mechanisms which arise from violation of these assumptions.

Hardy-Weinberg Assumption	Opposing mechanism
Random mating	Sexual selection/inbreeding
Infinite population size	Genetic drift/inbreeding
No mutation	Mutation
No emmigration/immigration	Geneflow
Equal survival of individuals	Natural Selection

The assumptions for the Hardy-Weinberg model are rarely met and one, infinite population size, is functionally impossible to achieve. However, the Hardy-Weinberg equilibrium model is still worth studying, as we can learn a lot about populations from how they fail to meet these conditions. Deviations from the assumption of the Hardy-Weinberg model lead to deviations from the predicted genotype ratios, which is readily measured by modern techniques. The different

ways in which each assumption can be violated each have a different name, and
each is the foundation for a different mechanism of population dynamics.

The Hard-Weinberg equilibrium model treats the species as belonging to a single
infinitely large panmictic population. Although populations can never truly be
infinite, with a large enough population the Hardy-Weinberg model can serve as
an accurate approximation. The problem is that the Hardy-Weinberg model also
assumes panmixis, freely mixing populations with no barriers to geneflow. This
assumption is often subverted by subdivided populations, and from these
divisions is derived genetic structure. These subdivisions take place at many
different scales, and this forms the foundation of what we term a population
index. As mentioned above, a population index is a single value ranging from 0 to
1 that describes the degree of population structuring at a given level. These
levels may vary based on the data set, but often include the following scales;
individual (I), subpopulation (S), region (R), and total population (T).
Comparisons between two levels are generally denoted as two letter subscripts
of a given index (eg. F_{SR} examines discrepancy between a subpopulation and
the larger region).

Despite all of these deviations from the original Hardy-Weinberg equilibrium
model, it still serves as a null model to compare against. In fact, the population
indices which we are about to examine are effectively just indicators of how the
heterozygosity of a population differs from the value predicted by the Hardy-

Weinberg model. In the above example (F_{sr}) the comparison between a subpopulation and the larger region can also be interpreted as the amount of heterozygosity lost because of structure between those two levels.

For our dataset we have three levels of organization; individual, subpopulation and total. As such, we will omit further discussion of any regional index values (eg. F_{SR}; F_{RT}). The underlying principles behind these values are the same, change in heterogeneity against expected Hardy-Weinberg values, and thus our discussion of the remaining indices should serve well in interpretation of regional indices.

A Review of Major Population Indices

A suite of population indices known as F-statistics was the first attempt at formulating an index to express the degree of population structuring. The original population index F_{ST}, along with the accompanying parameters F_{IS} and F_{IT}, was formalized by Dr. Sewell Wright in 1949 (Wright, 1949). Wright's three parameters each examine a different aspect of population structure, but all three F-statistics are concerned with level of heterozygosity, and how it deviates from the predictions of Hardy-Weinberg equilibrium. This focus on deviation from predicted values of heterozygosity is so central to Wright's model that later population indices, often called F_{ST} analogues, will also be formulated based on ratios of predicted and observed heterozygosity.

A brief overview of F_{IS}, F_{ST}, and F_{IT} is available below (see table 3.2). We will examine each of these values in turn, beginning with F_{IS}.

Table 3.2. A summary of the three major F-statistics

Index	Comparison	Alternate name
F_{IS}	Reduction in heterozygosity caused by inbreeding	Inbreeding coefficient
F_{ST}	Reduction in heterozygosity caused by population structure	Fixation index
F_{IT}	Total reduction in heterozygosity from both mechanisms	

F_{IS}, is a population parameter that describes the probability that for a given diploid individual the two copies of an allele are identical by descent. Because this probability is also the probability of inbreeding, F_{IS} is often referred to as the inbreeding coefficient of the population. The subscript "IS" indicates a comparison between the individual and the local sub-population to which that individual belongs. Because F_{IS} is determined within subpopulations, it does not take population structure into account; F_{IS} only informs us about levels of inbreeding. In terms of heterozygosity, F_{IS} is the reduction in the proportion of heterozygotes caused by inbreeding. An F_{IS} of zero indicates a completely outcrossing population, where sexual reproduction always occurs with unrelated individuals. Low inbreeding coefficients can be a result of extremely large populations (less likely to mate with related individuals), high geneflow (influx of unrelated individuals) or other factors that promote outcrossing. An F_{IS} of 1

indicates an extreme case of inbreeding, or potentially self-fertilization. The F_{IS} index tends to fall between these extreme cases, but it can give us an overall sense of the state of the population with regard to the risk of inbreeding depression.

F_{ST}, the most commonly reported of the three F-statistics, is also known as the fixation index. The fixation index is the probability that two alleles chosen from a population will be identical by descent. In terms of heterozygosity, the F_{ST} index indicates the reduction of heterozygotes in the population as a result of total (T) population being subdivided into subpopulations (S) (Hartl & Clark, 2007; Whitlock, 2011). An F_{ST} value of zero indicates complete panmixis of individuals, effectively meaning that no division into subpopulations exist (or that there is only one subpopulation, which contains everything). And F_{ST} index of one indicates complete separation of subpopulations, with no contact between them.

As an example of how a high F_{ST} index affects heterozygosity, consider this extreme example: We have two completely isolated subpopulations with two alleles, A and a. subpopulation 1 consists entirely of AA individuals, while subpopulation 2 consists entirely of aa individuals. The allele frequency of both p and q are 0.5, so according to the Hardy-Weinberg model we would predict a heterozygosity of 2pq, 2*0.5*0.5, or 50% of the population. Instead, because population structure prevents mixing of the alleles, we see zero heterozygotes.

This complete loss of heterozygosity between the two subpopulations gives us an F_{ST} index of 1.

F_{ST} compares subpopulations against the total population, and as such does not give us information about any activity within the subpopulations, such as inbreeding. F_{IS} only looks at the smallest units of population, subpopulations, and thus no information about population structure is possible. The F_{IT} index, however, combines both of these indices. The variation expressed in F_{IT} can be partitioned out between the other two F-statistics; part of F_{IT} can be explained by F_{ST} and the rest by F_{IS}. Conceptually, F_{ST} can be thought of as the reduction in heterozygosity explained by population structure (as described by the Wahlund effect) while F_{IS} can be thought of as the reduction in heterozygosity due to inbreeding pressures. This means that F_{IT} can be interpreted as the combined effect of inbreeding and population structuring. Both of these effects perturb the Hardy-Weinberg equilibrium levels of heterozygosity, leading to a deviation from the genotype frequencies predicted by that model.

Being the original formulation, Wright's F-statistics were derived for a simple model. Many more population indices were developed in the following decades, and an overview of the major indices can be found below in Table 3.3. The development of these later indices were necessary because, despite the major importance of Wright's work, the F-statistics made several simplifying

assumptions and could not apply to all real world situations. We will discuss

these assumptions, and how later authors corrected for them, in the following.

Table 3.3. An overview of the major population indices and their characteristics.

Index	Original publication	Calculation based on	Description
F_{ST}	Wright, 1949	Heterozygosity	Original index, limited to 1 locus and 2 alleles
G_{ST}	Nei, 1973	Heterozygosity	Allows for more than 2 alleles, otherwise the same
G'_{ST} (N)	Nei, 1987	Heterozygosity	Correction of Gst for cases with a small number of populations
G'_{ST} (H)	Hedrick, 2005	Heterozygosity	Correction of Gst for when populations have non-overlapping alleles
G''_{ST}	Meirmans and Hedrick, 2011	Heterozygosity	Correction of Hedrick's G'st with a small number of populations
R_{ST}	Slatkin, 1995	Heterozygosity	Derived for use with microsatellites, assumes stepwise mutation
Phi_{PT}	Excoffier et al., 1992	Nucleotide Diversity	Based on AMOVA tables
D_{est}	Jost, 2008	Effective Number of Alleles	Not an Fst analog

One of the major flaws of F_{ST} is that in its original form the index can only be applied to situations at a single locus with two alleles (Meirmans & Hedrick, 2011; Wright, 1949). The loci limitation can be readily compensated for by analyzing each locus separately and then averaging the resulting indices together. The allele problem, however, requires a different formulation of the F-statistics. This restriction lead directly to the development of G-statistics by Masatoshi Nei (Nei, 1973).

The family of G-statistics developed by Masatoshi Nei closely matches Wright's F-statistics in derivation, but does so in a manner which is far more general. Nei formulation closely matches Wright's in that it examines how a population's observed heterozygosity value deviates from the predicted heterozygosity predicted under Hardy-Weinberg equilibrium (Nei, 1978). In this way, despite being developed later, we can say that Wright's original F-statistics are effectively a special case of Nei's more general G-statistics (Hartl & Clark, 2007). In fact, the connection between Wright's F_{ST} and Nei's G_{ST} is so strong that many population genetics programs use the terms interchangeably, performing Nei's G_{ST} calculations and calling the output F_{ST}.

Masatoshi Nei later revisited his G_{ST} index value to account for limitations caused by application to datasets with a small number of populations. This minor adjustment accounted for circumstances where the G_{ST} index would underestimated (Nei, 1987). The corrected G_{ST} index was denoted by adding a

prime mark, resulting in G'_{ST}. However, as we are about to see, the name G'_{ST}

would also be used by Hedrick. To resolve the confusion Nei's G'_{ST} will

henceforth be denoted $G'_{ST}(N)$.

In 2005 Philip Hedrick developed his own derivative of the G_{ST} index value,

hereafter refered to as $G'_{ST}(H)$ (Hedrick, 2005). Hedrick was concerned with a

different error of the G_{ST} index than Masatoshi Nei was. The problem that

Hedrick saw was that two populations could often have non-overlapping alleles,

also referred to as private alleles (Hartl & Clark, 2007). This was important

because under such conditions the heterozygosity calculations of G_{ST} would

generate a maximum possible value that fell below 1. The maximum possible

value of G_{ST} is defined as (1- H_S) where H_S is the within population

heterozygosity (Hedrick, 2005; Ryman & Leimar, 2009). If the within population

heterozygosity were high enough the maximum the value could fall far below 1,

which would drastically affect interpretation. Hedrick accounted for this problem

by normalizing the G_{ST} index against its maximum possible value under the given

conditions (see Table 3.4 below for examples of how G_{ST} max effects these

values). This normalization effect increased the calculated values of the

population index so that there would always be full coverage of the range from 0

to 1 (Hedrick, 2005). This normalized index is what we refer to as G'_{ST} (H).

Although the two versions of G'_{ST} can be confusing, the conflict between Nei and

Hedrick's different G'_{ST} indices was resolved in 2011 by Meirmans and Hedrick.

In this paper Meirmans and Hedrick combined the low population correction from Nei's G'_{ST} and the private allele correction from Hedrick's G'_{ST} into a new index called G''_{ST}. The resulting index is, by a convoluted path, the most up to date version of Wright's original F_{ST} index. We shall address several other indices shortly, but it is important to note that G''_{ST} is the most recently developed index that continues to use Wright's original comparison of observed heterozygosity vs. expected values based on the Hardy-Weinberg model.

Table 3.4. An example of how a declining G_{ST} maximum can drastically affect interpretation of the fixation index.

G_{ST}	G'_{ST} (H)	G_{ST} max
0.05	0.050	1.0
0.05	0.056	0.9
0.05	0.063	0.8
0.05	0.071	0.7
0.05	0.083	0.6
0.05	0.100	0.5
0.05	0.125	0.4
0.05	0.167	0.3
0.05	0.250	0.2
0.05	0.500	0.1

In the mid-1990's a parallel line of development by Slatkin resulted in a specialized F_{ST} analog known as R_{ST} (Slatkin, 1995). Slatkin's work was in

response to the rising popularity of microsatellite markers for us in population genetics. Microsatellite regions, also known as short tandem repeats (STRs), are non-coding regions of DNA where mitotic errors cause regular subunits to be replicated some number of times (Eisen, 1999). Microsatellite techniques rose to popularity in genetics because of their adaptability to many timescales, and are now widely used in ecological fields (Selkoe & Toonen, 2006). In population genetics specifically they are widely used because they have the added advantage of being, for the most part, selectively neutral (Aldrich et al., 2010; Fischer & Kleunen, 2002; Hartl & Clark, 2007; Meirmans & Hedrick, 2011; Prati & Schmid, 2000). Despite these strengths, microsatellites posed an unusual problem for population genetics; under the existing methods for calculating F_{ST} or G_{ST} the value of the resulting index was consistently low (F Balloux, Brünner, Lugon-Moulin, Hausser, & Goudet, 2000; François Balloux & Lugon-Moulin, 2002; Slatkin, 1995). This is caused by Wright's underlying assumption that mutation rates would be much lower than migration (Hedrick, 2005). Not only would the maximum index value for microsatellite data would never reach 1, the maximum would often be extremely low. This is similar to the problem we encountered above with G'_{ST} and G''_{ST}, but even more severe. The cause of this problem was that the modular components of microsatellites make them highly variable, and that the number of replicated units in a microsatellite region can effectively be considered the allele type. In some cases as many as 40 alleles have been observed for a single locus (Peijnenburg, Fauvelot, Breeuwer, & Menken, 2006). This combination of extremely high mutation rate and large

number of alleles results in a low maximum index value (F Balloux et al., 2000;

Hedrick, 2005).

Slatkin's solution for this problem was to directly tackle the cause of the conflict,

the high mutation rate. The modular construction of microsatellite regions

combined with the high mutation rate lead to the development of the stepwise

mutation model (Ellegren, 2004). The stepwise mutation model assumes that

each time a microsatellite region undergoes replication one of three things will

occur; the number of repeated units will stay the same, the number will increase

by one, or the number will decrease by one. Effectively, the model assumes that

the number of repeat units will shift by one "step", hence the name "stepwise

mutation" model. Based on this model, Slatkin developed an F_{ST} analog known

as R_{ST}. The major weakness of R_{ST} is that the underlying assumptions of the

index make it highly specialized. R_{ST} was designed for information from

microsatellite regions that use the stepwise mutation models. Any deviation from

either assumption severely impairs the accuracy of the R_{ST} index. Although some

evidence does support the use of stepwise mutation model (Weber & Wong,

1993; Whitlock, 2011), in the last decade R_{ST} has fallen out of use. Evidence has

shown that it is not uncommon for microsatellites to violate the stepwise mutation

assumption (François Balloux & Lugon-Moulin, 2002; Innan, Terauchi, &

Miyashita, 1997; Meirmans & Hedrick, 2011), and a small number of random,

non-stepwise, mutations can drastically change R_{ST} estimates (F Balloux et al.,

2000). Even when the stepwise mutation assumption has been found to be true,

the R_{ST} index does not necessarily perform better than other options (François Balloux & Lugon-Moulin, 2002).

The current most popular population index is the Phi_{PT} index, which was developed by Laurent Excoffier, Peter Smouse, and Joseph Quattro in the early 1990's (L. Excoffier et al., 1992). Excoffier's work was based on the Analysis of Molecular Variance (AMOVA) model which was developed some years earlier by Weir and Cockerham (Weir & Cockerham, 1984). To understand how AMOVA works, it helps to think of F-statistics as an attempt to partition the variation of a population across different structural levels (eg. F_{IS}, F_{ST}, etc.). AMOVA also attempts to partition population variation, but adapts the ANOVA method (e.g. partitioning variation between and within treatments) for use in population structure. More specifically, the AMOVA table is based on a matrix of squared Euclidean distances between DNA haplotypes (L. Excoffier et al., 1992; Meirmans & Hedrick, 2011). The AMOVA would later lead to the development of programs such as Arlequin and GenAlEx (created by Excoffier and Smouse respectively).

The use of the AMOVA approach gives Phi-statistics many intrinsic benefits. Because of its ANOVA origins, AMOVA is extremely flexible and can accommodate many different datasets and models (L. Excoffier et al., 1992; Holsinger & Weir, 2009). AMOVA can also accommodate the high mutation rates of microsatellite markers, making them a viable alternative to Slatkin's R_{ST} index

(Michalakis & Excoffier, 1996; Whitlock, 2011). Significance testing is also quite easy, as AMOVA lends itself well to testing against data permutations with little to no assumptions (L. Excoffier et al., 1992).

The last population index we will discuss is Jost's D, also denoted as D_{est}. (The subscript is important, as D is also used as a symbol for linkage disequilibrium) It is important to note that, unlike the other population indices we have examined, D_{est} is not an F_{ST} analog. F_{ST} analogs are concerned with loss of heterozygosity as compared to Hardy-Weinberg assumptions, but D_{est} was derived based on the effective number of alleles (Jost, 2008).

The origins of D_{est} are based on the fact that, by focusing on heterozygosity, G_{st} and related measures do not actually measure the differentiation of genes. Jost noted that, although heterozygosity is often used to infer genetic diversity, there is not always a direct connection between the two. The assumed correlation between genetic diversity and heterozygosity works well when the total diversity of the populations is limited, but it breaks down as genetic diversity increases (Jost, 2008). The original population indices seemed to describe genetic differentiation because they were derived based on low diversity systems (Nei, 1972; Wright, 1949). Remember that Sewell Wright worked with a system of only 2 alleles. As our models were applied to more complex datasets situations arose where the old assumptions could cause problems with data interpretation.

The root of the problem is that for a population with extremely high genetic diversity the value of G_{ST} will actually decline, and a high enough diversity can potentially drive G_{ST} to near zero values (Jost, 2008). Reduction of the maximum possible G_{ST} index is a problem we have seen addressed before. Slatkin found that high microsatellite diversity caused a mutation driven reduction of G_{ST}, and attempted to correct it by developing the R_{ST} index (Slatkin, 1995). Hedrick also found that a reduction of G_{ST} values could be caused by alleles not being shared between populations, and derived his own G'_{ST} to fix the problem (Hedrick, 2005). Both of these examples, high mutation rate and private alleles, show situations where the genetic differentiation of populations do not match the relationship predicted by F- and G-statistics. This divergence of genetic diversity and heterozygosity means that it is actually possible for two complete isolated populations to have a near-zero G_{ST} index, an index value which would normally imply panmixis. This is not just a mathematical abstraction, a direct example of this can be found in work by Carreras-Carbonell *et al*., where two populations known to be completely isolated were found to have an F_{ST} of 0.05 (Carreras-Carbonell, Macpherson, & Pascual, 2006).

As we have seen, many solutions for this problem have been proposed. Hedrick's solution was to normalize the G_{ST} index against its maximum possible value. Slatkin created a new F_{ST}-analog based on assumptions specific to microsatellite systems. Jost, however, took a complete different approach and created an index which was not related to Wright's F-statistics. This new index,

called Jost's D or D_{est}, is based on the effective number of alleles (Jost, 2008; Meirmans & Hedrick, 2011). D_{est} can be thought of as the relative differentiation between two populations, which makes it quite easy to interpret; a D_{est} of 0 represents completely identical populations, while D_{est} of 1 represents completely distinct populations (Whitlock, 2011). This is in contrast to F_{ST} analogs, which represent reduction in heterozygosity caused by population structuring. The most extreme example of the divergence of these two measures would be two isolated, but otherwise identical populations. Under such a situation the populations would be highly structured (F_{ST}=1) but have no differentiation (D_{est}=0). Clearly then, we must keep in mind that D_{est} is telling us something quite different than the various F_{ST} analogs.

Despite the easy of interpreting the index, the use of D_{est} is quite limited. D_{est} is limited to a single locus, and is unable to separate purely genetic effects (such as mutation) from population level effects (such as drift and geneflow) (Ryman & Leimar, 2009). D_{est} also should not be used to derive information about migration between populations, as it was not designed for such uses (Jost, 2009; Meirmans & Hedrick, 2011; Whitlock, 2011). Some authors have also argued that D_{est} is not independent of effects from heterozygosity and mutation (Ryman & Leimar, 2009). In his 2009 response, Jost defends the D_{est} index by explaining that D_{est} and F_{ST} analogs are each suited to answer different questions (Jost, 2009). D_{est} measures "actual relative degree of differentiation of allele frequencies" while F_{ST} analogs are suited to estimating structuring effects caused

by migration. The problem is that relative population differentiation is not featured in any existing evolutionary models, limiting its current utility (Whitlock, 2011).

In the end, D_{est} tells us specific information about differentiation, but provides little information about actual population structure (Whitlock, 2011). This is not surprising though, since Jost did not derive D_{est} to be used in such a way (Jost, 2008, 2009; Whitlock, 2011). D_{est} has some potential use in conservation (Jost, 2008, 2009), but is not particularly informative for inferring large scale dynamics.

Ailanthus altsissima and Population Structure

All of these indices were developed to help characterize the complex mechanisms of population structure into a single representative parameter. This allows us to use population index to compare very different habitats, such as urban brownfields and exurban refuges. In our work we focused on the role of urban development, and how anthropogenic factors influence the population structures of the invasive tree *Ailanthus altissima*. Land-use legacy plays a dual conflicting role in these dynamics; fragmenting habitats and isolating populations (increasing population structuring), while simultaneously increasing migration through human traffic (reducing population structure). Based on the extremely prolific seed development of *Ailanthus altissima* (Aldrich et al., 2010; Kowarik & Säumel, 2007; Martin & Canham, 2010; Virginia Department of Forestry, 2009), we propose that human mediated dispersal of propagules plays a larger role and

that human development of a site acts to reduce the level of population structuring for *Ailanthus altissima*.

> **Hypothesis 3.1:** Sites with heavy human impact will display reduced levels of population structuring, measured as lower G''_{ST} and Phi_{PT} indices.

This condition would be true if the effects of anthropogenic transport of propagules is sufficient to overcome the fragmentation caused by human development. Support for this model comes from the high mobility of *Ailanthus altissima* propagules, as observed in both terrestrial and aquatic systems (Aldrich et al., 2010; Delgado et al., 2009; Kowarik & Säumel, 2007, 2008; Landenberger et al., 2007).

We also investigate the role of clonal reproduction in such a system. As discussed in chapter 1, human development and fragmentation of sites results in heterogeneous environments, both spatially and temporally. We also discussed that such environmental heterogeneity tends to promote higher levels of clonality in many plant species. This is important to understanding population structure, as clonality in plants tends to promote higher levels of heterozygotes than would be observed under sexual systems (Francois Balloux, Lehmann, & de Meeûs, 2003; Hartl & Clark, 2007). Population indices are formulated to represent the reduction

of heterozygosity as a departure from Hardy-Weinberg equilibrium, thus clonality tends to reduce the value of population indices.

The convergence of these two mechanisms means that both clonality and human mediated geneflow should result in a lower degree of population structuring in heavily urban sites. However, from the perspective of the plant these two mechanisms act in different directions; anthropogenic transport favors investment of seeds, while clonality shifts resources to vegetative development (Cheplick, 1995; Gardner & Mangel, 1999; F. Liu et al., 2008; Méndez, 1999; Sakai, 1995; Winkler & Fischer, 2002). We have already seen reduced viability of seeds under heavy human development (under greenhouse conditions, see chapter 2), but the ecological impact of this finding remains unknown. This reduced seed viability should decrease the relative reproductive contribution of sexual allocation, thereby increasing the relative level of clonal reproduction. From this we derive our second hypothesis:

Hypothesis 3.2: The proportion of clonal reproduction at urban sites will be higher than at exurban sites

Fortunately this is simple to verify, as clonal offspring are, by definition, genetically identical to their parent. Testing hypothesis 3.2 will allow us to partition out the relative effect of geneflow and clonal growth, and to determine how reproductive strategy is affected by human land-use effects.

As mentioned above, *Ailanthus altissima* is well adapted for seed dispersal. Because of this, we tested a null hypothesis, based on a simple geometric assumption that geneflow attenuates as populations become more distant. If propagules are randomly distributed, and human development plays no role in population structuring, then the number of migrant propagules should attenuate based on the distance between sites. Sites located in close proximity should show high levels of geneflow, while more distant sites will have much lower levels. Long range human mediated transport, however, may bypass this assumption and reduce the correlation between distance and geneflow.

Hypothesis 3.3: Geneflow between sites is correlated to geographic distance between those sites.

This null model also acts as another method of disentangling the relationship between the clonality and propagule transport mechanisms. This hypothesis is based on a background rate of passive dispersal, whereas falsification of this hypothesis would be evidence in favor of human transport mechanism.

Methods: Ailanthus protocol

Samples of *Ailanthus altissima* were collected from six sites in the central New Jersey and Southern New York area. Sites were chosen to represent a diversity of environmental habitats, with varying degrees of human impact to serve as one

of our treatments. We performed collections at two sites for each treatment; urban, suburban, and exurban.

Two urban sites were chosen for their high levels of human impact, Branch Brook Park in Newark and Liberty State Park in Jersey City. Branch Brook Park is a large public park (~150 hectares) that runs approximately 3 miles north to south in the northern half of Newark (in Essex County). The site is heavily managed, has several high traffic access roads through it, and is in the middle of one of the largest high density population centers in the state of New Jersey. Liberty State Park was our second urban site. Located in Hudson County, Liberty State Park is approximately 1,212 acres (490 ha) in size. Similar to Branch Brook Park, Liberty State Park is also located in an area of high population density, although the study area that we chose has little to no direct human traffic. Instead, we worked in the 100 ha restricted access brownfield. This brownfield has not been managed since abandonment in the 60s and therefore gave us a representation of post-industrial impact as separate from concurrent human activity (Gallagher et al., 2008b; Gallagher, Pechmann, Holzapfel, & Grabosky, 2011). The soil at this part of Liberty State Park is contaminated with heavy metals including, chromium, zinc, copper, lead, and arsenic. These two urban sites provide a good foundation for a comparison of human impact against the relatively less disturbed sites.

Our exurban sites were chosen in opposition to the above sites, with an emphasis on limited human intervention in the environment. Due to our study being localized to New Jersey, we could not make use of a proper "rural" site, as most of the state has long since been developed. We were, however, able to make use of sites which has previously been developed and then returned to a more natural state, hence the choice of "exurban" to describe these locations. We made use of two exurban sites; Hutcheson Memorial Forest and the Watchung Reservation. Hutcheson Memorial Forest is a 200 hectare plot of land located in Somerset County, New Jersey which is privately owned by Rutgers University for use in ecological studies. The majority of Hutcheson Memorial Forest is maintained in a natural state, although a small region near the entrance is set aside for use in successional plot studies. The only human traffic through this site is on foot, and due to the land being private property this disturbance is kept to a minimum. The Watchung Reservation is a public nature preserve of approximately 790 hectares being maintained by Union county. It is open to the public and has many hiking trails, but also has large regions of the park with no direct access roads. To ensure that our exurban site had as little human impact as possible we chose these interior locations to collect our samples at. Overall both sites had limited human intrusion and were chosen to be the least impacted locations possible in New Jersey.

Our suburban sites were more difficult to define. We eventually settled on a definition that included a moderate human population density combined with

close proximity to major roadways. Two sites were found to meet this criteria, which we will call Plainfield and Rockland County. The Plainfield site chosen was located in Somerset County, not far from the Watchung Reserve. The study site was located along county highway 531 in the town of North Plainfield. The site had a steep embankment of approximately 20 feet, reducing human foot traffic. Overall classification was considered to be suburban as this site was located between a moderately sized county highway and zone of single-family housing. The Rockland County site is the one site not located in New Jersey, instead being just across the state line in New York. Similar to our Plainfield site, the Rockland County site was located on the edge between a single-family residential zone and a moderately sized county highway. A major criteria for both suburban sites was the presence of human traffic with little to no industrial impact. Thus an optimal suburban site would provide the potential for geneflow into and out of the local population without the added physiological stress of heavy metals or other contaminants.

With regard to our three treatments, each pair of sites was chosen for specific conditions. Urban sites had high human population density and additional physiological stress from heavy metals and other contaminants. Exurban sites were chosen based on limited human development, little to no traffic and otherwise limited impact. Suburban sites focused on proximity to roadways and low density residential dwellings, while avoiding any possible industrial impact.

Suburban sites, due to their acting as a border between residential buildings and highway, were of limited area, often only a few hectares. This is in comparison to sites like Branch Brook Park which measure in at 150 hectares or Liberty State Park (490 hectares). This did not affect our data collection, however, because of the patchy distribution of *Ailanthus altissima* in its environment. *Ailanthus altissima* is found in dense stands, and the size of these stands was independent of site, so our data surveying was kept uniform by collecting from these subpopulations.

Tissue samples were collected during the summer of 2012. Tissue collection focused on green leaves which were intact, avoiding any leaves with obvious physiological stress or herbivore damage. Specifically we wanted to avoid damage caused by insects which may have left genetic material behind, particularly eggs or leaf miner insects. Sites were broken up into sub-populations based on contiguous proximity to other individuals of *Ailanthus altissima*, with new sub-populations being assigned to any group that was isolated by at least 20 meters. All sites were assigned at least two sub-populations. For the two suburban sites these two sub-populations were separated by a road.

All samples were immediately stored on ice before being placed for long-term storage in a -80°C freezer on campus. Unfortunately, this freezer had a failure and samples had to be transferred to an alternate freezer in the Ware lab. The freeze-thaw cycles of this event resulted in tissue damage, and the integrity of

the leaf tissue showed a marked decline by the end of the project. This limited our ability to re-extract DNA, reducing the number of samples per site.

DNA extraction was performed using a Qiagen Blood and Tissue DNEasy Extraction Kit (Qiagen, Venlo, Netherlands). Tissue disruption prior to extraction was performed with tools that were heat sterilized to between $200^{o}C$ and $250^{o}C$. Leaf surface was washed with a dilute bleach solution to remove surface contaminants. The dilute bleach solution used in this step was approximately 3% NaClO by volume. After wash the tissue sample was cut into small pieces with the heat sterilized tools before being placed in an Eppendorf Tube full of Qiagen lysis buffer. We extended the duration of the incubation period for our leaf tissue due to the kit being nominally designed for animal tissue. An incubation time of 18 hours was sufficient for both cell wall and membranes. After finishing extraction the final DNA was transferred to a labeled Eppendorf tube and placed into long-term storage. Similar to the fresh leaf samples, extracted DNA was initially stored in a $-80^{o}C$ freezer, until a mechanical failure necessitated a transfer to the Ware lab freezer. Long-term storage in a freezer with a freeze-thaw cycle may have resulted in some degradation of DNA, but overall integrity was verified through PCR results. Similar verification was done to primers which were also stored under the same conditions.

PCR was conducted using a combination of 9.5µL millipore H_2O, 12.5 µL of Qiagen Taq Mastermix, 1µL of forward primer, 1µL of reverse primer, and 1-2µL

of our DNA template (Table 3.5). The Primers used for the *Ailanthus altissima*

samples were microsatellite markers specifically developed for this species

(Dallas, Leitch, & Hulme, 2005). These same primers have also previously been

used by Aldrich *et al*. in their own study and shown to perform well (Aldrich et al.,

2010). The reactions were run for two sets of 30 cycles, with the temperature for

the annealing step being defined as T_m-2^oC for the first set of cycles and T_m-1^oC

for the second set. PCR product was stored under the same conditions was DNA

extract above.

Table 3.5. Composition of 25 µL *Ailanthus altissima* PCR reactions.

	Volume
Millipore Water	9.5 µL
TAQ Mastermix	12.5 µL
Forward Primer	1 µL
Reverse Primer	1 µL
Template DNA	1-2µuL

Gel electrophoresis was conducted using a 1.2% agarose gel. The standard

0.8% agarose gel was initially used, however the small size of the STR

fragments made it necessary to use a higher concentration gel. We tested

multiple methods of visualization, including ethidium bromide, Genscript brand

GelRed, and both in combination. Ethidium bromide is a well established

technique but the material is a known carcinogen and teratogen. It also requires

a wash in $MgSO_4$ to remove excess ethidium bromide from the gel, resulting in a

large quantity of hazardous waste. We settled on using GelRed on its own, as visualization results were similar to those of ethidium bromide, but did generated far less hazardous waste. All gels were run using a Genscript 100-bp ladder for standardization. Gels were visualized under ultraviolet light and the resulting fluorescence was recorded using a Canon PowerShot A590 IS digital camera.

Gel image analysis was performed using ImageJ software developed by the National Institutes of Health (Abràmoff, Magalhães, & Ram, 2004). Gel bands were characterized in one-dimensional lanes through a histogram of fluorescence intensity. Peaks in the histogram were identified based on local maxima in the regions predicted by Dallas *et al.* (Dallas et al., 2005). These histogram peaks were then exported to a spreadsheet for further calculations. OpenOffice Calc (an open source equivalent of Microsoft Excel) was used to calculate R_f values for the bands. A linear regression of the 100-bp ladder standard was used to estimate the size of PCR product fragments. The results of this regression equation were again curated against predictions from Dallas *et al.* (Dallas et al., 2005). These final molecular weight values were then used to infer the number of microsatellite repeats. Given that our microsatellite markers were STRs (Short Tandem Repeats) all of the repeated units consisted of two base pairs, making the conversion from base pairs to repeat count rather simple. The dataset with a final number of repeat units was then exported in an .XLS file for analysis in GenAlEx.

Methods: GenAlEx

Datasets were combined into a single .XLS file for analysis in GenAlEx. Standard headers were added to the spreadsheet, in accordance with (Peakall & Smouse, 2006, 2012). Samples were organized into six regions, each corresponding to a specific field site. AMOVA tests were performed assuming co-dominance. The co-dominance assumption was justified in our study because microsatellite alleles are not expressed, therefore they exhibit equivalent degrees of dominance. AMOVA tests were performed for the Phi_{PT} and R_{ST} indices, the former being a widely used index developed by Excouffier (L. Excoffier et al., 1992; Laurent Excoffier & Excoffier, 2008) and the latter being a microsatellite specific index developed by Slatkin (Slatkin, 1995). Although R_{ST} is rarely used anymore the underlying assumptions of the R_{ST} model, namely the stepwise mutation model, may help us to interpret the Phi_{PT} results. Nm values were also calculated as part of the Phi_{PT} model. Nm represents the number of individuals from a specific population who become migrants in a given generation. For this reason, Nm is an important parameter in expressing geneflow between otherwise isolated sites.

The search for the correct population index to use is a difficult one with no clear answers (Meirmans & Hedrick, 2011; Whitlock, 2011). No index currently developed is able to account for all of the effects of mutation (Ryman & Leimar, 2009). Despite this, we must choose some indices to report our findings. Our work uses microsatellite markers with a high mutation rate, which precludes us

from using G_{ST} and Nei's G'_{ST}. R_{ST}, though specifically designed for microsatellite

systems, has recently fallen out of favor for the reasons enumerated above.

Jost's D_{est} parameter does not provide us with useful information on population

structure, and so does not provide answers the questions we are after. As a

result we are left using Meirmans and Hedrick's G''_{ST} and Excoffier's Phi_{PT} as our

reported statistics. D_{est} was also calculated due to its high correlation with G''_{St}

(Heller & Siegismund, 2009).

Methods: STRUCTURE

Haplotype analysis was performed using the program STRUCTURE, developed

by the Pritchard Lab at Stanford (Pritchard, Stephens, & Donnelly, 2000). The

data fed into this program was the same microsatellite marker data used above,

with minor formatting adjustments to convert it into an appropriate STRUCTURE

input file. STRUCTURE utilizes a Markov-chain Monte Carlo (MCMC) simulation

to assign individuals to a previously defined number of haplotype groups. Initial

iterations of a MCMC simulation will have low accuracy, due to randomized

starting parameters, and are designated as a "burn-in" phase. The output of each

step of a burn-in phase is discarded, and used only to generate the next step in

the Markov Chain. STRUCTURE documentation recommends a burn-in phase of

10,000 to 100,000 iterations to allow models to converge to reasonable values

(Pritchard et al., 2000). Having too short of a burn-in phase can severely affect

the reliability of models, but excessively long burn-in has little downside (apart

from the increased data processing time). As a result, we chose to use a 100,000 burn-in phase followed by a 100,000 sampling phase.

STRUCTURE is assessing the likelihood of multiple models, and for a model of K possible haplotypes each individual will have K values, representing the relative probability of belonging to each one of these groups. The total sum of all K probabilities will equal 1. Unfortunately, we have no prior knowledge of the number of haplotypes present in our study sites. Although we have six discrete sites, there is not enough information to assume any such correlation with haplotype numbers; a given site may have multiple haplotypes, or a single haplotype can be spread across many sites. In order to determine the correct number of haplotypes (K) we performed multiple simulations with K ranging from 1 to 20 in replicates of 10. Maximum log-likelihood values from each model were recorded in a scatter plot, as see in figure 3.2 and 3.3. The optimal model for the number of haplotypes was chosen based on this maximum likelihood comparison.

A figure based on this optimal k value was then generated, with each *Ailanthus altissima* individual being assigned a probability value for belonging to each haplotype group. A final color-coded figure was then generated, allowing us to visualize population level trends in haplotype distribution across all 6 sites.

Methods: MIGRATE-N

The population dynamics between our six study sites was examined using the program MIGRATE-N developed by Peter Beerli from Florida State University (Beerli & Palczewski, 2010). MIGRATE-N was configured to use Bayesian inference and Markov-chain Monte Carlo simulations to infer posterior probability distributions for population and migration parameters. Parameters reported by MIGRATE-N are mutation scaled, introducing two new terms; Θ and M. Θ, the mutation scaled population term, is equal to the effective population size multiplied by the mutation rate (N*μ). M, the mutation scaled migration rate, is equal to the probability of individual migration per generation divided by the mutation rate (M=m/μ). Despite the unusual scaling, the mutation constants cancel each other out, and we can readily calculate the number of migrant individuals (Nm) by multiplying the two terms together. It is also possible to estimate the original parameters, effective population size (N) or migration probability (m), is you have a known mutation constant (μ).

Our dataset was quite large (276 individuals across 6 populations at 8 loci) so we utilized the Brownian motion estimate model for our simulations. The Brownian motion estimates perform as well as the singlestep model, and run in a much more reasonable timeframe (P. Beerli, personal communication). Given the complexity of our models (a single run of our data under the simplest models could easily take more than 24 hours on a high performance personal computer), and the need to generate multiple replicates, we made use of the CIPRES

computer cluster to run our simulations (Miller, Pfeiffer, & Schwartz, 2010). Our 8 loci data set was run across 9 nodes (1 node per locus, plus 1 head node to coordinate the parallel computing), greatly reducing the amount of time needed to complete a simulation. This enabled us to perform exploratory runs, tweaking parameters of the model to improve the distributions of our posterior probabilities.

Initial runs of MIGRATE-N using our full dataset were run for a relatively small number of short chains. These initial runs were meant to verify proper function of the program, correct data formatting, and to find a rough estimate of the population and migration parameters. These rough estimates could then be fed back into MIGRATE-N as starting prior values for our next run. Beginning our MCMC analysis closer to the true value should, according to Bayesian theory, make the values ultimately converge at the true value more often. Posterior distributions from such simulations should be more consistent, as well as more narrow. Once the prior parameters had been settled on we ran the simulations for a longer number of chains, giving the simulations more time to finally settle on the values for a posterior parameter.

Irregularities in the posterior distributions occurred as a result of local maxima in the genealogy landscape. To correct for this we introduced a heating element to the simulation, enabling for a more effect exploration of the possibility landscape. Initial heating test was performed using 4 chains under a bounded adaptive setting to identify optimal heating values. Under the "bounded adaptive"

configuration heating elements were allowed to vary their values based on performance of the simulation (Beerli & Palczewski, 2010). During these trials we saw very minor variance from the starting default heating parameters, indicating that we would be justified in using the static heating model. All further models were run using a 4-chain, 1-step swap with [1, 1.5, 3, 100000] as the heating parameters.

Once the prior parameters and heating parameters had been estimated we were finally able to run the full analysis. Due to the probabilistic nature of Bayesian and Markov-chain Monte Carlo simulations we ran multiple replicates of our simulations and examined the combined probabilities of all runs.

Results: Effective Population Size

Estimates of the effective population size of *Ailanthus altissima* at our study sites showed that the sub-populations we studied were quite small, indicating that the extent of invasion was limited (see figure 3.1). Effective Sample Size (ESS) of these simulations provides evidence of convergence (see Table 3.6). Estimates of mutation scaled migration rates (not shown) were inconclusive, as simulations did not converge to consistent parameter estimates.

Table 3.6 Mutation scaled population size (Θ) and effective sample size (ESS) for six *Ailanthus altissima* populations.

Site	Θ	ESS
Branchbrook Park	0.0095	173
Hutcheson Memorial Forest	0.0111	176
Liberty State Park	0.0154	410
Plainfield	0.0108	105
Rockland County	0.0134	228
Watchung Reservation	0.0037	119
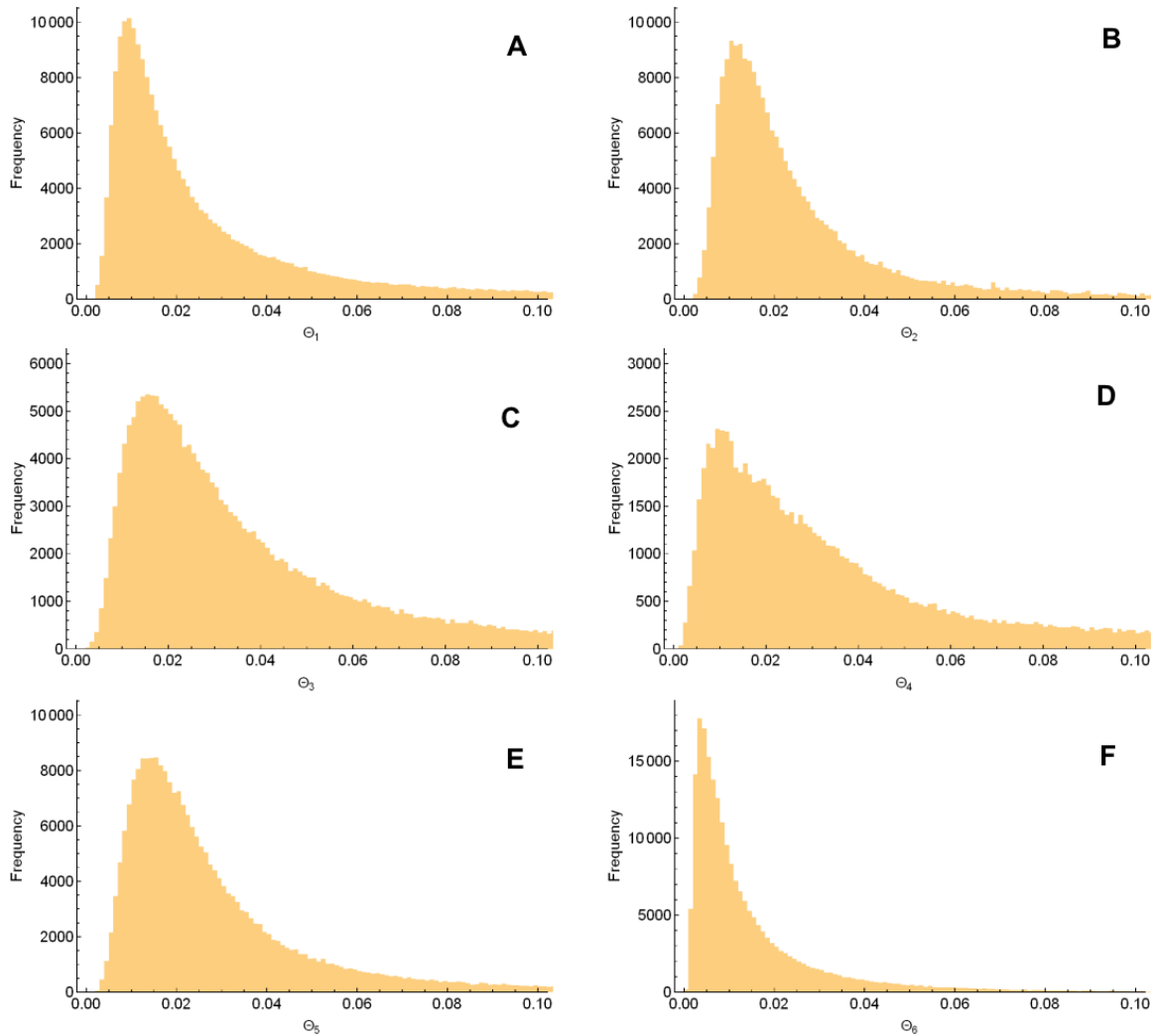
Figure 3.1. Posterior probability distributions representing estimates of the mutation scaled effective population size for *Ailanthus altissima* at: Branchbrook Park (A), Hutcheson Memorial Forest (B), Liberty State Park (C), Plainfield (D), Rockland County (E), Watchung Reservation (F).

Results: Haplotype Estimation

Due to the variable nature of Markov Chain Monte Carlo simulations, we ran multiple rounds of tests to estimate the number of haplotypes (K) from our data. The optimum number of possible haplotype groups in our *Ailanthus altissima* data were consistently found to be around K=5 to K=7. Our initial rough

simulations consisted of a burn-in phase of 10,000 generations and a sampling phase of another 50,000 generations (a burn-in equal to 20% of the sample size). Simulations were run with ranges from k=1 (a single haplotype, no differentiation between groups) to K=20 (highly fragmented, with an exceedingly high number of haplotypes). Each MCMC simulation was replicates 20 times to provide a measure of consistency for the estimates of K, ensuring that the Markov Chain simulations did not get stuck in a local maxima and feed out aberrant parameter estimates. A plot of these initial estimates can be seen in figure 3.2.
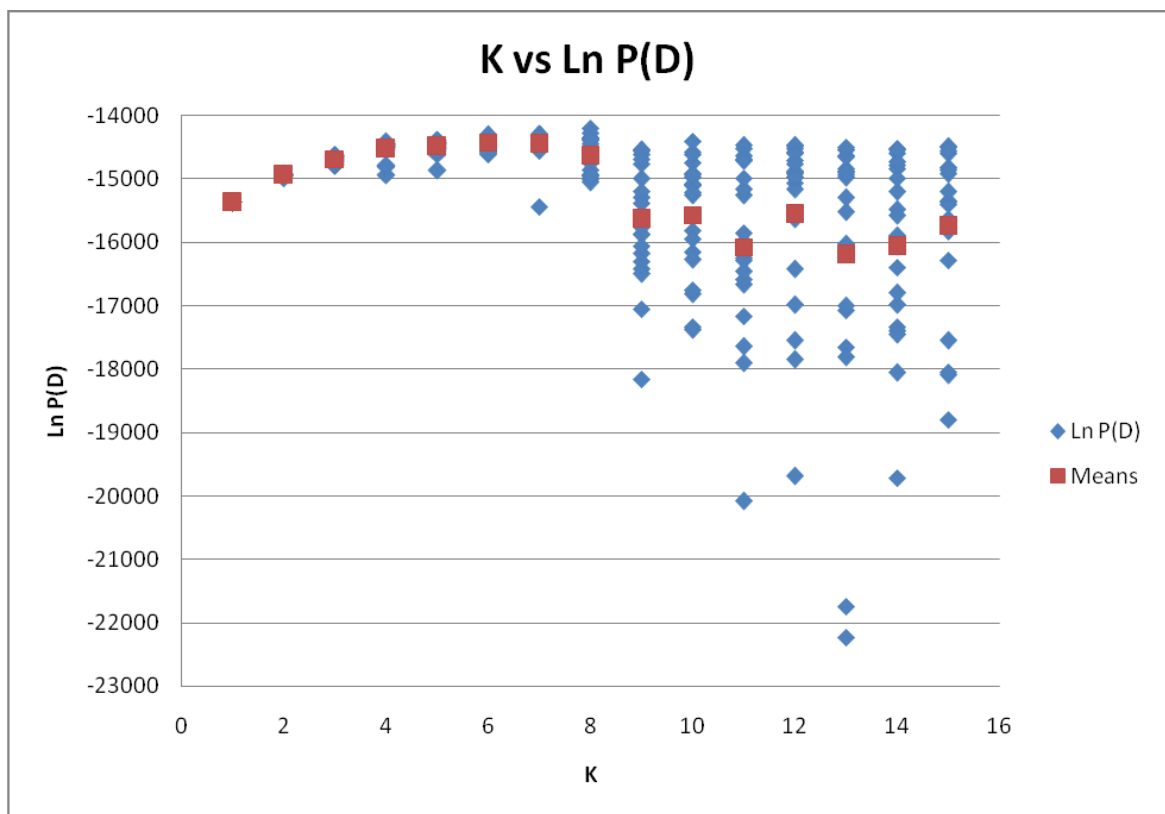
Figure 3.2 Probability estimates of haplotype number (K) for *Ailanthus altissima* acrosss 6 sites. Data were geneated through MCMC with 10k burn-in, 50k sampling, and 20 replicates.

In the above figure we can see that the probability of our models increases uniformly for low values of K. Variation of these estimates was also extremely low. The optimum model appears to be found when K has a value of between 5 and 8. As K approaches higher values than 8 we see a sharp decrease in the probability of the models. More importantly, we see a rapid increase in the variance of the estimates, indicating a non-convergence of the Markov chains. Based on this quick preliminary simulation we estimated the most likely number of haplotypes (K=5 to K=8) for our next round of simulations.

Our next round of simulations included a much longer burn-in of 100,000 generations and data collection of a further 100,000 generations. The longer simulations are important because they would allow more time for the Markov chains to converge, and could potentially reduce the variance of the higher K estimates. A scatter plot of log-likelihoods of the resulting models can be seen in figure 3.3. Once again, 20 replicates were run for each model, showing a high consistency at low K values and declining as the models become more complex.
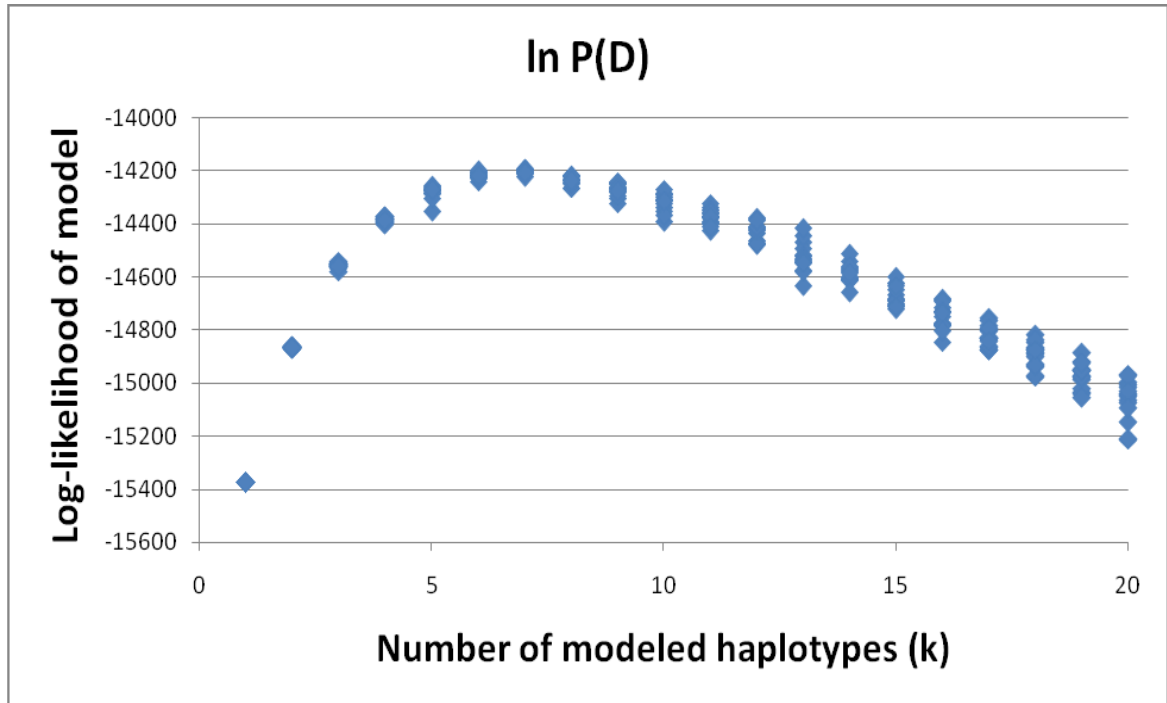
Figure 3.3. A comparison of Markov Chain Monte Carlo models predicting the number *Ailanthus altissima* haplotypes across all 6 sites. 10 replicates were run for each value of k.

As can be seen in the figure, model outputs for a given K were consistent, indicating reliability of the STRUCTURE simulations. The continuous decline of log-likelihood on the right tail of the figure indicates that highly complex models with many haplotypes do not perform any better than models with intermediate numbers of haplotypes. Although we could potentially have simulated even larger numbers of haplotypes, this clear and continuous decline was evidence that no further models were required. The maximum likelihood peak of these models consistently fell between 6 and 7 haplotypes. Additionally, these models also had the smallest variance between log-likelihood values for their set of 10 replicates.

Based on these two tests we were able to conclude two important details about our model systems; 1) that we had sufficient burn-in time for the model parameters to stabilize and 2) that the most probable value for k fell between 3 and 10 haplotypes. We used this information to run our final analysis where we examined the range of k between 3 and 10, using a 50,000 step burn-in phase and 500,000 recorded simulations.
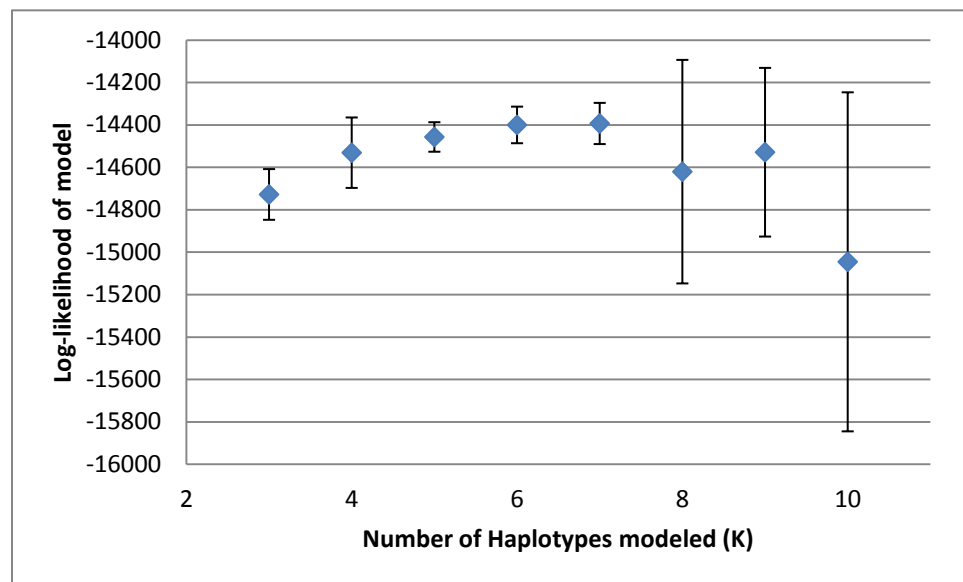
Figure 3.4 Probability estimates of haplotype number (K) for *Ailanthus altissima* acrosss 6 sites. Data were geneated through MCMC with 50k burn-in, 500k sampling, and 20 replicates.

As you can see in figure 3.4, the results of our full-scale simulation were consistent with the previous 3 tests. Maximum likelihood rose and then fell, with a peak in the range of k = 4 to k = 7. The variance of the simulations rose dramatically after k to 8 haplotypes or higher, indicating a lack of convergence in higher k models. We took this to indicate that the maximum value of k was 7, and

disregarded any models with a higher haplotype count, even if those models showed a higher likelihood score. We verified the optimum haplotype number using the ΔK method developed by Evanno et al. (Evanno, Regnaut, & Goudet, 2005). ΔK values showed a clear peak at k = 7, in agreement with our maximum likelihood analysis (see figure 3.5 below). From these two methods we concluded that our data shows evidence of 7 major haplotype groups.
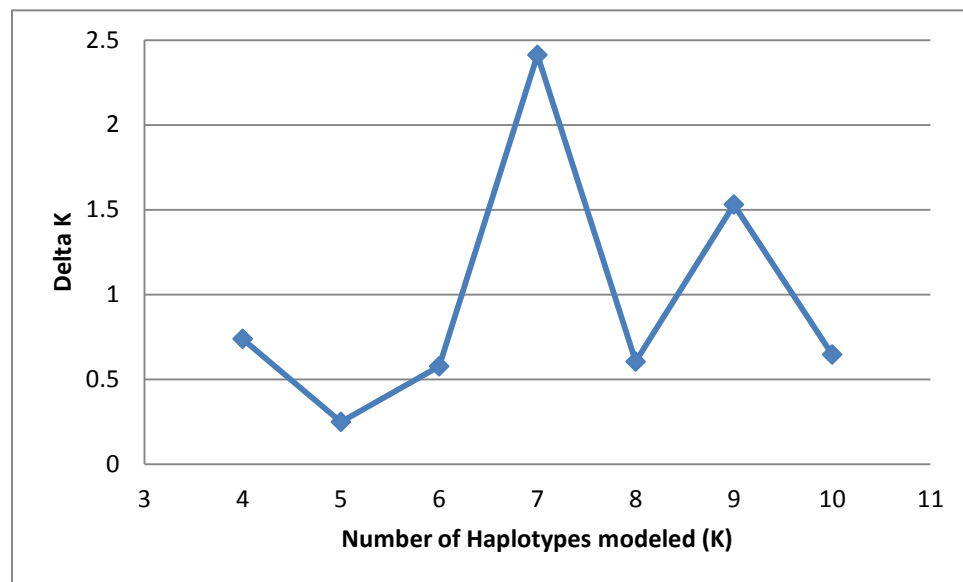
Figure 3.5 ΔK values for *Ailanthus altissima* models. Data were geneated through MCMC with 50k burn-in, 500k sampling, and 20 replicates.

The actual output of the STRUCTURE MCMC simulations is a set of data points where each individual is assigned a relative probability value of belonging to each of the k haplotype groups. Probability values for belonging to each of these haplotypes were assessed based on feeding the microsatellite marker information from our populations into the MCMC modeling software of

STRUCTURE. Following a 50,000 iteration burn-in phase, the simulations were then run for 500,000 iterations (10% burn-in), enough to ensure that consensus was reached.

An individual must belong to one group, the value of all probabilities sums to 1.0, so strong assignment to one haplotype means a reduced probability of belonging to any other. Each narrow bar represents one *Ailanthus altissima* individual, and each color represents a different haplotype it can belong to. Individuals are grouped by site and then ordered by sample number along a transect. This allows us some degree of geographic information, despite not having exact GPS coordinates.
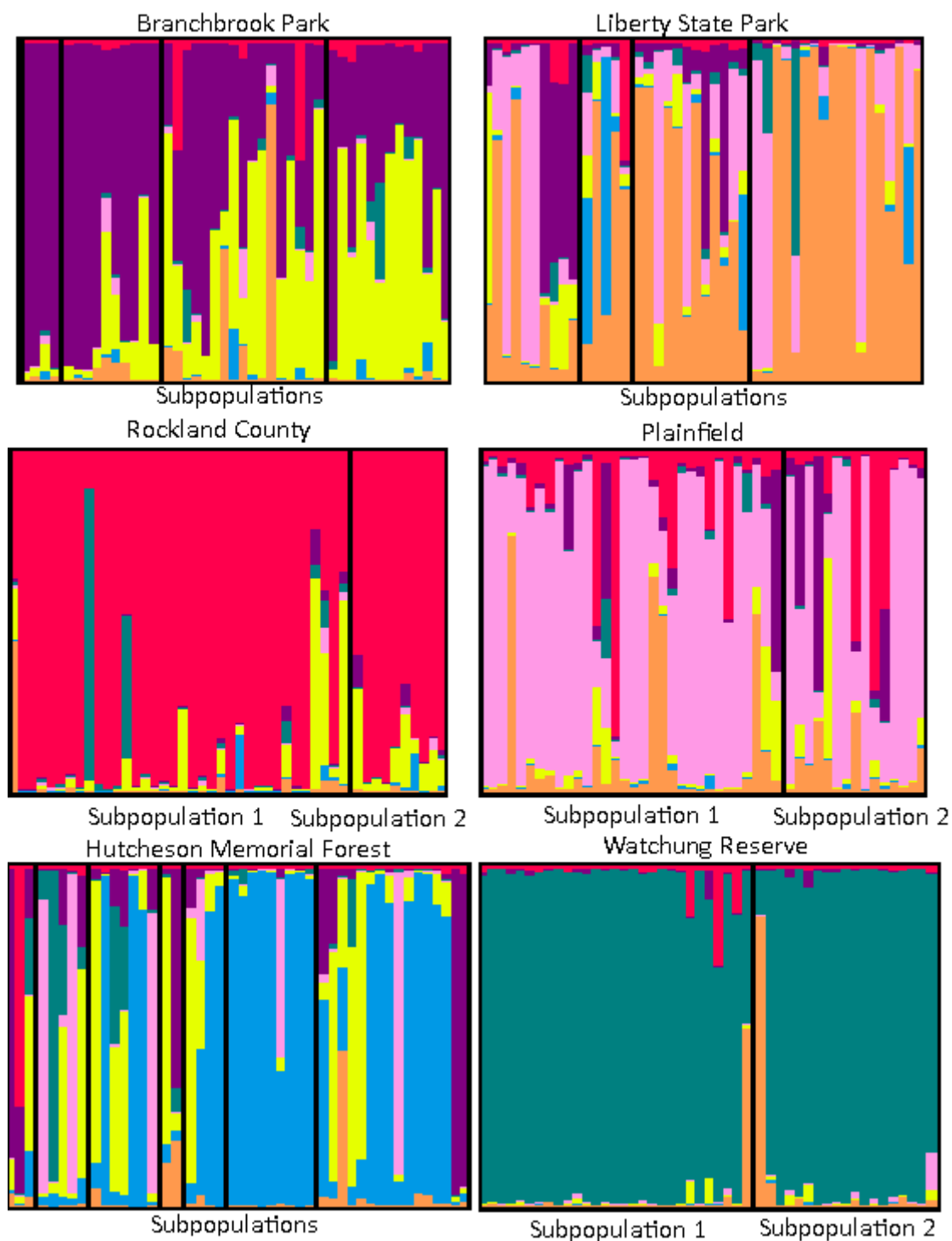
Figure 3.6 STRUCTURE output of haplotype probabilities for 278 Ailanthus altissima individuals across 6 sites. Figure prepared in DISTRUCT.

A visual representation of this information can be seen in figure 3.6; figure generated using DISTRUCT (Rosenberg, 2003). The large amount of variation and lack of contiguous color bands in several regions indicates a strong amount of geneflow, which corroborates the results of our GenAlEx analysis. Despite this, several regions do separate out by haplotypes. Rockland County and the Watching Reserve both are largely dominated by a single haplotype, indicating a higher level of population structuring. This is in agreement with our GenAlEx results in the following section.

In addition to haplotype probability modeling, STRUCTURE also generates probability distributions for the likelihood and alpha values found during the Markov Chain Monte Carlo simulations. These parameters are important for verification that the MCMC simulations do not end up at widely divergent estimates. Dataplots of the likelihood over time values can be found in Figure 3.7, while the estimated alpha values can be seen in Figure 3.8. In both figures you can see that these parameters have a clear normal distribution, indicating strong convergence of parameters by the MCMC simulations. Based on these figures we can be confident that Figure 3.6 is a strong estimate of the actual haplotype probabilities.
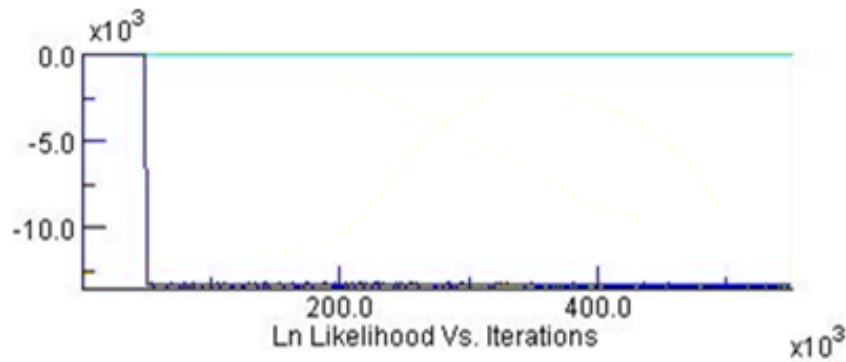
Figure 3.7 The likelihood of MCMC estimates over time for the 7 haplotype model of *Ailanthus altissima*. The flat trajectory indicates convergence.
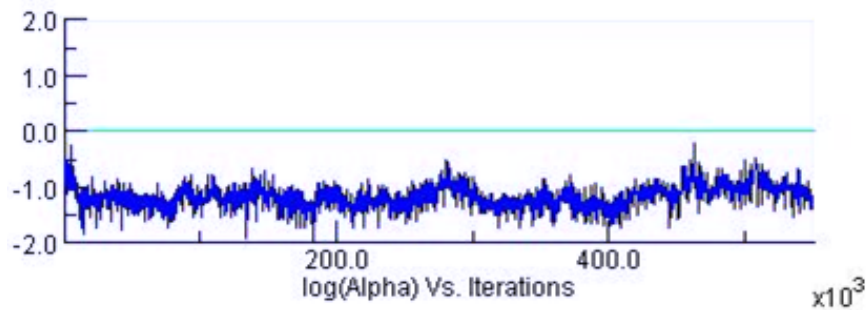
Figure 3.8 The logarithm of the Alpha value over time for MCMC estimates of the 7 haplotype model of *Ailanthus altissima*. The "fuzzy caterpillar" indicates convergence.

Results: GenAlEx

We utilized the Excel package GenAlEx (Genetic Analysis in Excel) to estimate population indices and migrant numbers between our 6 sites (Peakall & Smouse, 2006, 2012). Much like how ANOVA partitions variation between and within a treatment, AMOVA partitions genetic variation, breaking it up into variation between sites and variation within sites. From this information we can derive a population index value, giving us a sense of how well structured the populations are.

Multiple population indices exists, as explained above, and disagreement over which index to use still exists. We display several of them below in Table 3.10.

One of the most popular, and the most recent, population index is Phi_{PT}, developed by Excoffier and Smouse (L. Excoffier et al., 1992). Phi_{PT} can be expressed as a proportion of molecular variance between sites out of the total amount of molecular variance. A comparison of 8 loci across 6 sites showed that *Ailanthus altissima* has a Phi_{PT} index value of 0.03, corresponding to 3% of the molecular variance being found between sites (see Figure 3.9). An overwhelming 97% of the molecular variance was found within populations, indicating a high amount of variation even on the local scale. A more detailed breakdown of Phi_{PT} comparisons can be found in Table 3.7, which shows the pairwise combinations of each site and the accompanying Phi_{PT} value.

Percentages of Molecular Variance

Among Pops 3%
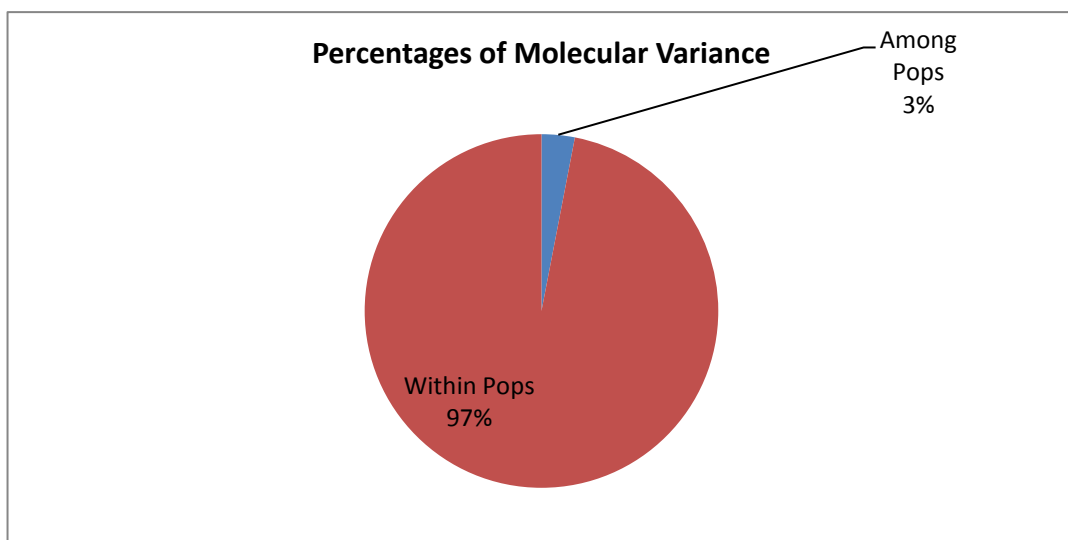
Within Pops 97%

Figure 3.9 Partitioning of molecular variance for all 6 *Ailanthus altissima* populations under Phi_{PT}

demonstrates an overwhelming amount of variation occurs at the small scale (within populations).

Pairwise Population Phi_{PT} Values

Table 3.7 A comparison of pairwise Phi_{PT} index values (ranging from 0 to 1) for *Ailanthus*

altissima across all 6 populations.

BB	LSP	HMF	W	PL	RC		
0						BB	Urban
0.024	0					LSP	
0.019	0.016	0				HMF	Exurban
0.027	0.025	0.027	0			W	
0.023	0.016	0.029	0.033	0		PL	Suburban
0.048	0.037	0.049	0.053	0.027	0	RC	

The extremely low values found for Phi_{PT} raised some concern. Microsatellite

markers are known for having extremely high levels of variation (F Balloux et al.,

2000; François Balloux & Lugon-Moulin, 2002; Slatkin, 1995). This is important

because high variation can reduced the maximum possible value of an index

below 1, which may drastically change the interpretation of our results. One

method of compensating for this is the R_{ST} index developed by Slatkin et al.

(Slatkin, 1995). Despite falling out of favor (see discussion for R_{ST} assumptions

about mutation models) we report the values here as a point of comparison

against the newer Phi_{PT} measures. Figure 3.10 shows AMOVA partitioning of

Ailanthus altissima data under R_{ST} assumptions, while Table 3.8 shows pairwise R_{ST} estimates.
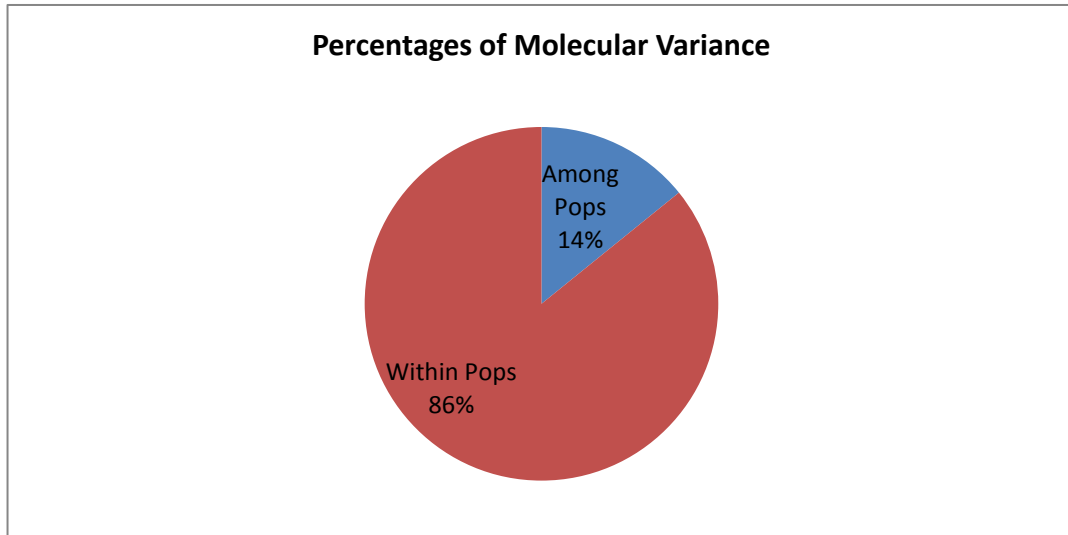
Figure 3.10 Partitioning of molecular variance for all 6 *Ailanthus altissima* populations under R_{ST} has a much higher variation between populations.

Pairwise Population G"$_{ST}$ Values

Table 3.8 A comparison of pairwise G"$_{ST}$ index values (ranging from 0 to 1) for *Ailanthus altissima* across all 6 populations.

BB	LSP	HMF	W	PL	RC		
	0.001	0.001	0.001	0.001	0.001	**BB**	Urban
0.452		0.001	0.001	0.001	0.001	**LSP**	
0.373	0.302		0.001	0.001	0.001	**HMF**	Exurban
0.518	0.385	0.466		0.001	0.001	**W**	
0.376	0.233	0.401	0.465		0.001	**PL**	Suburban
0.485	0.509	0.580	0.450	0.382		**RC**	

G"$_{ST}$ index values were much higher than Phi$_{PT}$ index values in Table 3.7. This can be attributed to the high heterozygousity of microsatellite markers, and the heterozygousity correction of the G"$_{ST}$ index. Desipte these differences, similar trends are visible, as depicted in the heatmap coloration.

Pairwise Population R$_{ST}$ Values

Table 3.9 A comparison of pairwise R$_{ST}$ index values (ranging from 0 to 1) for *Ailanthus altissima* across all 6 populations.

BB	LSP	HMF	W	PL	RC		
0						BB	Urban
0.142	0					LSP	
0.139	0.074	0				HMF	Exurban
0.208	0.103	0.246	0			W	
0.136	0.064	0.178	0.073	0		PL	Suburban
0.264	0.104	0.223	0.188	0.074	0	RC	

R$_{ST}$ estimates were uniformly higher than Phi$_{PT}$ estimates by an order of magnitude, yet 1/2 to 1/5 the size of G"$_{ST}$ index estimates. This demonstrates both the importance of accounting for the highly variable nature of microsatellites, and the importance of mutation model assumptions, which can drastically shift index estimates.

To help resolve these differences we also examined the entire suite of G-statistics, which can be found in Table 3.9.

Table 3.10 F_{ST}, Dest and G-statistics as estimated by GenAlEx. Associated p-values are based on 999 permutations.

	Index value	P-value
F_{ST}	0.032	0.001
G_{ST}	0.015	0.001
$G'_{ST}(N)$	0.018	0.001
$G'_{ST}(H)$	0.426	0.001
G''_{ST}	0.427	0.001
D_{est}	0.417	0.001

Of particular note is that these different indices divide into two distinct groups, either having values that are extremely low or values near 0.4. The former (F_{ST}, G_{ST} and G'_{ST} (as formulated by Nei) are in general agreement with Phi_{PT}. All of these index values are based on an assumed maximum index of 1. The latter two G-statistic indices (G'_{ST} (as formulated by Hedrick) and G''_{ST}) are normalized indices, which take maximum values lower than 1 and scale them up appropriately. The role of this assumption, and the implications of such a large divergence in index values, can be found in the discussion below.

GenAlEx was also used to estimate the number of migrant individuals per generation (Nm) for each pairwise comparison of sites. Nm is an absolute

measure of geneflow. Values of Nm can be found below in table 3.11. When acting in isolation the Nm value should be inversely related to the geographic distance between sites (i.e. more distant sites will exchange fewer migrant individuals). This null hypothesis was tested through simple linear regression, the results of which can be seen in figure 3.11. Geographic distance between sites had little to no effect on the number of migrant individuals exchanged between those sites, indicating that the null model was incorrect. The correlation coefficient was 0.0266, as demonstrated by the nearly horizontal trend line in figure 3.11, and the p-value was 0.561, indicating non-significance. Similar results were also found when comparing the G''_{ST} and D_{est} index values (Figure 3.12, p=0.751; Figure 3.13, p=0.745 respectively). The non-significance of these tests was taken as evidence that geneflow between sites is primarily driven by factor beyond simple geometry. Distant sites were observed to experience higher levels of geneflow than one would expect under the null model.

Table 3.11 Pairwise estimates of the number of migrant individuals (Nm) between sites. Values were estimates through AMOVA analysis in GenAlEx.

Pop1	Pop2	Nm	Distance (km)
BB	HMF	13.247	44.7
BB	LSP	10.034	12.6
HMF	LSP	15.213	49.3
BB	PL	10.775	27.3
HMF	PL	8.351	18.2
LSP	PL	15.452	34.2
BB	RC	4.956	36.3
HMF	RC	4.841	80.5
LSP	RC	6.468	38.2
PL	RC	9.014	62.5
BB	W	8.852	20.3
HMF	W	9.171	25.4
LSP	W	9.807	28.3
PL	W	7.310	7.3
RC	W	4.442	55.2
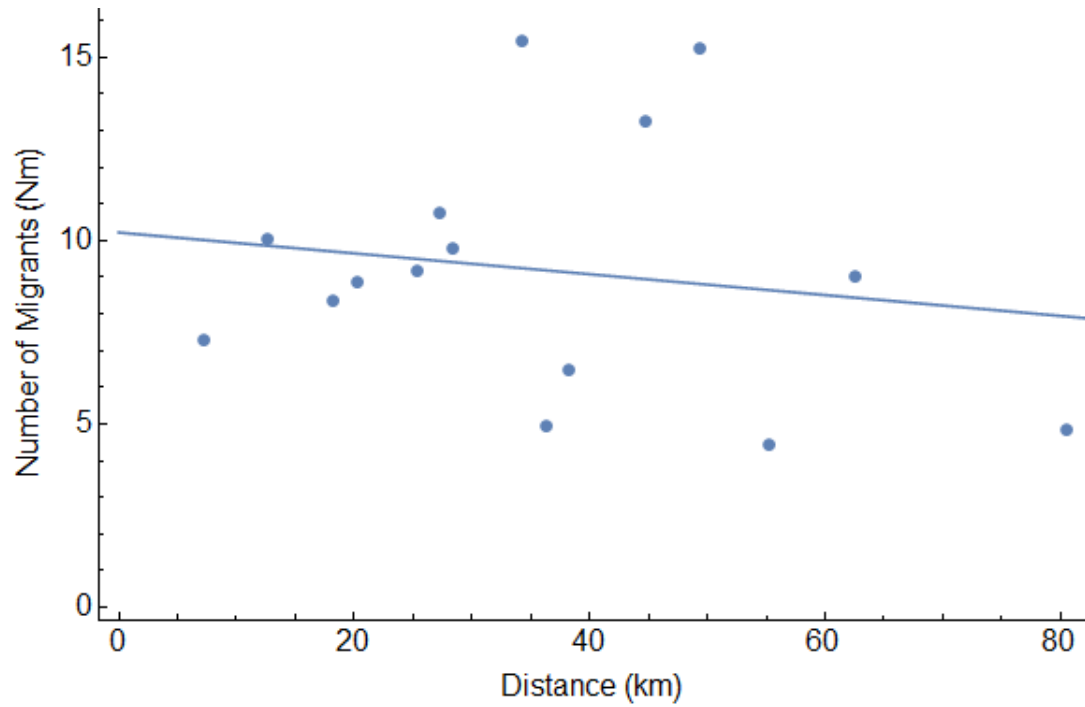
Figure 3.11 The number of migrant individuals between sites shows no correlation with the geographic distance between sites. (R^2=0.0266; p=0.561)
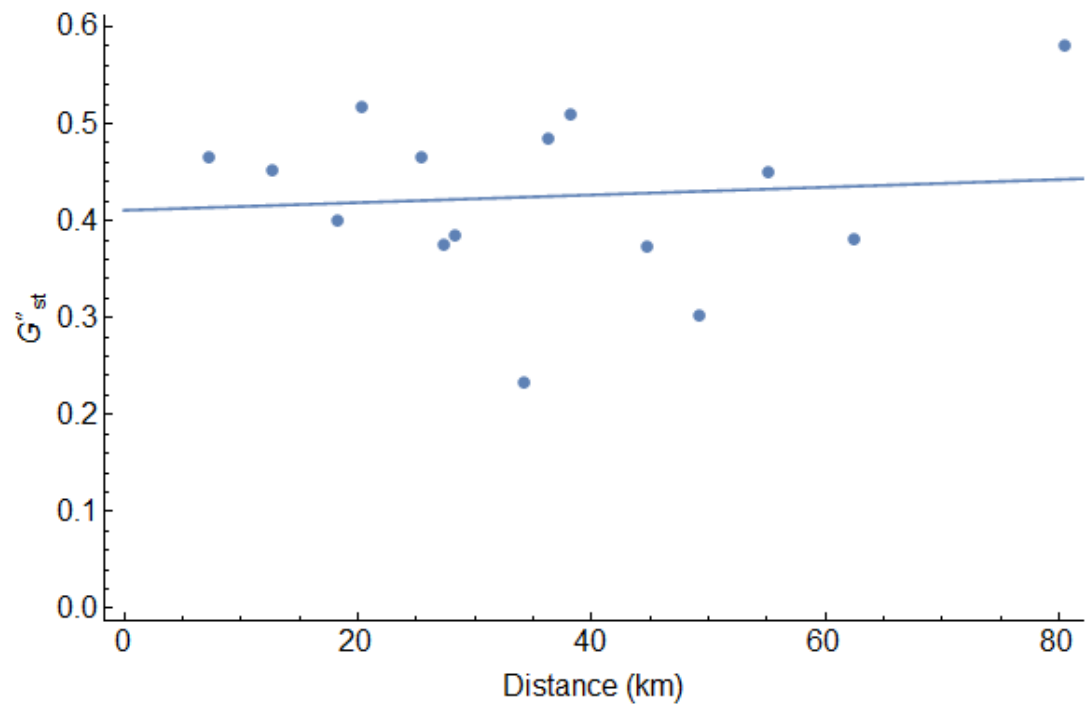
Figure 3.12. The pairwise G''_{ST} index between sites shows no correlation with the geographic distance between sites. (R^2=0.008; p=0.752)
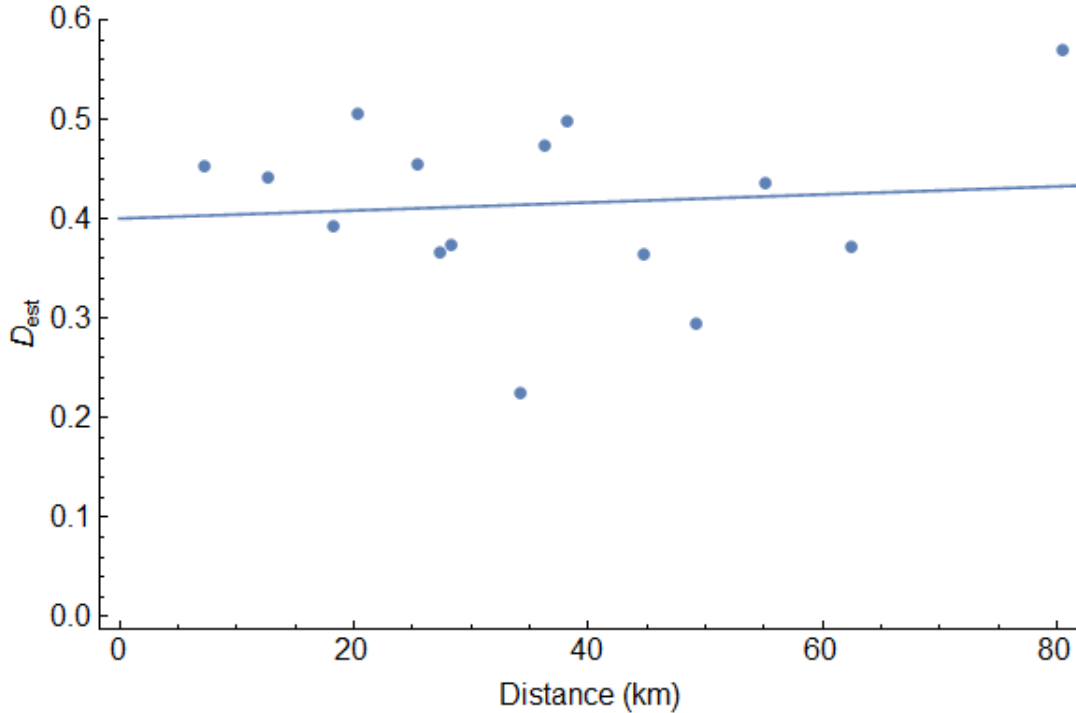
Figure 3.13 The pairwise D_{est} index between sites shows no correlation with the geographic distance between sites. (R^2=0.008; p=0.745)

Discussion

Taken as a whole, our work with *Ailanthus altissima* demonstrates that population structure is likely influenced by anthropogenic effects, but the story is more complicated than we originally thought. We originally hypothesized hypothesis 3.1 that environmental heterogeneity caused by human development would influence the reproductive method of *Ailanthus altissma*, shifting the equilibrium point between sexual and asexual reproduction. Not only did we fail to support this model, we actually found no measurable levels of clonality at the population scale for any of our sites. Despite observations of aggressive root sucker growth, the population dynamics of *Ailanthus altissima* at our sites was

completely dominated by sexual reproduction. This unexpected conflict between initial observation and eventual results could indicate that the aggressive root sucker growth is not actually being utilized for reproduction, so much as it is being utilized to increase individual survival rates. This would explain the discrepancy for such a frequent field observation not being observed at the population scale. Vegetative root sucker growth would still be beneficial to an invasive plant, increasing the resiliency of the organism during the high risk early establishment phase of founder populations. Vegetative growth from the root also increases the resiliency against human control efforts, making it difficult to remove established colonies of *Ailanthus*. Ultimately though, root stock seems to be acting to replace a damaged individual rather than contributing to the spread of multiple individuals. This is extremely useful information for control efforts; it predicts that resources would be better spent focusing on controlling the spread of propagules rather than on preventing vegetative growth.

Having failed to find any evidence of clonality effects at the population scale, we shifted our focus to investigating the population structure of *Ailanthus altissima*. We investigating two models; one model hypothesis 3.2 based on anthropogenic effects predicted that human development level of a site would increase propagule pressure, thereby increasing geneflow and reducing population structuring. The other model hypothesis 3.3 predicted that the population structure of Ailanthus altissima was independent of human land use, and was instead determined by geographic distance. This latter hypothesis was based on

a geometric model of radial dispersal, where propagules become more sparsely distributed as you get farther from the source. The former hypothesis showed some support, although it was not as clear cut as we had expected. The mixed results of hypothesis 3.2 were verified by two different approaches; haplotype modeling through Markov Chain Monte Carlo simulations and partitioning of genetic variation through AMOVA.

Estimation of Haplotype Structure

The results of the MCMC program STRUCTURE software showed some limited, but mixed, results regarding our land-use hypothesis linking the level of urban development to the degree of genetic structuring in a population. Liberty State Park, the most strongly urban of our sites, demonstrated extremely high levels of haplotype variation. This is in keeping with the predicted model of anthropogenically mediated geneflow, where high human development and traffic lead to the dispersal of *Ailanthus altissima* propagules. However, we did not see the same results at our other urban site, Branchbrook Park. This was unexpected because Branchbrook Park is located in the center of Newark, with high levels of both foot and vehicle traffic. Branchbrook Park seems to be dominated by two different haplotypes, with assorted minor sites present in small numbers.

Among the exurban sites, the Watchung Reserve matched our predictions, as the population was heavily dominated by a single haplotype. Hutcheson Memorial Forest, however, showed only moderate levels of its most common

haplotype. Similar results were seen in the suburban sites, with Rockland County consisting mostly of its own haplotype despite extremely close proximity to local roads (approximately 2-3 meters). The Plainfield site, also located along a highway, showed the expected levels of higher variation associated with geneflow.

Regarding haplotype assignment, the Markov-Chain Monte Carlo simulations were consistent across all three tests that we performed. Simulations modeling 8 haplotypes or more displayed a severe lack of convergence, indicating a lack of support for such models. The delta K approach (Evanno et al., 2005) in conjunction with maximum likelihood values helped us to narrow down the number of haplotypes to k=7. Given that we had a total of 6 sites, a model with 7 haplotypes means that the sites cannot separate out cleanly. Either a site is subdivided, with a secondary haplotype existing in a subpopulation, or the haplotypes are spread across multiple sites, indicating geneflow. The STRUCTURE output (shown in figure 3.6) gives support to the latter conclusion. Two sites, Rockland County and Watchung Reserve, were largely dominated by their own haplotypes (red and green respectively). These sites did still show some evidence of geneflow, with foreign haplotypes being probable in some individuals, but Rockland County and Watchung Reserve overall seem to be relatively isolated. The remaining sites all showed a variety of different haplotypes, with Liberty State Park having a particularly large amount of variation. These observations about the Liberty State Park, Rockland County and

Watchung Reserve sites are corroborated by our GenAlEx results (Table 3.7-

3.9). Rockland County and Watchung Reserve were both found to display the

highest levels of population structuring among the 6 sites, from which we can

infer limited geneflow at those sites. Limited geneflow would also preserve the

prevalence of the local haplotype, which we see occurring. The least structured

site, Liberty State Park, showed a corresponding lack of a dominant haplotype,

with extremely irregular patterns in figure 3.6.

 Even in the more diverse sites, some minor clustering was observed between

similar haplotypes. This was likely driven by our sampling method, as we moved

along a transect the plants in closer proximity are more likely to be related.

Ailanthus altissima is quite capable of long range dispersal, particularly around

sources of flowing water (Kaproth & Mcgraw, 2008; Kowarik & Säumel, 2008;

Säumel & Kowarik, 2010). Our sites, however, were selected in drier

environments without any regular source of flowing water in the proximity.

Dispersal, therefore, was largely limited to the distance that the *Ailanthus*

altissima samaras could glide. This helps to explain the small scale haplotype

clumping seen in 3.6.

Previous work with *Ailanthus altissima* microsatellites provide us with a useful

comparison (Aldrich et al., 2010). In comparison to Aldrich's work, we see that

many of our sites have much higher levels of internal variation. The Rockland

County and Watchung Reservation sites were similar to the sites in Aldrich's

work, being largely a single haplotype with some scattered individuals of a different haplotype. The remainder of our sites, however, demonstrated much higher variation indicating that several of our New Jersey sites were not as cleanly structured.

While Aldrich discovered that there was no clean east-west gradient in haplotypes, we have found evidence that landscape level phenomena may be preventing even site-based haplotypes. The most probable driving factor for these differences is the level of human traffic. Many of Aldrich's sites were located in West Virginia, a state known for extremely low population density. New Jersey, by contrast, is extremely densely populated. Even our exurban sites, such as the Hutcheson Memorial Forest, were not far distant from human development. High human traffic is well known for driving dispersal of propagules, resulting in geneflow and mixing of haplotypes. *Ailanthus altissima* fruits in particular are well adapted to dispersal with their flat double-winged samara (Illustration 3.1). This structure allows the fruits to become wedged in motor vehicles, as well as to disperse through waterways (Kowarik & Säumel, 2008; Säumel & Kowarik, 2010).
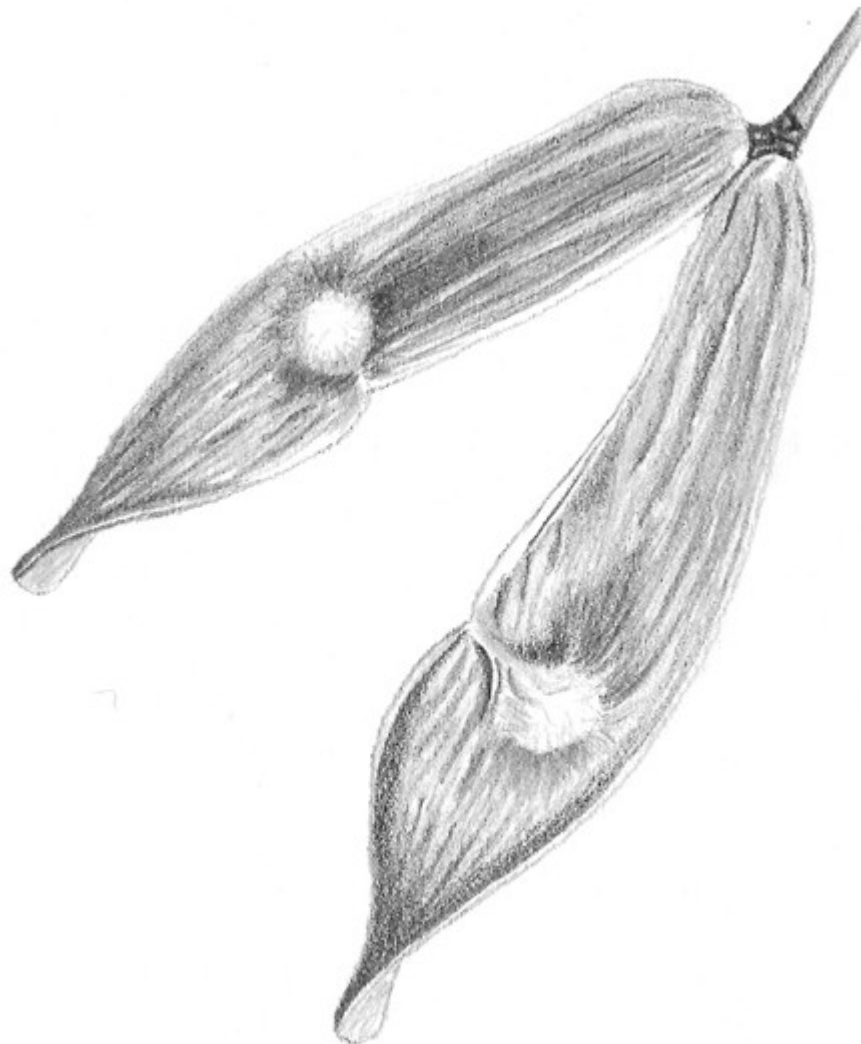
Illustration 3.1. An illustration of the double-winged samara structure of *Ailanthus altissima* fruits. Image © Yvonne Roelofs.

Additionally, in Aldrich's 2010 work the *Ailanthus altissima* sites were only sampled for 20 individuals while we sampled 50 individuals. By the nature of sampling 2.5x as many individuals, we performed our sampling across a larger geographic range. If we subsampled our larger Ailanthus altissima populations into smaller groups of contiguous individuals we can actually see similar results

to Aldrich's work (see figure 3.14). This implies a more patchy distribution of *Ailanthus altissima*, where multiple subpopulations may exist within a given location, some closely related and others more strongly influenced by geneflow.

This is important because it shows the importance of scale when conducting sampling to determine the population structure and genetic origins of invasive species. There more heterogeneous a species is, the more risk there is that a sample will not be truly representative of the population. Rural areas with limited anthropogenic geneflow, such as West Virginia, may be accurately estimated with lower numbers of individuals. More heterogeneous locations will require more samples, and more distance between samples, to get an accurate image of what the population looks like.
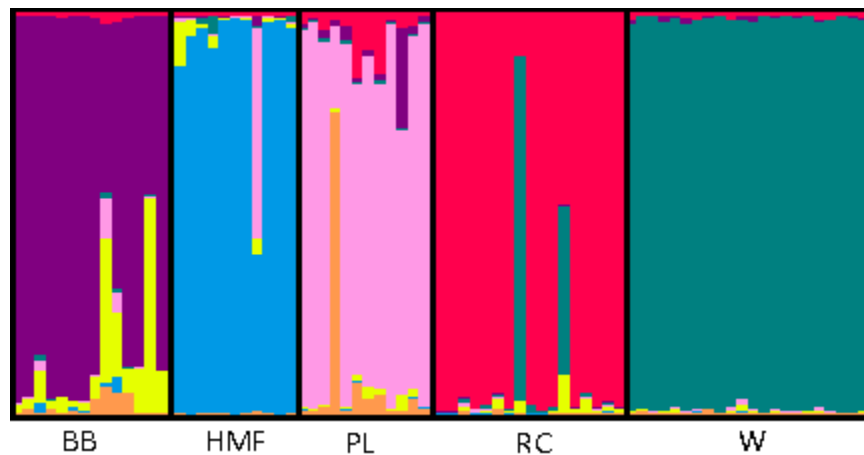
Figure 3.14. Estimates based on 50 individuals show a large degree of variation, but some sub-populations (N= 10-20) are more consistent. These sub-populations are similar in structure to the results of Aldrich et al., 2010.

Partitioning of Genetic Variation

AMOVA analysis performed through GenAlEx was used to corroborate the results of STRUCTURE, and also to estimate geneflow between sites. Pairwise comparisons of estimated population indices were compared to our primary hypothesis, with conflicting results. The pairwise population index tables (Tables 3.7-3.9) showed a similar conflicting result to what we saw in STRUCTURE (see above). Although Liberty State Park, our strongly urban site, showed the weakest amount of population structuring, we did not see a corresponding lack of structure in Branchbrook Park. Watchung Reserve and Rockland County showed relatively high levels of population structuring, as indicated by elevated population index values, but Hutcheson Memorial Forest and Plainfield did not. Although the amount of support for our hypothesis was conflicting, the agreement between GenAlEx and STRUCTURE indicate that our data is an accurate representation of what is occurring in nature.

We used GenAlEx to compare multiple population index types. The exact value of a given population index was dependent upon the specific assumptions of that index (e.g. R_{ST} assumes stepwise mutation), but relationships between the sites remained the same. The extreme heterozygosity of microsatellite regions needs to be corrected for when determining absolute measures of a population index, but relative estimates between sites were consistent across all population indices.

Our unmanaged urban site showed a lower degree of structuring, in agreement with our hypothesis that human traffic promotes geneflow. The managed urban site, however, did not show the same result. From this were saw evidence that human management of a site can offset some of the anthropogenic propagule pressure effects at the population level. One of our exurban sites showed a higher degree of structuring, with an extreme situation of the population being almost entirely dominated by one haplotype. This supports our hypothesis, as lower human land use results in reduced propagule pressure, a higher degree of population structuring, and preservation of the dominant haplotype. Unfortunately, these findings were not mirrored in the other exurban site, which showed a moderate degree of both population structure and haplotype diversity. Suburban sites were very similar to exurban sites, with one suburban site exhibiting a higher degree of structuring and a corresponding dominance of the population by a single haplotype. This population actually fit our predicted model of an exurban site, indicating a need to reconsider our urban-exurban categorization standards. The remaining suburban site demonstrated moderate values for both structuring and haplotype composition, in agreement with our hypothesis. Across all six sites the overall support for hypothesis 3.2 was that three sites supported our hypothesis, while the remaining three sites were inconclusive. It should be noted that despite the inconsistencies, we found no evidence that directly contradicted the proposed model. Human development level of a site did correlated to lower population structuring and higher geneflow,

but it was not sufficient to explain the entirety of the phenomena. Based on the mixed support for hypothesis 3.2 we concluded that human land-use does impact population structure, but that there are other factors affecting population structure that we did not account for.

Geneflow Between Sites

To assist in untangling these results we tested a geometric dispersal model of Ailanthus altissima samara dispersal (hypothesis 3.3). This hypothesis represented a null model, with population structuring being independent of human development and land use patterns. Our Bayesian MCMC models, run under the MIGRATE-N software, provided us with estimates of effective population size but were unable to converge to a reliable estimation of migration parameters (Figure 3.1). Due to this difficulty, all estimates of migration rates were analyzed based on the estimates obtained from AMOVA tables.

When tested, the geneflow between sites, measured as number of individual migrants per generation (Nm), showed no significant correlation when compared against the corresponding pairwise geographic distances (Figure 3.11; p=0.561). This lack of significant relationship was also observed when comparing the $G"_{ST}$ population index vs. geographic distance and Jost's D vs. geographic distance (Figure 3.12, p=0.752; Figure 3.13, p=0.745 respectively). The lack of support for a link between distance and geneflow is important because it violates one of the basic assumptions about dispersal between isolated populations. It is tempting to

think of individual populations of invasive species as similar to the well known island biogeography models, but our work shows that this is not a valid comparison. Biologically invasive plants, such as *Ailanthus altissima*, may not be sufficiently isolated for such models to apply. Our work presents evidence that, to some significant degree, human impact has resulted in a decoupling of the distance vs. geneflow interaction. It is important to keep in mind that *Ailanthus altissima* is known for extremely high propagule pressure, even among invasive plant species (Aldrich et al., 2010; Kowarik & Säumel, 2007; Martin & Canham, 2010; Virginia Department of Forestry, 2009). This high propagation rate may allow for more ready isolation breaking than in other species. Nevertheless, the geneflow vs. distance decoupling means that we should be cautious about what assumptions we make when modeling the spread of invasive species populations.

We should also consider the fact that this failure the verify the geometric distance hypothesis tells us a lot about the potential magnitude of anthropogenic effects on the environment. The predicted relationship between distance and geneflow, being based on basic geometric principles, does not have any particularly complicated mechanisms to bypass. The fact that the geometric signal has been lost in our dataset indicates that another mechanism is at play here, and this alternate mechanism is at least an order of magnitude higher in impact than the null model. While human development levels of our sites did not cleanly correlate

to population structure (see above) it did play some role, and the effect of this human interaction was enough to disrupt the underlying geometric model.

The incomplete success of hypothesis 3.2 lead to our testing of hypothesis 3.3 based on the geometric distribution of propagules. If the population structure was not being affected by anthropogenic distribution of propagules then we would expect that the geographic distance between sites would determine the degree of connection between them. This was, effectively, a null hypothesis based on the inverse square law of basic geometry. Propagules radiating out from a central point, the parent population, will display a higher propagule pressure on nearby sites than on sites farther away. We tested this secondary hypothesis by using GenAlEx to estimate pairwise values for Nm, indicating the number of individuals immigrating between sites per generation. By its very definition, Nm is an excellent indicator of propagule pressure. Based on geometry our null model then would predict a high Nm value at short geographic distances, which would then attenuate as the distance between sites increased. However, figure 3.11 above demonstrated that this was not the case. The slope of the relationship was only slightly negative, and results were not significant (R^2 = 0.0266; p = 0.561). This is a very important result, as a number of basic population modeling approaches treat individual populations as "islands" with geneflow between them (Hartl & Clark, 2007). Our results suggest that the simpler models, with geneflow rate between all islands being equal may be more accurate that the more complicated models that attempt to introduce distance matrices.

Since our null model was shown to be incorrect, there must be some factor which exerts an effect which is far more powerful than the underlying geometric relationship between sites. From testing our primary hypothesis we know that this factor is not simply human development level, as the results of that test were mixed. Human development certainly plays a role, but the specifics of that role remain unknown. Based on our analysis of pairwise Nm values, the likely cause is some form of human-mediated geneflow. In broad terms this makes sense, as invasive species tend to spread in the wake of human traffic. If the geneflow between sites is determined primarily by human traffic, this could explain the inconsistent results we found in testing our primary hypothesis. Human development level and human traffic are often strongly correlated, and even used as proxies for the other, but they are distinct phenomena. A low development site may still have roads providing access, such as a rural field near a busy highway, while high development sites may have restricted access, such as hazardous materials remediation sites. It is possible that a future study directly investigating human traffic, either vehicular or on foot, may provide a better prediction of geneflow between sites and a better estimate of the population structure as a whole.

Human management of sites may also play a role in the discrepancies that we saw. Branchbrook Park, despite being located in the center of the city of Newark, showed lower than expected levels of geneflow. Even taking into account human

use didn't explain this discrepancy, as Branchbrook Park has high levels of foot traffic and vehicle access roads. One possible explanation is that Branchbrook Park is a heavily managed park, with specifically manicured plots of land. Heavy human management limits the number of possible sites for immigrant propagules to establish. Seedlings growing in areas used by humans, and hence with the majority of foot traffic, are likely to be removed by grounds crews before they can contribute to the genepool. This effect is particularly important for invasive trees, as they take much longer to contribute reproductively than herbaceous species. A lucky invasive herb may escape culling long enough to reproduce, but most woody species do not have this option. Instead, *Ailanthus altissima* immigrants need to establish in the unmanaged periphery of the park, a comparatively small target for a propagule to hit. Liberty State Park, by comparison, is another heavily urban site. Our samples were taken from the hazardous materials restriction zone where human management is limited to simply putting up a fence. Individuals can potentially establish anywhere within the site, limited only by niche availability and competition. This is actually an encouraging result, the results from Branchbrook Park show that human management of a site does reduce the ability of *Ailanthus altissima* to establish and reproduce in new areas.

Final Thoughts

Ultimately, the partial success of our land use hypothesis and the failure of the geometric distance hypothesis can both be explained by the same mechanism, unexpectedly high levels of anthropogenically assisted propagule spread. The

scale of vehicle based transportation by humans can potentially be orders of

magnitudes higher than the normal propagule dispersal range of *Ailanthus*

altissima seeds. Samara dispersal by gravity is limited to a few hundred feet,

while occasional water dispersal may transport propagules a few kilometers

(Delgado et al., 2009; Kaproth & Mcgraw, 2008; Kowarik & Säumel, 2008;

Landenberger et al., 2007). By comparison, a samara wedged in the trunk or

windshield of a car can potentially travel many tens of kilometers in an hour.

Even samaras along the side of a highway can potentially be kicked back up into

the air for further dispersal by a passing vehicle. High levels of this type of

dispersal could easily swamp out any signal that would come from the geometric

distance model, explaining the failure of hypothesis 3.3. The partial success of

our land use hypothesis (hypothesis 3.2) can also be explained by high levels of

anthropogenic geneflow. Human land use is often correlated with traffic levels. If

human traffic is actually the driving factor behind our results, our partial success

can be explained by the fact that we were testing for a correlate of the true factor,

rather than the underlying cause of the phenomena. Further work investigating

the role of anthropogenic traffic as dispersal corridors for *Ailanthus altissima*

would help to elucidate the mechanisms that we believe caused our conflicting

results.

Separate from human traffic, human management of sites may also have played

a role in at least one of our study locations. One of our two urban sites,

Branchbrook Park, was a highly managed municipal park with a focus on

maintaining open ground for human recreation. This targeted removal of propagules, despite only being performed at sites with active recreation, did cause a noticeable reduction in the amount of haplotypes present. We take this as evidence that directed human management can offset, at least partially, the unintentional propagule spread cause by human traffic. The success of invasive species management is usually framed as eradication or containment efforts, but our work demonstrates another metric which can be used to determine the impact of management efforts.

Chapter 4: Internal Geneflow Prevents Ecotype Formation in *Schismus arabicus*: A Comparison of Sonoran and Mojave Populations

Introduction

We have addressed the effect of heterogeneous environments on plant development and reproductive strategies back in Chapter 1. The majority of such examples were concerned with plants capable of reproductive versatility, namely sexual (e.g., pollen mediated) and clonal methods of reproduction. Changes in environmental and resource distribution also affect annual (i.e. non-clonal) plants as well. If the divisions between these heterogeneous microclimates are strong enough, it is possible that divergent ecotypes may arise. Ecotypes are a subgroup of a population possessing traits that give it an advantage under specific habitat. Much recent work has examined characteristics that can drive the formation of ecotypes. Ecotype divergence may be driven by factors such as drought stress (Chakhchar et al., 2016), freezing tolerance (Fabbri, Ploschuk, López, Insausti, & Rua, 2016), salinity (Ruiz et al., 2016) and elevated carbon-dioxide levels (Runion, Prior, Capo-Chichi, Torbert, & Van Santen, 2016). Generally the traits in question provide a fitness benefit, although it is possible for the formation of ecotypes to be driven by population dynamics and genetic drift (Vander Mijnsbrugge, Le Clercq, & Michiels, 2016).

The effect of drought stress and salinity are of particular interest for our work. Shrubs in regions of the American Southwest often act as nurse plants, creating

localized areas of shade and increased fertility (and therefore potentially reduced stress) beneath their canopy (Holzapfel, Tielbörger, Parag, Kigel, & Sternberg, 2006; Schafer et al., 2012). This creates a heterogeneous landscape, with fertile areas around nurse plants separated by regions of open ground (Mudrak, Schafer, Fuentes-Ramirez, Holzapfel, & Moloney, 2014). The area localized around these nurse plants have been compared to "islands of fertility" in the midst of the arid desert region, either because of elevated Nitrogen and Potassium levels (Mudrak et al., 2014; Schafer et al., 2012) or due to water availability (Holzapfel et al., 2006). The microhabitats caused by these nurse plants allow for the establishment and growth of smaller herbaceous species. As the population increases, inhibition from density dependent mechanisms will offset the fitness benefits of close proximity to the nurse plant. The trade-off of local resource abundance and density dependent mechanisms results in a carrying capacity for the nurse plant microhabitat. The balance of this system can be broken, however, by reducing the severity of the environmental heterogeneity between the nurse plant and the surrounding environment. This is supported by modeling work showing that under uniform environmental conditions selection favors increased dispersal to reduce competition between descendent and parent plants (Hamilton & May, 1977). Indeed, Holzapfel et al. added water to experimental treatments, which broke the heterogeneous barriers caused by water stress, shifting the balance in favor of dispersal, and resulting in an observed spread of *Schismus arabicus* into open ground (personal comm.).

The grasses growing under these nurse plants are primarily annuals, however the mechanisms we discussed have also been observed to function in non-annual plants as well. The nurse plant phenomenon has been observed in *Opuntia rastrera*, a species of prickly pear cactus found in the Chihuahuan Desert. *Opuntia* is capable of both sexual and asexual reproduction, and the relative success of each method is determined by environmental conditions. Under similar patchy conditions to the above studies *Opuntia* seedlings have a higher survival rate when growing in immediate proximity to a nurse plant (Mandujano, Montana, Mendez, & Golubov, 1998). The opposite effect was also observed in areas with no canopy for protection: survival rates were significantly higher for clonal offspring than for seedlings (Mandujano et al., 1998).

Selection for dispersal could favor the development of ecotypes, since dispersed propagules will most likely end up in the drastically different open ground habitat. An ecotype specialized for open ground has many potential benefits if it disperses rather than if it stays under the canopy. The open ground between nurse plants is largely free from competition, while individuals under the nurse plant will eventually suffer reduced fitness from crowding and intraspecific competition. The effects of this competition are particularly strong because of the close relationship between the individuals. When both individuals are genetically related, the negative effects of competition reduced the fitness of that individual twice; once for direct competition and the other for reduced fitness of the kin. The pressure for dispersal is also increased by the fact that, regardless of habitat

heterogeneity, there is always a strong pressure acting in favor of dispersal, even under conditions that would be considered "suicidal" (Hamilton & May, 1977). Clearly "suicidal" propagation, such as ecotypes adapted to growing under nurse plant canopies expanding into open ground, has severe fitness costs. Factors acting against dispersal, such as severe heterogeneous distribution of critical resources, nurse plants, high mortality, etc. may restrict the spread of propagules and reduce dispersal range, but dispersal persists even under the most severe of conditions (Dytham, 2009; Hamilton & May, 1977; Talavera, Arista, & Ortiz, 2012). High stress arid environments, such as the locations in our study, have been directly linked to a shift toward short-range dispersal strategies (Kéfi, van Baalen, Rietkerk, & Loreau, 2008). For dispersal to persist under such severe conditions, adaptations that improve the fitness of dispersed propagules into relatively harsher new environments, should be selected for by natural selection. When the habitat of the dispersed and non-dispersed offspring vary dramatically, disruptive selection may select for divergent characteristics, resulting in different ecotypes.

In our study we proposed that the short range contrast between nurse plant canopies and open ground is enough to cause divergent ecotypes in the invasive grass *Schismus arabicus*. *Schismus arabicus* is a Eurasian grass introduced during the 1930's that has spread rapidly across the American Southwest due to its severe drought tolerance (Gutterman, Gendler, & Rachmilevitch, 2010). It is a short-lived annual, between 2 and 12cm in height, which exploits harsh

environments that even other desert plants are unable to utilize (Gutterman, 2003; Sánchez-Flores, 2007). *Schismus arabicus* grows in small clumps (Illustration 4.1) and are located both under nurse plants and across open ground, connecting otherwise isolated nurse plant communities. We propose that *Schismus arabicus* individuals growing under a nurse plant are subjected to lower levels of water stress but face greater levels of crowding, both intraspecific and interspecific. *Schismus* growing in open patches face less crowding and competition, but must deal with both increased water stress and lower nutrient levels. The differences in environmental factors create a disruptive selection pressure. If this disruption is strong enough, the division into ecotypes should be reflected in the underlying genetics of the population.

Illustration 4.1 Growth habit of *Schismus arabicus*. Image © Yvonne Roelofs.

Based on the impact of high contrast heterogeneous environments, we developed two contrasting hypotheses regarding the populations of *Schismus arabicus*. Our primary hypothesis concerns the development of specialized ecotypes better suited to either the nurse plant canopy or open ground environments.

Hypothesis 4.1: A division between the two ecotypes is reflected in the genetic structure of the population. (a) Canopy individuals are more closely related to other canopy individuals than to open ground individuals. (b) Open ground individuals are more closely related to other open ground individuals than to canopy individuals.

The existence of two ecotypes would indicate disruptive selection, and would create a bimodal genetic distribution within the two sites. The division of the population into these two groups would result in a larger amount of variation away from the central population mean. If sufficiently large, the variation within sites should be large enough to overcome the natural variation caused by geographic distance.

Hypothesis 4.2: Genetic variation within the Sonoran and Mojave sites will be higher than the variation between sites.

A higher within site variation is expected due to the contrasting environmental conditions which should favor a disruptive selection of phenotypes. Sonoran and Mojave sites will have genetic difference due to geographic separation of the sites, but we predict that environmental factors exhibit a selective effect and should create a stronger signal.

If these hypotheses are falsified, the question then arises of what dynamics underlie the population. Being wind pollinated, a panmictic population is very possible, which would prevent the divergence of different ecotypes due to the freely random mating. Most populations show at least some degree of structuring due to the physical geography, where distant individuals show a higher amount of polymorphisms than nearby individuals. From this model we derive our null hypothesis:

Hypothesis 4.3: Pairwise comparisons of *Schismus arabicus* individuals within a given site will show a positive correlation between the number of segregating SNPs and the large-scale geographic distance between those individuals.

A lack of such correlation would be considered evidence of long distance pollen dispersal with enough range to overcome geographic limitations within the local site. If so, we could conclude that the *Schismus arabicus* site in question is either panmictic, or very close to being so.

Methods

Plant samples for the study were collected by Hadas Parag and Dr. Marjolein Schat during the spring of 2013. Collection of *Schismus arabicus* was conducted at two sites; Fort Irwin, California (a part of the Mojave Desert) and Gila Bend, Arizona (a part of the Sonoran Desert). Each site consisted of several sub-populations, defined by their proximity to shrubs of the species *Larrea tridentata*. The landscape at both of these sites was extremely barren, such that these shrubs were one of the few sources of shade. This created a strong division between two microclimates, which we describe in our treatments as "shade" and "open" conditions. *Schismus* growing under the shaded conditions, found under the shrubs, suffer less from direct exposure to harsh sunlight, a major physiological stress of in the deserts of the American Southwest. The trade off, however, is an increase in interspecific competition caused by close proximity to *L. tridentata*. Individuals growing on open ground avoid this competition, but are directly exposed to harsh sunlight throughout the entire day. These open ground individuals are also of greater ecological impact than the shade individuals, as they bridge the gap between otherwise isolated shrubs. The connection of otherwise discrete sub-populations is responsible for a major shift in the region's fire regime, as the short-lived annual grass provides a ready supply of fuel and avenue of spread for wildfires. To compare these two growing conditions, and possible ecotypes, we worked with 5 samples from shade and 5 from open ground for each of the 10 sampled shrubs. The sampling design is illustrated

below in figure 4.1. Collection sites were geo-referenced using a sub-meter

Trimble Geo-XT 2003 GPS. Specific locations of these shrubs at each site are

illustrated in figure 4.2 and 4.3.
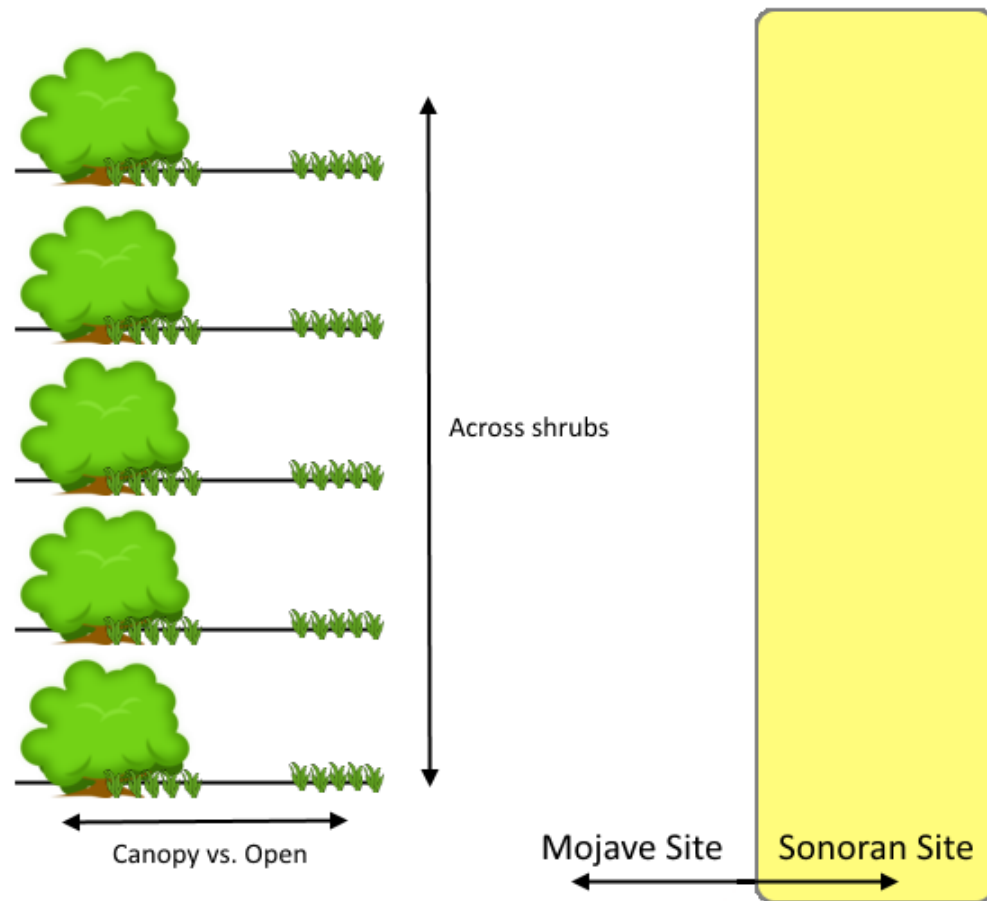
Figure 4.1 Sampling regime for *Schismus arabicus*. Comparisons were made at 3 levels; around

a single shrub, between several shrub sub-populations, and cross two geographically distinct

sites.
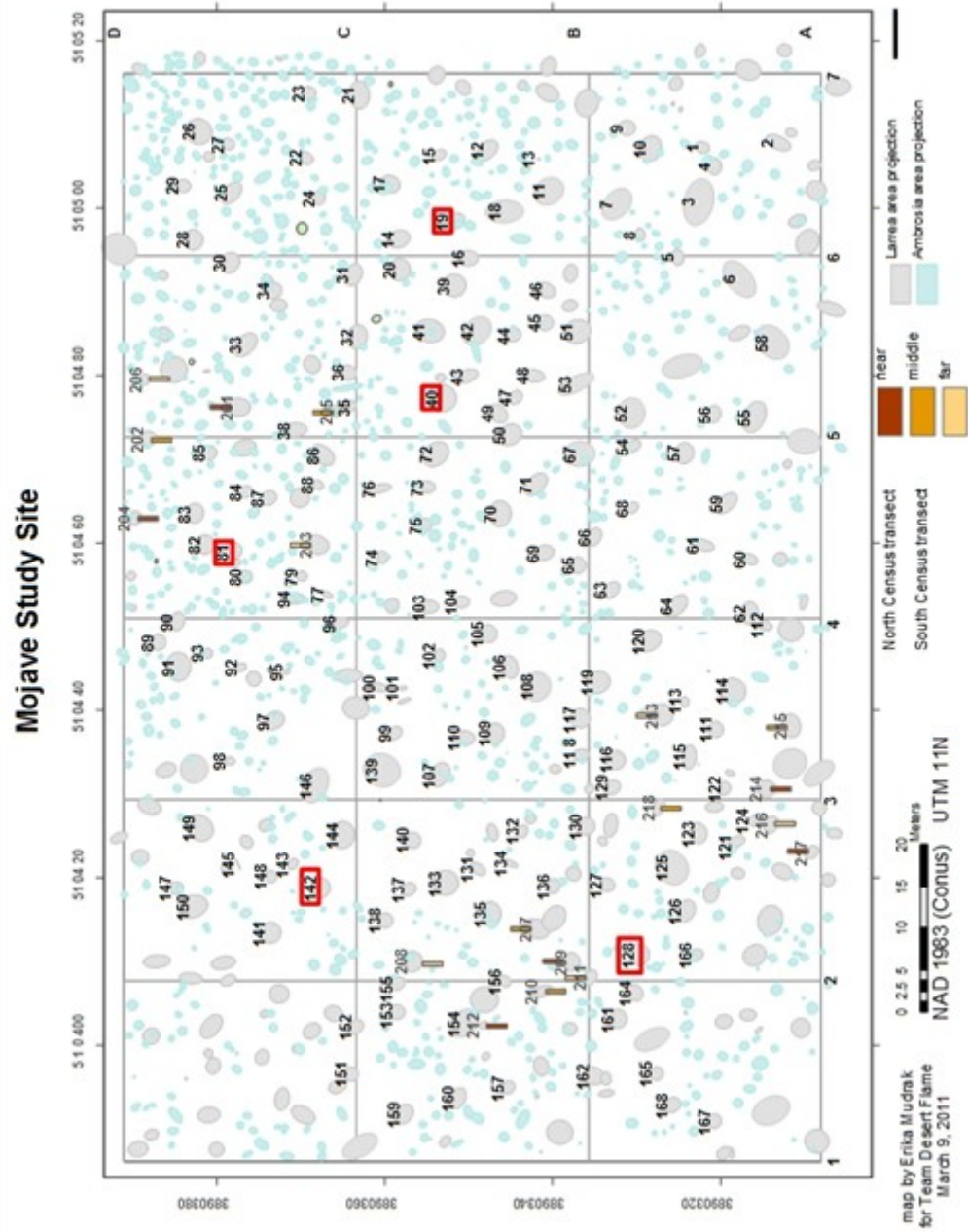
Figure 4.2. Collection of samples of *Schismus arabicus* at the Mojave site was conducted around five shrubs, represented by red boxes.
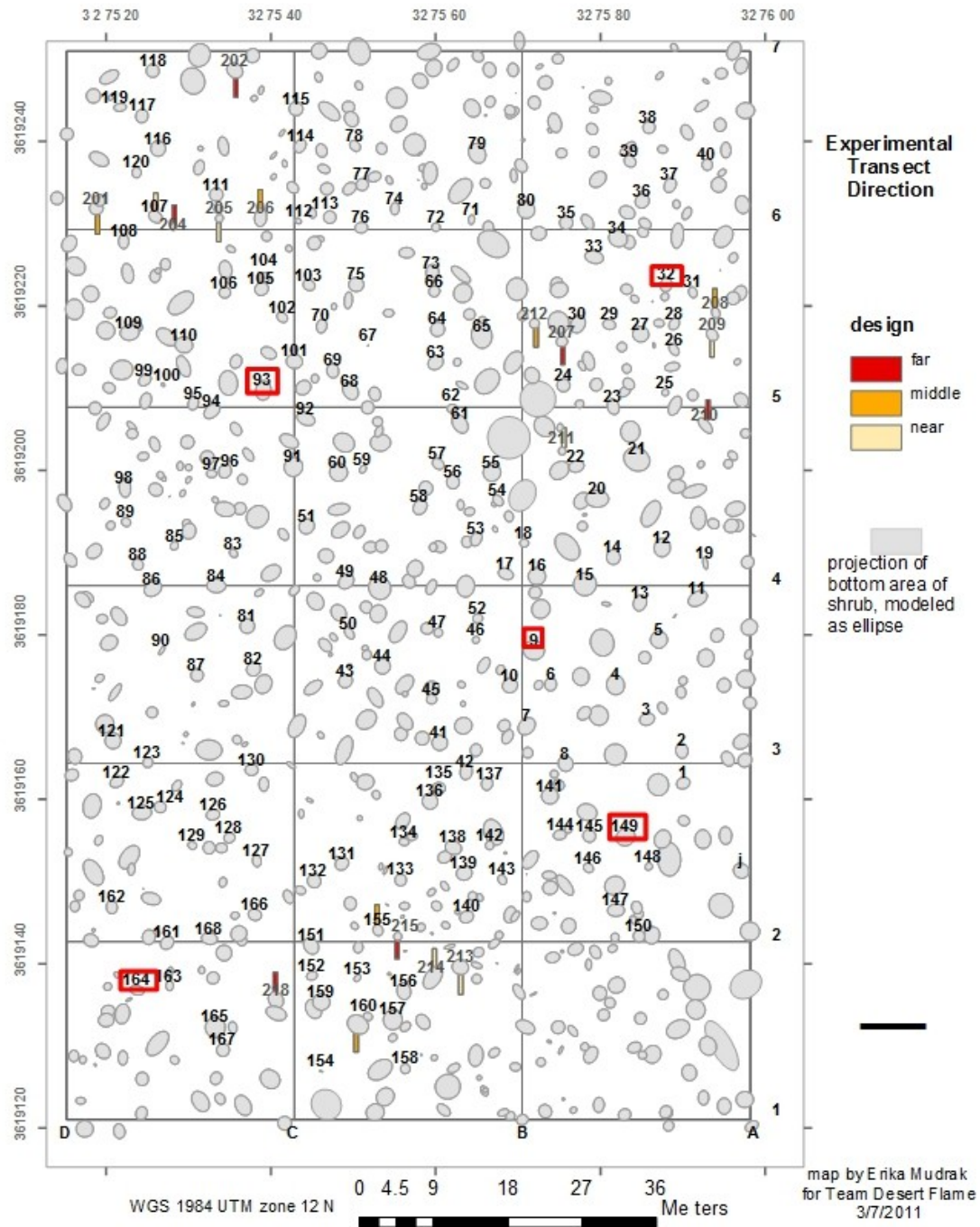
Figure 4.3 Collection of samples of *Schismus arabicus* at the Sonoran site was conducted around

five shrubs, represented by red boxes.

The sites we used were well characterized from previous work performed by Dr. Holzapfel. Natural occurring *Schismus arabicus* densities in the two desert sites varied from year to year depending on ambient precipitation but on average were fairly similar in the two deserts sites. In the years 2011 to 2013, 20 to 350 individuals were found per m^2 in the Mojave Desert while in the same period the Sonoran Desert showed densities ranging from 40 to 200 individuals per m^2 (Holzapfel et al., unpublished data).

There currently exist no developed microsatellite markers for *Schismus arabicus* or *Schismus barbatus* in the NCBI BLAST library (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Although microsatellites are well suited to asses genetic population structure within species , we did not possess sufficient prior information to search for such microsatellite regions and develop these primers. In the search for an alternative marker we looked to the existing library of sequences available through the NBCI BLAST database (http://blast.ncbi.nlm.nih.gov/). Several genetic markers were tested for use in our *Schismus* study. The NCBI BLAST database showed limited amount of sequences for *Schismus arabicus*, only 4 nucleotide sequences had been reported, however a closely related species, *Schismus barbatus*, had a larger library, consisting of 30 sequences in the NBCI database. We examined both species when considering which genetic markers to use in the study.

We initially chose to use primers for the beta-amylase gene, based on the work by Mason-Gamer (2005) on the family *Poaceae*. Eventually we discarded this marker; since being a functional gene its rate of conservation likely is too high for the analysis of population structure. However, during this work we notice an odd discrepancy in the behavior of one of the outermost primers. While primers for loci 3-for, 4-bac and 5-bac all functioned normally, the primers for locus 2-for did not properly anneal. A replacement tube of the 2-for primer was also tested and we varied the PCR cycle with respect to Tm, both continued to give the same result. The beta-amylase primers were developed for broad use on the family *Poaceae*, which may account for this. We present this as preliminary evidence that the *Schismus* genus may be divergent from the rest of *Poaceae* with regard to the sequence that Mason-Gamer's 2-for primer attaches to. If flanking regions could be identified then this could be an interesting prospect to examine, however it was beyond the purview of our project.

Leaving beta-amylase behind, we examined two pairs of primers commonly used in DNA bar-coding for plants. The first was the large sub-unit of Ribulose-1,5-bisphosphate carboxylase/oxygenase. The second bar-coding primer we examined was the Internal Transcribed Spacer (ITS) for the region adjacent to the 5.8S ribosome (B. G. Baldwin et al., 1995). We initially discounted these primers because their use was primarily at the phylogenetic scale (B. G. Baldwin et al., 1995; Catalán, Torrecilla, López Rodríguez, & Olmstead, 2004; Quintanar, Castroviejo, & Catalan, 2015), but small scale testing was promising as the

Rubisco Large Subunit gene, hereafter denoted as rbcL, showed discernible variation between individuals of the same local population. Unfortunately rbcL also showed a high degree of non-specific binding, which interfered with the sequencing reactions of Genewiz (Genewiz, South Plainfield, NJ, USA). The primer pair that we ultimately decided to use, was the Internal Transcribed Spacer element directly adjacent to the 5.8S ribosome gene, here after referred to as ITS. Minimal non-specific binding was observed and did not noticeably interfere with the sequencing reactions. ITS2, a related region downstream of the 5.8S ribosome also performed well. We chose to use ITS rather than ITS2 due to the larger size of the ITS region (~650bp vs. ~200bp), which would allow for more potential variation in the sequence to analyze.

DNA extraction was conducted in the same method at in the *Ailanthus altissima* work. We used a Qiagen DNEasy Blood and Tissue Extraction Kit (Qiagen, Valencia, California, USA), allowing for additional incubation time to allow the cell walls to lyse. Total incubation period for samples was approximately 18 hours. Samples were stored using the elution (AE) buffer to maintain pH, and long-term storage was done using a -80°C freezer. PCR was conducted using a combination of 9.5μL millipore H$_2$O, 12.5 μL of Qiagen Taq Mastermix, 1μL of forward primer, 1μL of reverse primer, and 1-2μL of our DNA template (Table 4.1). The reactions were run for two sets of 30 cycles, with the temperature for the annealing step being defined as T$_m$-2°C for the first set of cycles and T$_m$-1°C

for the second set. PCR product was stored under the same conditions was DNA extract above.

Table 4.1 Composition of 25 µL *Schismus arabicus* PCR reactions.

	Volume
Millipore Water	9.5 µL
TAQ Mastermix	12.5 µL
Forward Primer	1 µL
Reverse Primer	1 µL
Template DNA	1-2µuL

Gel electrophoresis was conducted using a 1.2% agarose gel due to the small size of the ITS fragments. Visualization of DNA fragments was provided by adding GelRed to the loading buffer, and fluorescing the gel under UV light. Images were captured using a Canon PowerShot A590 IS digital camera. All gels were run using a Genscript 100-bp ladder for standardization. Gel electrophoresis verification of PCR product showed a high success rate, as well as a high concentration of DNA product. Out of 120 samples of *Schismus arabicus*, 115 produced usable DNA.

PCR product was purified before sequencing using a Qiagen PCR Product Purification Kit. The final elution was conducted using 30µL of buffer, and resulted in a very high concentration of PCR product (>100ng/µL). The high concentration of DNA caused problems with the sequencing reactions,

necessitating a dilution factor of 1:1 for all samples. All dilutions were conducted using RNase and DNase-free water from the Rutgers-Newark millipore system.

The necessary equipment to run sequencing reactions was not available on the Rutgers campus, so all sequencing was outsourced to the Genewiz company (Genewiz, South Plainfield, NJ, USA). Our sequencing mix (10µL DNA + 5µL primer) was picked up and delivered to Genewiz processing same day, minimizing exposure to room temperature conditions. This appears to have been effective, as no DNA degradation was visible in the sequencing results.

Sequencing data received from Genewiz was processed using the SnapGene Viewer program. Bases marked as ambiguous (N) were curated manually, by examining nucleotide trace data from the chromatogram to estimate dominant peak at a given nucleotide position. The resulting sequences were then aligned using ClustalOmega (Goujon et al., 2010; Sievers et al., 2011). Alignments were verified using Mesquite (Maddison & Maddison, 2015), and converted into a simplified Nexus format for greater to compatibility with the remaining programs. Haplotype networks based on minimum spanning distances were generated using the software PopART (Bandelt, Forster, & Röhl, 1999) (http://popart.otago.ac.nz). PopART allows us to assign clusters based on the sequence data and geotag locations using the k-means method. From this we made three predictions based on the relative strength of ecotype and geographic origin signals.

Prediction 1: If ecotype signal is strong there will be 2 total

clusters, taking up 50% of individuals from each site.

Prediction 2: If ecotype and geography signals are both strong

then then each site will separate out into two clusters of equal size.

There will be a total of 4 clusters, with 2 in each site.

Prediction 3: If the geographic origin signal is strong then we will

find 2 clusters, each of which contains the entire population of a

given site.

These three permutations give us full coverage of the two factors of interest,

ecotype divergence and geographic origin, and give us the framework to

investigate the overall signal strength of these factors relative to each other.

Geneflow modeling and population size estimates were performed by running

Migrate-N on the CIRPES computing cluster (Beerli & Palczewski, 2010; Miller et

al., 2010). 10 replicates were run with the following parameters: 500,000

recorded steps, a 200 step increment between recorded steps, and 100,000

discarded trees (1% burn-in). Static heating was performed with the following

temperatures: 1, 1.5, 3, and 1,000,000. Results were verified both through trace

diagrams and through Effective Sample Size (ESS) estimates.

We also performed a basic analysis of relatedness by generating minimum spanning network trees using IQtree (Nguyen, Schmidt, Von Haeseler, & Minh, 2015), and examined the branches for a division between geographic and ecotype categories. IQtree was chosen because it compares a large number of different models on the basis of Akaike Information Criterion (AIC), Corrected Akaike Information Criterion (corrected AIC), and Bayesian Information Criterion (BIC). IQ tree results were visualized using FigTree (http://tree.bio.ed.ac.uk/software/figtree/). We also conducted a test for species delimitation to test for divergence between Sonoran and Mojave sites, this analysis was conducted using the software PTP (Zhang, Kapli, Pavlidis, & Stamatakis, 2013). PTP species delimtation was calibrated using ITS1/2 sequence from the genbank record of a related species, *Deschampsia nubigena* (GenBank: AM041246.1).

Results: Minimum Spanning Networks

The PopART software package was used to categorize the *Schismus* individuals according to the minimum spanning network. In accordance with our hypotheses, the minimum spanning networks were also separated into either two or four clusters through the k-means algorithm. Individuals clearly separated into two major haplotype clusters, closely matching their geographic origin (Fig. 4.4 and 4.5). Each site was dominated by a single haplotype, with several related minor

haplotypes also found. The Mojave site had a larger number of variant

haplotypes, indicating more diversity within the site.

When we applied the k-means cluster assignment with two clusters we found that

the resulting assignment exactly matched the geographic populations. The clear

division into two discrete geographic clusters is supporting evidence that the

geographic distance between the sites creates a strong isolation between

populations of *Schismus arabicus*, and that the populations have accumulated

enough genetic divergence to detect with our methods.
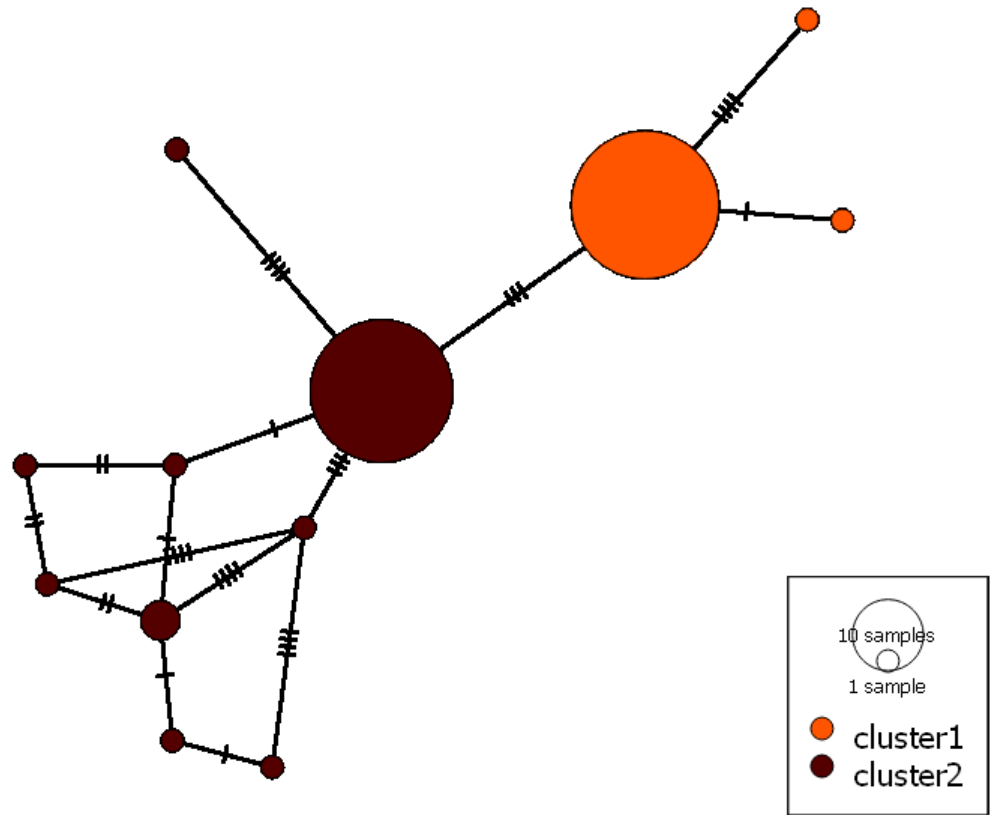
Figure 4.4 A minimum spanning network of haplotypes for *Schismus arabicus* across Sonoran and Mojave deserts inferring two clusters. No signal of ecotypes was detected. Instead, haplotype cluster assignment directly matches the geographic origin of the sample.

We also performed the same analysis letting the k-means protocol infer the optimal number of clusters based on the sequence data and geo-reference data of sampled shrubs. Our 4 cluster hypothesis predicted a 4 cluster model with equal ecotype divisions within each geographic location; 50-50 split of Mojave and 50-50 split of Sonoran. What we found instead was a substantial difference in structure of the two sites (figure 4.5). The geographic signal of the Sonoran site predominated, forming the entirety of a cluster, with no ecotype impact at all.

The Mojave site, having a larger number of haplotypes, contained the remaining three clusters.
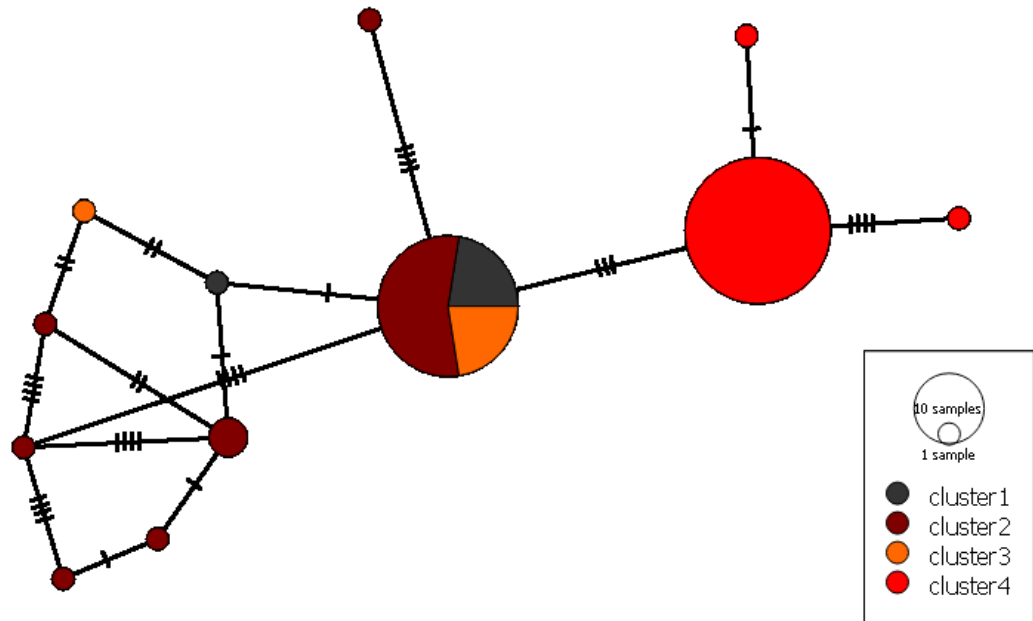
Figure 4.5 A minimum spanning network of haplotypes for *Schismus arabicus* across Sonoran and Mojave deserts. Haplotype cluster assignment inferred 4 clusters. The geographic origin of Sonoran samples is strong enough to form its own cluster.

Under the minimum spanning network model we saw no evidence of any ecotype separation in the haplotype network. Under the k-means clustering analysis the Sonoran Desert site, with a 50-50 mix of canopy and open individuals, separated out into one large cluster. The geographic distance between sites, and the corresponding divergence of the ITS region, had a far larger effect on haplotype assignment. The Mojave site was observed to be substantially more diverse than the Sonoran site, with the latter consistently separating out as its own cluster in both this (k=4) and the previous (k=2) analyses. Geographic region separated

out clearly, as expected, but we found no evidence to support the ecotype hypothesis. If an ecotype signal does exist, the effect of the ecotypes is far eclipsed by the impact of geographic region.

Results: Maximum Likelihood Trees

The sequence data for *Schismus arabicus* individuals was also analyzed using the IQtree maximum likelihood software (Nguyen et al., 2015). A total of 88 different models were compared across all three information criterion(AIC, corrected AIC, and BIC). The F81+I model showed the highest likelihood value across AIC, corrected AIC, and BIC. A total of 625,299 bootstrap trees were evaluated across 1,000 iterations. A cladogram of the results can be seen in figure 4.6 below.
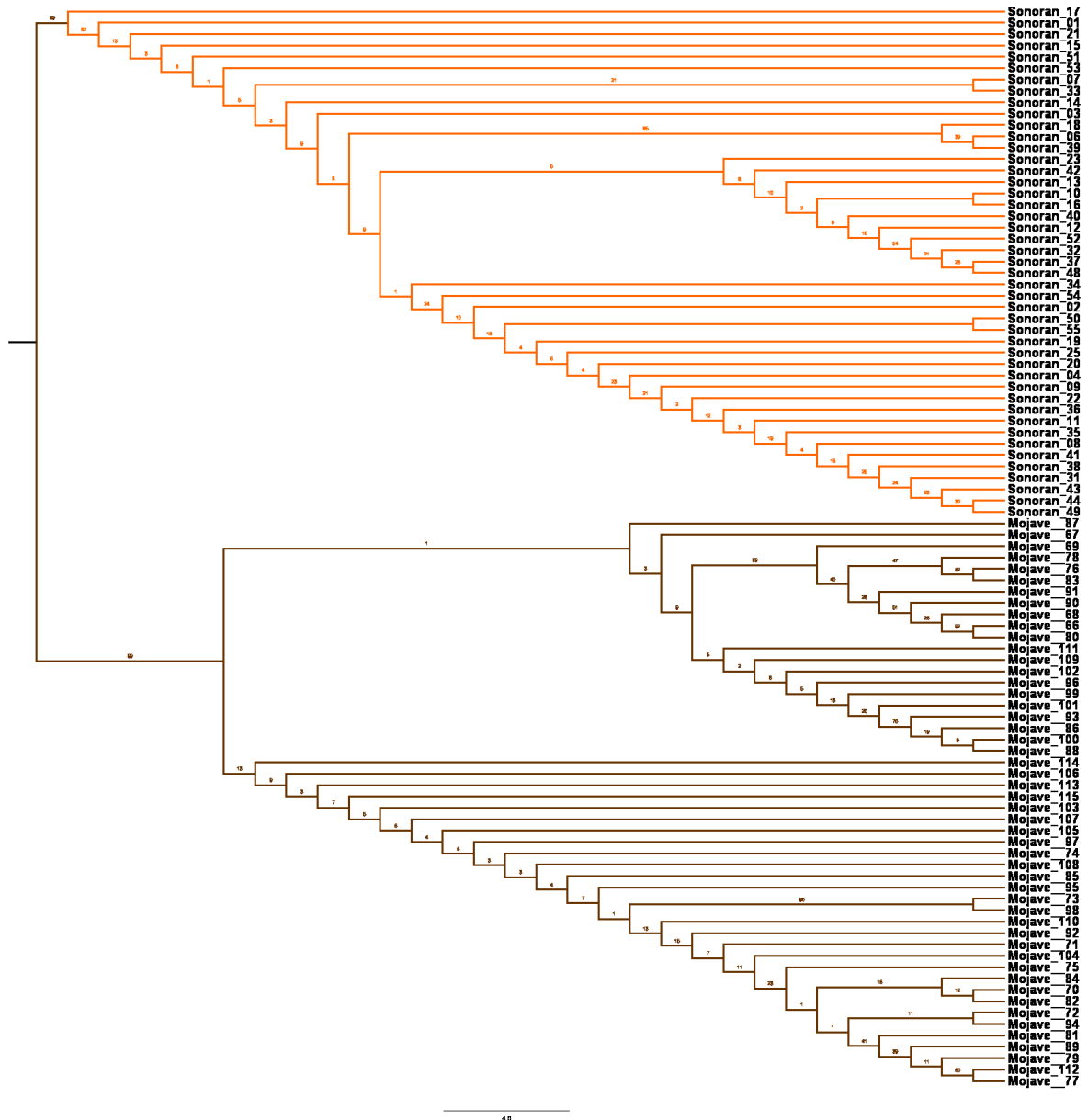
Figure 4.6 A cladogram of *Schismus arabicus* individuals based on the ITS region. A strong

geographic signal can be seen (Mojave in brown and Sonoran in orange).

The structure of the IQtree cladogram is in agreement with our results from the

PopART minimum spanning network, indicating a strong correlation with

geographic region. This division into geographic regions is strongly supported by

bootstrap values, occurring in 99% of permutations. These results support the

model of isolation of *Schismus* populations between the Sonoran and Mojave

deserts. The IQtree results do not support or disprove the existence of ecotypes

within the *Schismus* populations, but instead show a strong dominance of the

geographic distance which overwhelms any possible ecotype signal.

Investigation of individuals collected under canopy and open treatments was also

conducted internally, with the two desert sites serving as replicates. The Sonoran

desert site at Gila Bend showed no link between the environmental treatment

(canopy or shade) and the cladogram inferred from the ITS data. This can be

seen in figure 4.7, which uses color coding to clearly indicate that our proposed

ecotype is paraphyletic. The lack of monophyletic clades among treatments

indicates an overall lack of support for our proposed ecotype hypothesis.

Figure 4.7 A cladogram of the Sonoran *Schismus* samples inferred from the Internal Transcribed

Spacer (ITS) region. Cyan nodes indicate that the individual was collected from open area

between shrubs, while black indicates the individual was collected under the shrub canopy.

Similar results were also found at the Fort Irwin site in the Mojave desert. As illustrated in figure 4.8, canopy and open treatment individuals continued to be paraphyletic. Based on the irregular distribution of treatments among nodes, any ecotypic response does not seem to be linked by inheritance. Closely related individuals were just as likely to be found in either treatment, with only a single monophyletic group (of 3 individuals) being found.
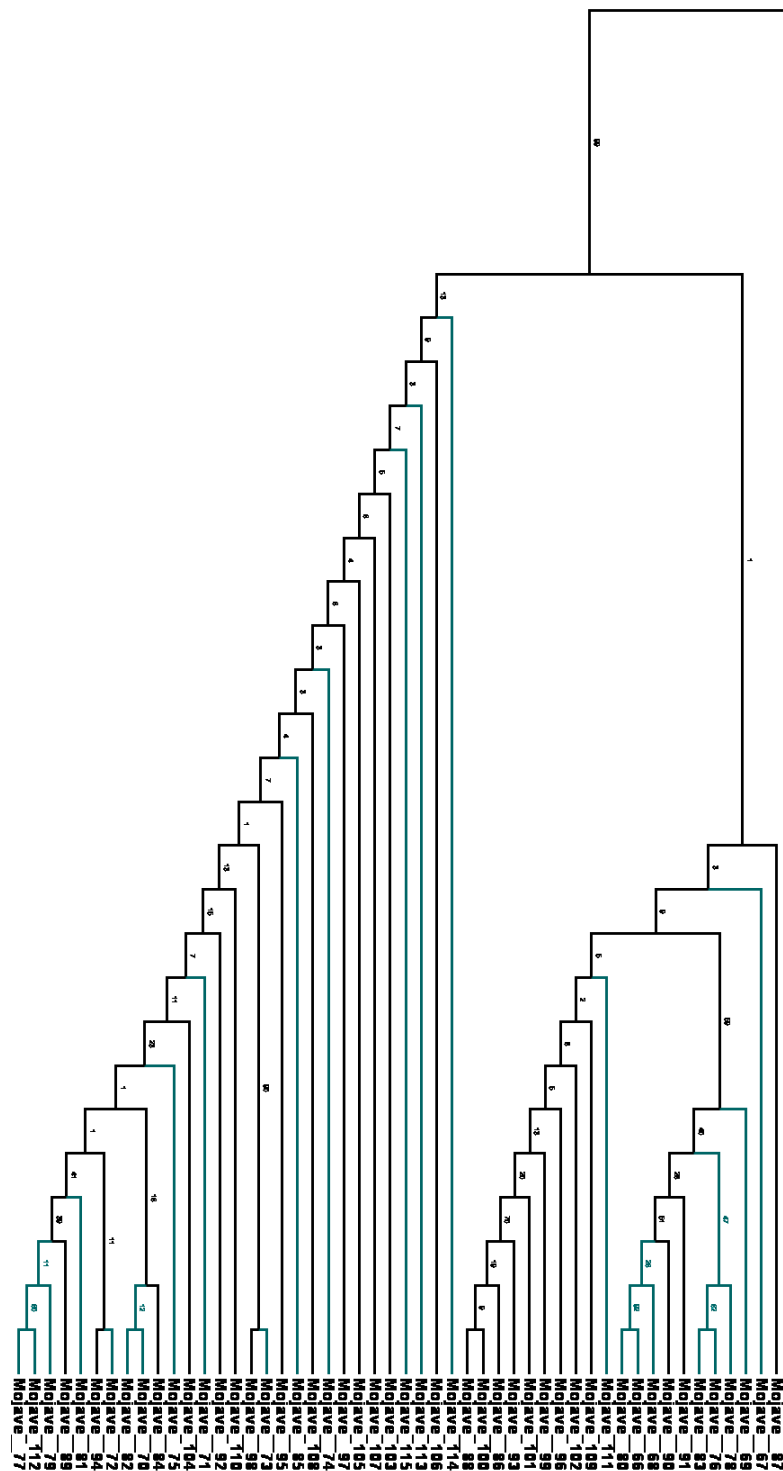
Figure 4.8 A cladogram of the Mojave *Schismus* samples inferred from the Internal Transcribed Spacer (ITS) region. Cyan nodes indicate that the sample was collected from open terrain, while black indicates the individual was collected from under the canopy.

Based on the failure to detect any type of monophyletic organization of treatments in either the Sonoran or Mojave populations, we conclude that the IQtree analysis of our data shows no support for the ecotype hypothesis.

Results: AMOVA partitioning

These results are also supported by our AMOVA analysis in GenAlEx. The partitioning of genetic variation into different hierarchical levels demonstrated that almost all of the genetic variation occurred at the within population level. This was observed in both regional and ecotype comparisons (Figures 4.9 and 4.10 respectively).

Percentages of Molecular Variance

Among Ecotypes
1%

Among Regions
0%
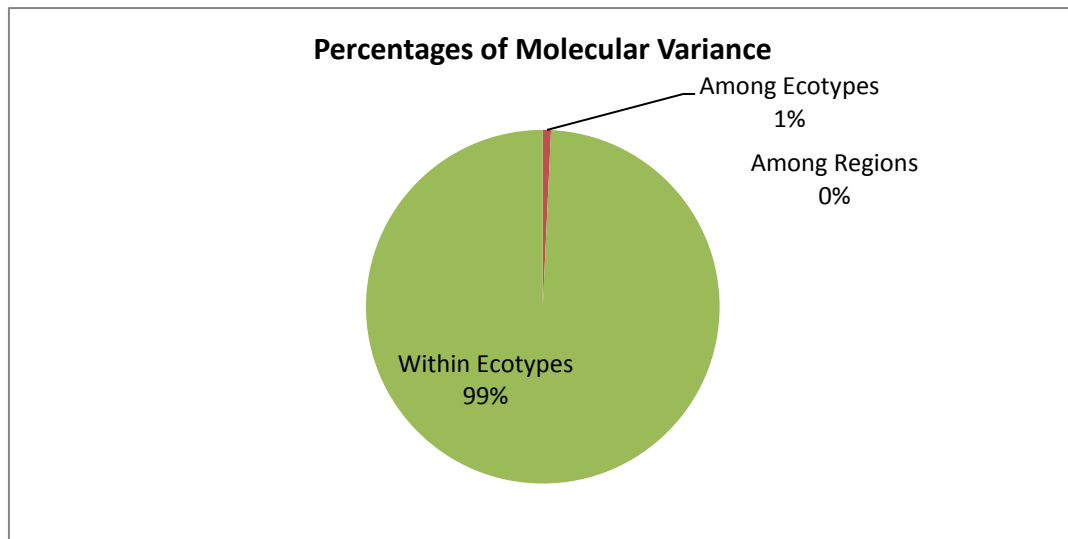
Within Ecotypes
99%

Figure 4.9 The results of AMOVA partitioning of molecular variance across region and ecotype groups, both Mojave and Sonoran populations.

Table 4.2 AMOVA summary of 3 structural levels, showing that 99% of genetic variation occurs within proposed ecotypes (e.g open ground or under canopy). Regional differences were non-significant (p=0.44).

Summary AMOVA Table

Source	df	SS	MS	Est. Var.	%
Mojave vs. Sonoran	1	0.568	0.568	0.000	0%
Open vs. Canopy	2	1.131	0.566	0.004	1%
Within Ecotypes	74	36.685	0.496	0.496	99%
Total	77	38.385		0.499	100%
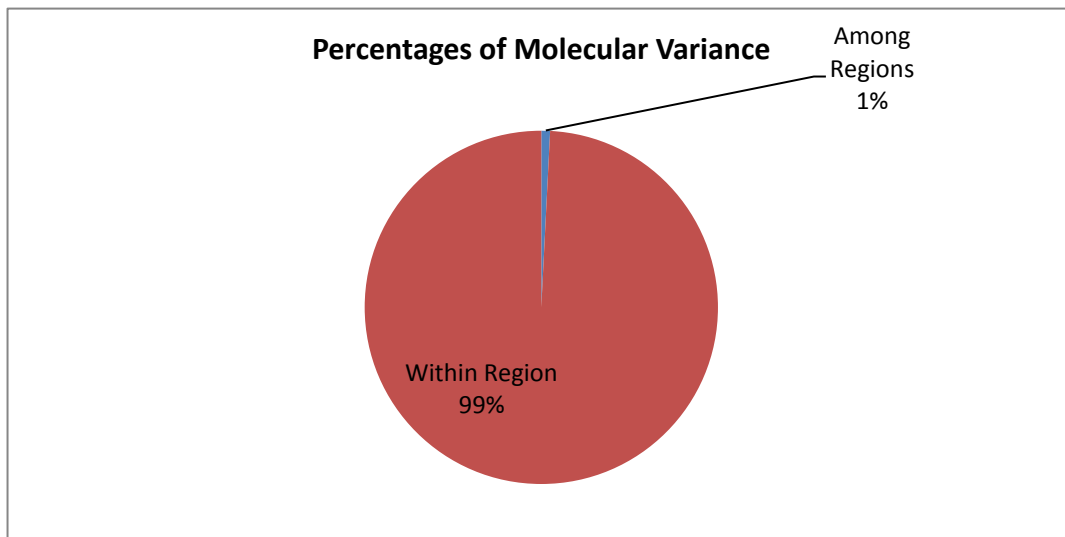
Figure 4.10 The results of AMOVA partitioning of molecular variance across region for Mojave and Sonoran populations.

Table 4.3 AMOVA summary of two structural levels, showing that 99% of genetic variation occurs with geographic regions.

Summary AMOVA Table

Source	df	SS	MS	Est. Var.	%
Among Regions	1	0.647	0.647	0.004	1%
Within Regions	76	37.738	0.497	0.497	99%
Total	77	38.385		0.501	100%

An AMOVA comparison at three structural levels (geographic region, open vs. canopy, and within ecotypes) shows that geographic origin explained 0% of the observed genetic variation in our samples (p=0.44), while the proposed ecotype differences only accounted for 1% of genetic variation (p=0.011)(Figure 4.9; Table 4.2). The vast majority of variation (99%) occurred within our hypothetical ecotype groups (p=0.004). These results present us with evidence of minimal population structuring (Phi_{PT}=0.007; p=0.008).

Similar results were found when comparing regions without regard to ecotypes (Figure 4.10, Table 4.3). Regional differences accounted for only 1% of the observed genetic variation, with the remaining 99% being attributed to variation within a given region. Overall structuring of the population was strong, once a Phi'_{PT} correction was applied (Phi_{PT}=0.008; Phi'_{PT}=1.0; p=0.001).

Results: Internal Geneflow

Based on this failure to confirm the ecotype hypothesis, we next examined the null model based on geographic distance between samples (see our *Ailanthus altissima* work in the previous chapter). For *Schismus arabicus* our null hypothesis is that the number of segregating nucleotide sites between any two individuals should be a positive function of the distance between those individuals. We tested for such a correlation in the scatter plot below (figure 4.11 and 4.12).
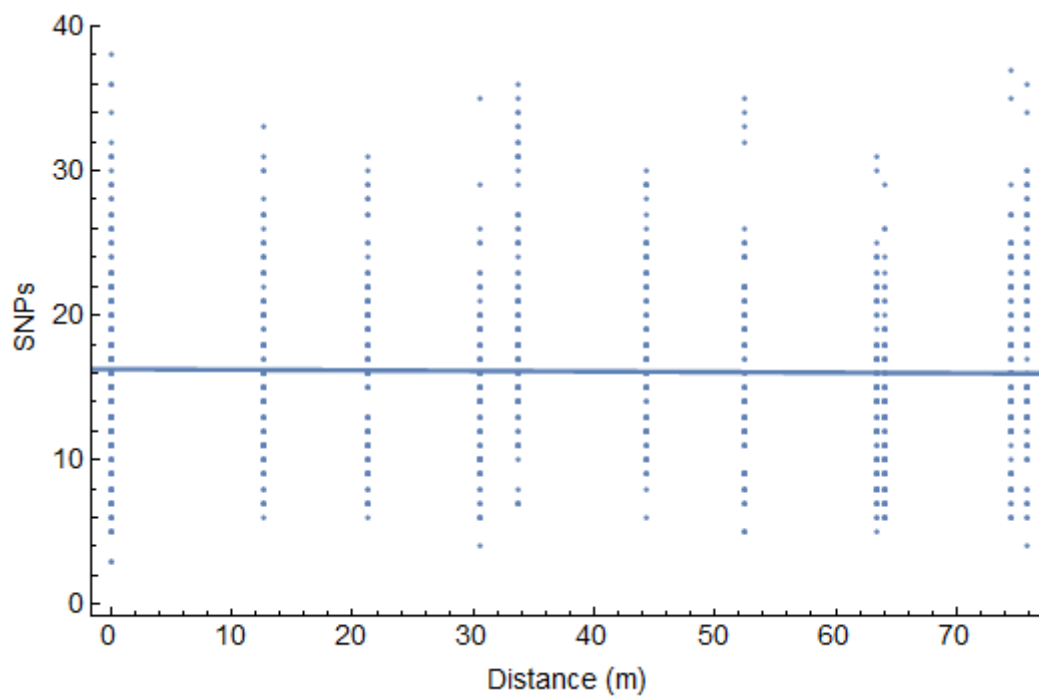
Figure 4.11. A pairwise comparison of segregating sites against geographic distance for all sampled individuals in the Mojave population. The lack of correlation shows that genetic differences are independent of physical distance for the scale being sampled.
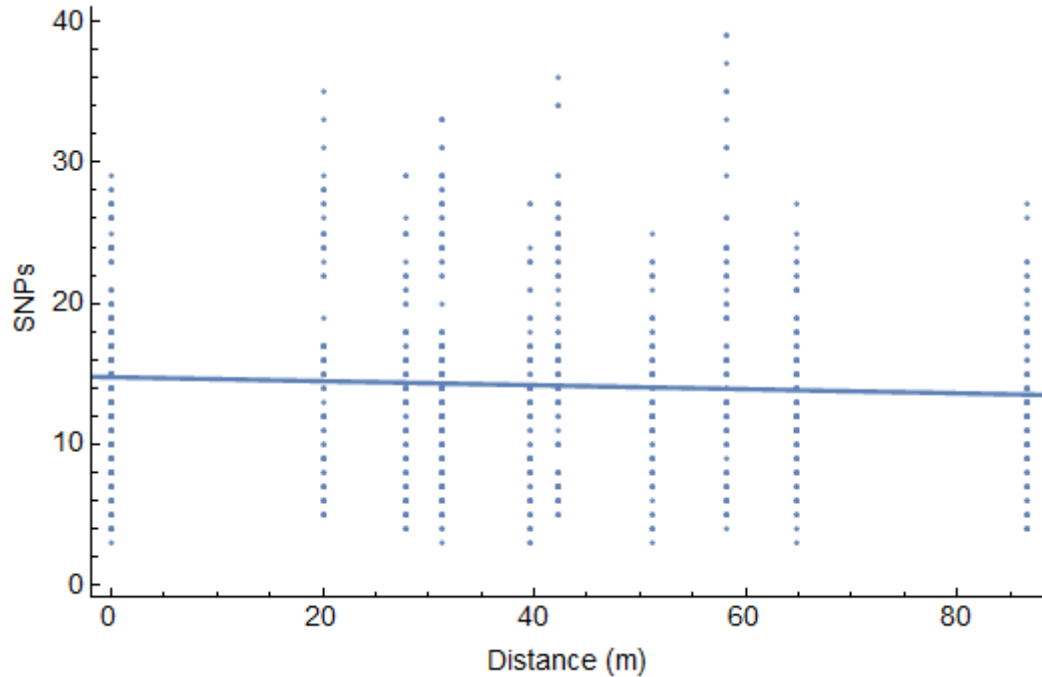
Figure 4.12 A pairwise comparison of segregating sites against geographic distance for all sampled individuals in the Sonoran population. The lack of correlation shows that genetic differences are independent of physical distance for the scale being sampled.

Linear regression shows that the number of segregating polymorphisms and geographic distance between samples has no significant correlation (Mojave $R^2=0.000273$; Sonoran $R^2=0.00285$). This contradicts the null model, and from this we conclude that these segregation and geographic distance are independent of each other for the populations sampled.

Results: Species Delimitation

Due to the strong geographic signal from both PopART and IQtree analyses (see above), we also performed a species delimitation analysis through PTP (Zhang

et al., 2013). The output of our PTP analysis can be seen below in figure 4.13.

Species delimitation results were strongly negative across the entirety of the tree.

Despite the strong geographic isolation, we found no evidence of speciation at

the present time.
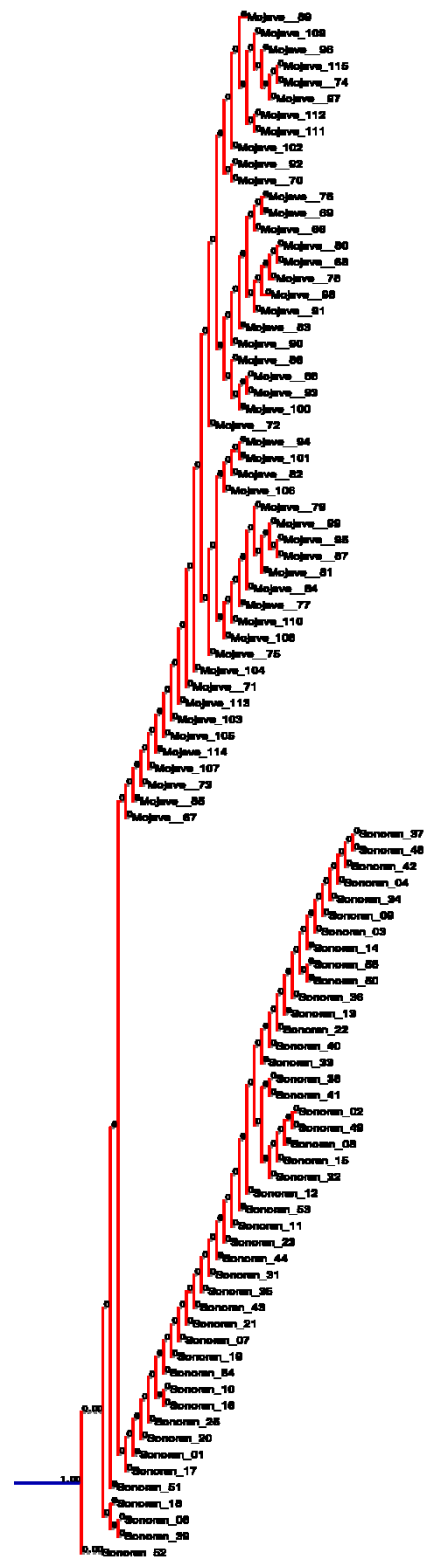
Figure 4.13 PTP species delimitation shows no evidence of speciation between the two *Schismus arabicus* populations

Results: Geneflow Between Sites

Geneflow modeling was also conducted using the Bayesian Inference software

MIGRATE-N. The posterior distribution of 10 replicates of our Markov Chain

Monte Carlo simulations can be seen below in figure 4.14.
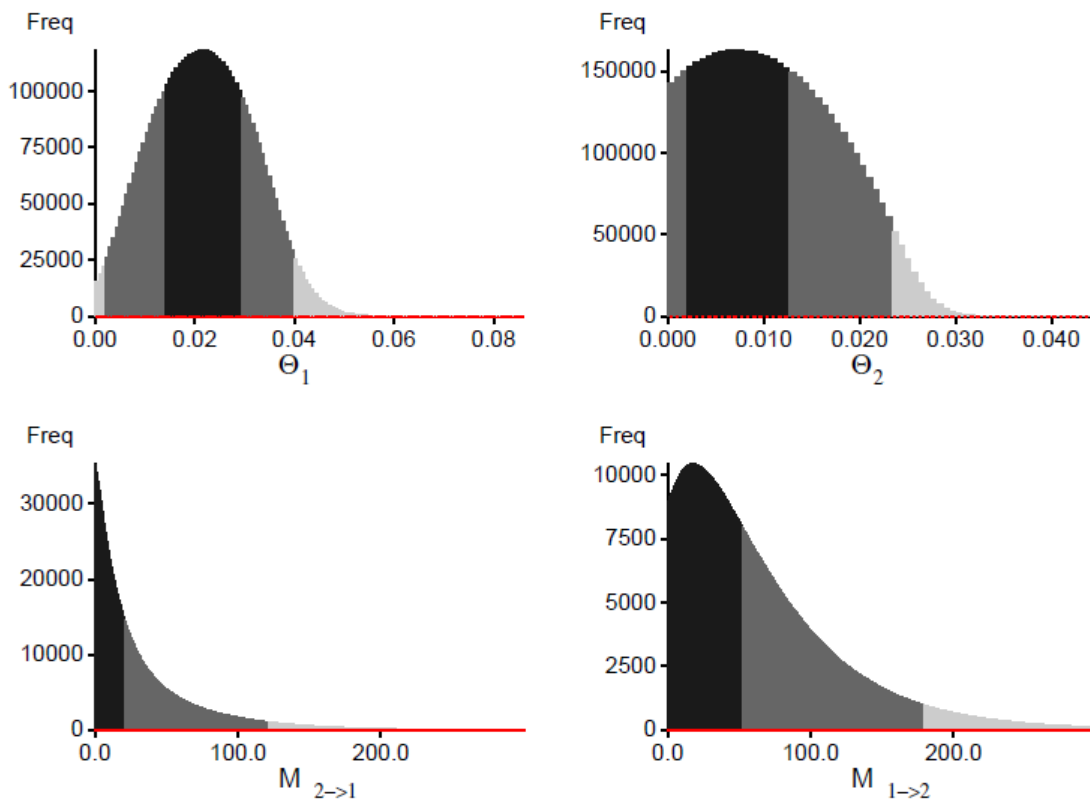
Figure 4.14 MIGRATE-N simulation of population size and geneflow between the Mojave and

Sonoran deserts.

Table 4.4 Effective Sample Size and autocorrelation estimates for 10 combined Migrate-N runs of *Schismus arabicus* ITS data. Run parameters were 500,000 iterations, 200 step interval, and 100,000 burn-in (1% burn-in).

Parameter	Autocorrelation	Effective Sample Size
Θ_1	0.65437	1,089,090.70
Θ_2	0.74477	733,956.97
$M_{2->1}$	0.66973	990,712.80
$M_{1->2}$	0.27243	2,858,287.38

In our MIGRATE-N models site number 1 corresponds to the Mojave desert region near Fort Irwin, California and site number 2 corresponds to the Sonoran desert site near Gila Bend, Arizona. The results of the MCMC simulations show that the *Schismus* population at the Mojave Desert is the larger of the two populations by a factor of two. Geneflow between the sites is unusual in that it appears to be unidirectional. The estimated geneflow from the Sonoran population to the Mojave population was effectively zero. The geneflow from the Mojave populations to the Sonoran populations had a non-zero mode that was consistent across all replicates, however the 95% confidence interval for Sonoran to Mojave migration did included zero.

Discussion

Our leading hypothesis was that the strongly contrasting environmental conditions found between nurse plant canopies and open ground in the harsh desert environments may promote the divergence of two distinct ecotypes of

Schismus arabicus. This divergence would create population structuring and small scale genetic isolation, resulting in a detectable signal at the population level. We did not detect any signs for such a divergence. We tested this model using genetic markers for the internal transcribed spacer (ITS) region. Analysis of the resulting sequences found an overall lack of support for the ecotype hypothesis. Haplotype network analysis through PopART showed that, while the two desert regions separated out strongly into two clusters, there was no internal haplotype signal at would match the proposed ecotypes. IQtree analysis also showed a very strong divergence of the two geographic regions, helping to corroborate the PopART results. Within site comparisons of open vs. canopy treatments resulted in polyphyletic distributions of the two ecotypes, indicating a lack of correlation between the genetic structure of a population and the environmental factors.

As a result of these findings we were forced to conclude that there is no evidence supporting the proposed ecotype hypothesis. Either this hypothesis is incorrect, or the ecotype effect is weak enough to have little to no impact on the populations as a whole. Our PopART and IQtree analyses demonstrated that the predominant factor effecting the populations is their geographic isolation. The impact of the geographic distance between the two populations completely swamped out any possible ecotype effect. Even if the ecotype principle is correct, we conclude that it must be orders of magnitude weaker than the geographic impact and thus largely irrelevant.

Haplotype Networks

The PopART MCMC simulations showed no sign of any ecotypes being reflected in the haplotype network. If ecotypes were dominant we would expect the signal to manifest in one of two ways. 1) If the ecotype signal were extremely strong, strong enough to swamp out the geographic origin of a sample, then we would expect division of the network into haplotypes the correspond to the shrub and open ecotypes. 2) Alternatively, a moderate ecotype signal would show division of the geographic regions into two haplotypes, corresponding to shrub and open ecotypes. This would give us 4 major haplotype groups; Sonoran-shrub, Sonoran-open, Mojave-shrub and Mojave-open. Neither of these models were supported by the evidence though, and no ecotype signal could be detected at the population level for either site.

The PopART MCMC results did, however, show a clear division between the geographic origin of an individual. All of the Sonoran and Mojave haplotypes segregated cleanly with no overlap. This indicates a degree of geographic isolation that we would expect from two populations located approximately 200 miles apart. This supports our null model, similar to the geometric model for *Ailanthus altissima* in the previous chapter, that geographic distance determines the population structure. In this case, our PopART simulations seem to indicate that the geographic origin of an individual is the predominant factor in predicting which haplotype group it belongs to.

We also note, however, that despite the clear division between the two geographic regions, the actual differences were not that strong. Only three polymorphism were different between the dominant Mojave and Sonoran haplotypes. This observation is also supported by our AMOVA results; high internal variation resulted in a Phi_{PT} value of 0.007 (p=0.008), but corrections for excess heterozygousity resulted in a Phi'_{PT} correction with a value of 1.0, indicating severe population structuring. While the sites are divergent and geographically isolated, they are also still quite similar. This could support a model of recent introduction. *Schismus arabicus* is known to be a relatively young invader, compared to invasive such as *Ailanthus altissima* (Kowarik & Säumel, 2007). The earliest boom of North American biological invasions came in the 1600's with early European settlers. The growth and industrialization of the United States resulted in increased immigration and trade, which brought with it more biological invasions throughout the late 1700's and 1800's. Plants such as *Artemisia vulgaris* (Mugwort) belong to the former group, while *Ailanthus altissima* belongs to this latter group. *Schismus arabicus*, however, belongs to a much more recent group on invasive species, arriving in the middle of the 1900's. Compared to the hundreds of years of time for divergence in these species, *Schismus arabicus* may only have been established in the United States for 70-80 years (Brooks, 2000). Taking into account the lag phase dynamic of biological invasions and the potential founder effect and subsequent genetic drift, the amount of similarity between the two sites is not unusual.

The two sites that we looked at did seem to have different levels of internal variation on their portion of the haplotype map. The Mojave site had many more sub-haplotypes within its geographic cluster than did the Sonoran cluster. These offshoot haplotypes were quite small, often only a single individual, which could have been taken to indicate that the threshold for assigning a haplotype was too sensitive. We decided that this was not the case, as the clusters were still dominated by two extremely large haplotypes. The level of variation within the Mojave population was unexpected since the Sonoran population was estimated to be the larger of the two groups by at least a factor of two (see MIGRATE-N above). When dealing with isolated populations the general assumption is that genetic drift will cause a reduction in genetic diversity over time, and that this is more severe in groups with smaller effective population size (N_e) (Hartl & Clark, 2007). This is not the case in our data, however, as the smaller site has many more haplotypes than the larger site. Given that they are the same species, we can safely assume that the mutation rate of ITS is the same for both populations. Then we can likely assume that this increased diversity is probably caused by geneflow from other sources which we did not measure. Another research team has been confirmed to be one source of geneflow between our two populations (see **Geneflow Between Sites** section below). However, Fort Irwin (Mojave) is also an active military base, which may be an additional source of the abnormally high haplotype variation we observed. Military vehicles returning from deployment during the second World War has been proposed as one possible

origin for the United States populations of *Schismus arabicus* (Cofrancesco,

Reaves, & Averett, 2007). The movement of personnel, supplies and vehicles

may still be serving as a method of dispersing *Schismus* propagules. The

PopART haplotype networks are not direct evidence of this, however, and future

work would be required to resolve this.

Maximum Likelihood Trees

The IQTree cladogram based on the F81+I model (Figure 4.6) showed results

similar to our PopART analysis, with a strong divergence between the Sonoran

and Mojave populations. Once again we had complete isolation between sites,

with no sign of any ecotype signal. We see extremely strong population

structuring between Mojave and Sonoran, but little to no internal population

structure. The single site cladograms (Figures 4.7 and 4.8) illustrate this behavior

clearly; all ecotypes were found to be polyphyletic, and the cladogram structure

showed no correlation to the predictions of the ecotype hypothesis. The IQTree

analysis served as a secondary confirmation to our PopART findings, that

evidence from our data shows the ecotype hypothesis to be falsified. From this

we have concluded that the response of *Schismus arabicus* to environmental

stress is not determined by a suite of fixed genetic traits.

Having failed to find evidence of a genetic signal of ecotype specialization, we

must assume that the adaptability of *Schismus arabicus* to the rapid small-scale

environmental shift caused by nurse plants must be due to another mechanism.

Ideas proposed include that individuals of *Schismus arabicus* may simply have higher overall robustness and be able to survive equally well under canopy as in the open. Without a difference in selection, and with no reproductive isolation, it is unlikely that a genetic difference will arise within a population. However, this model assumes equal relative fitness for plants growing in both environments. It is unlikely that the relative fitness of both groups would always be exactly equal to 1. Even relatively small differences is relative fitness (e.g. 0.95 vs. 1) can drive significant population level shifts over time (Hartl & Clark, 2007).

Additionally, it is unlikely that a given population of *Schismus arabicus* (or any species) will be equally well suited to such different environmental conditions. A perfect phenotype cannot exist, because many important environmental factors vary in opposition to each other (e.g. light and water) (Hutchings & Wijesinghe, 1997; J F Stuefer et al., 1996; Josef F Stuefer et al., 1994). These environmental contrasts require investment in specialized structures to exploit (Bloom, Chapin, & Mooney, 1985; Gleeson & Tilman, 1992; Iwasa & Roughgarden, 1984). Plants growing under the nurse shrub will receive less light for photosynthesis, but are also at less risk of desiccation. As an illustration, imagine a hypothetical transplant study where we swap mature *Schismus* individuals between canopy and open ground. An individual well suited to surviving out in the open may suffer from lack of light if grown under the canopy, while an individual grown under the canopy would dry out if moved into the open. A similar dynamic may also be at play for Nitrogen levels (Holzapfel et al., 2006; Pugnaire, Haase, &

Puigdefábregas, 1996; Schafer et al., 2012). This should drive specialization between the two habitats, our proposed ecotypes, and yet we do not see any evidence of this at the population level. Given the severity of the environment, how do we explain this lack of divergence?

Internal Geneflow

The most likely explanation is simply the lack of reproductive isolation within the given population. Panmictic populations are unlikely to show internal divergence, such as ecotype divisions, because they can freely recombine their genetic material between generations.

The lack of internal reproductive barriers is supported by the results of our GenAlEx analysis (Figures 4.9 and 4.10; Tables 4.2 and 4.3). Almost all of the genetic variation (99%) was observed to occur within the open ground and canopy populations, with only 1% of variation existing between these ecotypes. Such a low amount of variation being partitioned between these two groups shows that they have not diverged, and that no evidence of reproductive isolation between the two exists. The low Phi_{PT} value of our ecotype AMOVA (Phi_{PT}=0.007; p=0.008) is evidence in support of *Schimus arabicus* populations being panmictic within the geographic regions.

This explanation is also supported by our linear regression analysis, where we found that the number of segregating sites was independent of physical

separation (Figures 4.11 and 4.12). This lack of correlation shows that, for the scales we sampled at, the population appears to be panmictic. This conclusion is further supported by the fact that both Mojave and Sonoran sites exhibited the same results, with geographic distance explaining less than 1% of genetically segregating sites (Mojave R^2=0.000273; Sonoran R^2=0.00285).

The ecological explanation underlying the lack of ecotype isolation for this can be found in the life history of the species. *Schismus arabicus*, like most grasses, is wind pollinated and has little to no control over where pollen blows. Pollen can easily travel between individuals hundreds of meters apart. In comparison to pollen distribution range, the shift from canopy to open ground is extremely abrupt (Figure 4.15).

Figure 4.15 Landscape of the Mojave desert site. The distance between shrubs is short (a few meters) producing a highly heterogeneous environment. Image © Claus Holzapfel.

The distribution range of pollen is orders of magnitude larger than the small scale environmental heterogeneity that we based the ecotype hypothesis on. In attempting to pass on their specializations, any individual with traits well suited to their specific environment must compete with a large volume of non-specialized pollen. This is analogous to the Selection-Migration balance scenario, where the reduction in a given allele from natural selection is offset by the influx of additional copies of that allele through immigration of new individuals into the population. This mismatch of scale and the non-specific pollination strategy of *Schismus arabicus* creates a dynamic that likely precludes the development of any specialized ecotype sub-populations.

The success of the *Schismus arabicus* populations, then, can most plausibly be attributed to developmental plasticity of the individuals. Plasticity allows for individuals with similar genetic composition to exhibit divergent phenotypes based on environmental cues. This means that developmental plasticity of *Schismus arabicus* individuals could explain the lack of genetic differences observed in our work. Plasticity is extremely well known in plant species, both clonal and non-clonal, and may be one of the factors promoting biological invasions (Baker & Stebbins, 1965; Sultan, 2000).

Species Delimitation

Due to the strong isolation signal we observed during our PopART and IQTree

analyses, we felt it necessary to perform a species delimitation analysis using

PTP. The distance and lack of easy propagule transport between the Sonoran

and Mojave sites created reproductive isolation between the two populations,

which introduces the possibility that allopatric speciation could occur. Our PTP

analysis, however, shows that this is not the case (Figure 4.13). At least for the

current situation, there is insufficient differentiation between the two sites to infer

any degree of speciation. The genetic differences between the Sonoran and

Mojave sites fall into a very specific intermediate range. The two populations

demonstrate strong enough differentiation that they cleanly separate out in both

haplotype analysis (see PopART above) and maximum likelihood phylogenetic

analysis (see IQtree above). However, the differentiation between the sites is

also limited enough that the PTP species delimitation shows them as being

essentially the same.

This disagreement between the three methods arises because of the different

scale of analysis. PTP, being used for species delimitation, is concerned with a

much longer timescale than the other approaches. We can account for the

results of all three analyses by viewing it through the context of the historical

information we have regarding the *Schismus arabicus* populations at these two

sites.

The earliest recorded occurrence of *Schismus arabicus* in the United States was in California in 1935 (Brooks, 2000). Subsequent introductions are also likely to have occurred, particularly following the second World War when military vehicles deployed in Europe and Asia returned to the United States. This gives us a very specific timeframe of approximately 80 years, rather short compared with other invasive species such as Ailanthus altissima (1760's) or Artemesia vulgaris Mugwort (1600's). The PTP results, then, may simply indicate that the two populations have not had enough time to diverge or that populations are not sufficiently isolated to accumulate enough segregating sites to speciate.

An alternative explanation for the lack of speciation, despite the strong divisions found in PopART and IQtree, could be that the populations are still strongly influenced by the founder effect. The establishing individuals for the Sonoran and Mojave desert sites may have already shown divergence from the first generation, and our current measurements may just be seeing the continuation of this difference. As mentioned above, the combination of relatively recent introduction and reproductive isolation would contribute to maintaining this initial difference between populations.

Geneflow Between Sites

Interpretation of the MIGRATE-N results requires knowledge that the parameter estimates of Theta (Θ) and M from MIGRATE-N are the mutation scaled values for population size and migration values respectively. Theta is the population size

(N) multiplied by the mutation rate of the marker being modeled (μ), while M is the proportion of migrants divided by the mutation rate. The advantage of using these constructed parameters is that the MCMC models can be run independent of the mutation rate of a given marker. If the mutation rate for a marker is known then the estimated parameter can be converted into a real-world parameter, Θ and M can be converted into N or m respectively. However, if the mutation rate is not known, we can still perform MCMC simulations to estimate the mutation scaled parameters. This can be used to infer relative values, e.g. comparisons of relative population size. Mutation scaled parameters can also be used to directly calculate the real world parameter Nm, number of individuals immigrating between two sites during a single generation. This is possible because the mutation rate parameter cancels out when the two mutation scaled parameters are multiplied. This is demonstrated in equations 4.1-4.3 below.

$$\Theta = 4N \times \mu$$
$$M = m / \mu$$
$$\Theta \times M = 4Nm$$

Equations 4.1-4.3. Definitions of the mutation scaled parameters used by MIGRATE-N. Multiplication causes μ to drop out, allowing estimates of Nm independent of mutation rate.

Applying this equation we can calculate Nm values for each direction of geneflow in our dataset. Being a simple model, there are only two Nm values to estimate; Sonoran to Mojave and Mojave to Sonoran. The mode of the Sonoran to Mojave posterior distribution for $M_{2>1}$ was zero, which indicates no geneflow and an Nm equal to zero. The Mojave to Sonoran geneflow estimate $M_{1>2}$ had a mode of

17.5. When multipled by the Θ_1 estimate of 0.02167, this results in an Nm of approximately 1.52. When we consider that Schismus is an annual plant, we can estimate that going from the Sonoran to Mojave there is, on average, one and a half migrant propagules every year. This is an exceedingly low value, indicating a strong level of geographic isolation between the two sites.

In contradiction to the PopART and IQtree analyses above, our MIGRATE-N analysis potentially showed a small amount of unidirectional geneflow from the Sonoran to the Mojave population. The overlap of the 95% confidence interval with zero would normally be enough to dismiss the results as non-significant, however this relationship was verified to occur in every single trial throughout multiple runs (N=10), indicating that it is not merely an artifact of the Markov Chain Monte Carlo method. Estimates of the Θ and M parameters indicate that the number of migrant individuals (Nm) was 1.5, indicating that the impact of geneflow was equivalent to 1 and a half individuals moving from the Mojave site to the Sonoran site every year.

The unusual results prompted further investigation, where we discovered that another long-term study performed Sonoran site and the Mojave site may have been responsible for these findings. The researchers in question consistently visited the Sonoran desert before visiting the Mojave, and may have transported seeds between sites on their clothing and vehicles. This anthropogenic influx of seeds broke the isolation between sites in a single direction, which explains the

posterior distribution results above (Figure 4.14). The MIGRATE-N models

indicate that the cumulative effect of this geneflow on the population as a whole

was quite low, averaging 1.5 individuals per year. This indicates that the overall

impact of the anthropogenic interference was, while measureable, quite limited.

Small enough, even, to be non-significant despite observational confirmation that

geneflow did occur. Continued interference of this nature could increase the M

parameter estimates over time, resulting in higher Nm estimates for the

population as a whole. The study responsible for the geneflow, however, has

concluded and thus the anthropogenic influences should already have ceased.

Based on our PopART and IQtree models showing such strong site-based

divergence, and the lack of Mojave to Sonoran geneflow, over time the estimates

of Sonoran to Mojave transfer can be expected to return to zero.

Beyond the anthropogenic meddling, we can also use the MIGRATE-N posterior

distributions to estimate differences in the relative population size between the

Sonoran and Mojave desert sites. Existing records showed that form 2011 to

2013 the Mojave Desert had a density of 20 to 350 individuals per m^2; the

Sonoran Desert had a similar range of densities, being between 40 and 200

individuals per m^2 (Holzapfel et al., unpublished data). Despite the populations

having comparable population densities, the effective population size (N_e) of the

Mojave desert *Schismus* appears to be approximately 3 times the size of the

Sonoran population ($N_{Mojave}=0.02167$; $N_{Sonoran}=0.007$). The smaller population

size of the Sonoran group indicates that, in the long run, we would expect to see

more genetic divergence arise out of the Sonoran population. It is unfortunate, then, that the unidirectional flow acted toward the Sonoran population. The amount of geneflow needed to break genetic isolation of a population is actually quite small (Hartl & Clark, 2007) and the Mojave to Sonroan geneflow may have been sufficient to reduce the genetic divergence that had accumulated thus far. This is in agreement with the strong geographic differences (PopART and IQtree), the lack of species delimitation (PTP) and the limited unidirectional exchange of migrants (MIGRATE-N). Under these conditions, it is possible that the temporary isolation breaking caused by the other researchers may have acted to reduce the divergence between the two populations, resulting in two populations with distinct, but not completely divergent, genepools.

Conclusions

Ultimately we have concluded that the proposed ecotype hypothesis must be rejected, as we found no evidence to support this model. The genetic divergence observed was strongly driven by geographic origin, and analyses within a given site showed no correlation between habitat type and genetic background.

Despite falsifying the ecotype hypothesis, our data enabled us to infer other useful characteristics about the two populations. We found that, despite the strong isolation between the two sites, there was little to no evidence of divergent speciation. While the two populations appear to be well suited to allopatric speciation, the relatively recent introduction of the two populations means that

they have not accumulated sufficient differences between them to diverge into separate species. Anthropogenic interference from another research team is also know to have contributed to isolation breaking, as demonstrated in our MIGRATE-N analysis.

We also found that the Mojave population is substantially larger than the Sonoran population, by a factor of about three. From this we would expect the Sonoran population to be more severely affected by genetic drift and have a lower internal genetic diversity. Instead we observed that the Sonoran site actually has a much larger number of haplotypes as inferred by the MCMC simulations of PopART. This unusual discrepancy could be accounted for by increased geneflow, as immigrant individuals break isolation and introduce more variation. The unidirectional geneflow detected by MIGRATE-N, and later verified to be anthropogenic in origin, acts in the correct direction to create just such a result. Based on our Bayesian simulations in MIGRATE-N, we concluded that human interference has artificially increased the genetic diversity of the Mojave desert *Schismus arabicus* population.

In the end we conclude that the overall genetic structure of *Schismus arabicus* is largely driven by the long range dispersal mechanisms, both pollination syndrome and seeds dispersal. Being wind pollinated, the dispersal range of *Schismus arabicus* individuals is far wider than the relatively small microhabitat variations that would give rise to ecotypes. The seeds of *Schismus arabicus* are

likewise easily carried by the wind, allowing for wider dispersal. This lack of reproductive isolation within the populations creates a largely panmictic population, which precludes the development of specialized ecotypes based on genetics. The tenacity of *Schismus arabicus* under such severely contrasting environments is not mediated by contrasting genetic ecotypes, but rather by a highly plastic developmental response to the environment.
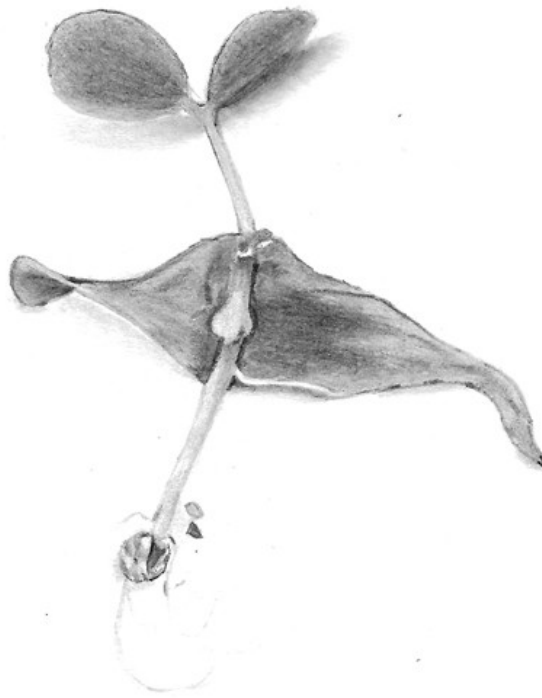
Image © Yvonne Roelofs.

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Appendix

Table 1: Raw data from experiment #1

Treatment	Flat #	AG-Biomass	Rhizome biomass	Flowers	Adventitious root count	Total root count
Het	1	5.72	1.32	13	68	95
Het	2	13.83	2.6	22	184	226
Het	3	14.02	3.85	0	337	366
Het	4	16.85	3.85	24	336	433
Het	5	1.84	0.42	0	50	88
Het	6	14.11	3.7	55	290	357
Het	7	10.18	2.55	33	169	214
Het	8	3.84	1.04	8	75	75
Het	9	15.8	3.91	26	259	342
Het	10	11.61	2.95	15	244	323
Hom	11	23.57	5.33	20	348	442
Hom	12	12.7	2.84	11	240	343
Hom	13	3.47	0.75	0	62	87
Hom	14	19.44	4.56	26	277	372
Hom	15	21.65	3.39	46	319	411
Hom	16	27.95	8.09	65	623	734
Hom	17	13.72	3.8	11	250	294

Hom	18	11.38	3.51	13	254	284
Hom	19	13.66	4.14	25	327	416
Hom	20	4.61	1.25	4	74	94
Con	21	8.03	1.74	4	126	208
Con	22	7.9	2.11	7	60	83
Con	23	16.14	5.56	6	270	332
Con	25	22.27	6.16	20	404	463
Con	26	2.88	0.78	0	85	119
Con	27	5.97	1.17	5	113	147
Con	28	4.6	1.35	5	117	147
Con	29	10.08	2.82	8	215	276
Con	30	6.31	1.7	3	96	135

Table 2: Descriptive statistics for experiment #1

	Treatments					
	Heterogeneous		Homogeneous		Control	
Parameters	Mean	SD	Mean	SD	Mean	SD
Above-ground-Biomass	10.78	7.10	15.22	7.37	9.35	3.09
Runner biomass	2.62	1.65	3.77	2.13	2.60	0.82
Number of Inflorescences	19.60	16.71	22.10	20.37	6.44	6.82

Adventitious root count	201.20	105.97	277.40	167.37	165.11	55.79
Total root count	251.90	136.76	347.70	191.44	212.22	66.79
Runner /biomass	0.24	0.02	0.25	0.05	0.27	0.03
flower/biomass	1.74	1.27	1.21	0.68	0.65	0.59

Table 3: Raw data for experiment #2

Flat #	Treatment	Leaf Mass	Horizontal Stem Mass	Adventitious Root Mass	Flower Count	Total Biomass
1	1	0.26	0.08	0.01	0	0.35
2	1	4.40	1.68	0.62	0	6.70
3	1	0.35	0.10	0.00	1	0.45
4	1	0.45	0.13	0.00	1	0.58
5	1	2.09	1.03	0.22	1	3.34
6	1	1.95	0.93	0.33	0	3.21
7	1	0.07	0.01	0.00	0	0.08
8	1	1.39	0.63	0.12	2	2.14
9	1	0.59	0.20	0.01	1	0.80
10	1	2.68	1.49	1.34	2	5.51
11	1	1.53	0.54	0.12	0	2.19

12	1	3.53	1.26	0.29	1	5.08
13	1	2.48	0.88	0.53	1	3.89
14	1	1.02	0.43	0.01	1	1.46
15	1	1.28	0.56	0.01	0	1.85
16	2	1.90	0.72	0.14	0	2.76
17	2	2.45	0.98	0.19	0	3.62
18	2	1.17	0.34	0.01	0	1.52
19	2	3.75	1.40	0.40	0	5.55
20	2	0.25	0.09	0.01	1	0.35
21	2	3.40	1.29	0.54	0	5.23
22	2	2.43	1.13	0.42	0	3.98
23	2	1.07	0.52	0.08	2	1.67
24	2	1.87	0.61	0.50	0	2.98
25	2	1.84	0.70	0.29	1	2.83
26	2	2.43	1.02	0.11	0	3.56
27	2	0.97	0.43	0.00	1	1.40
28	2	1.13	0.49	0.18	1	1.80
29	2	3.18	1.33	0.74	2	5.25
30	2	0.57	0.06	0.01	0	0.64
31	3	1.41	0.60	0.07	1	2.08
32	3	2.22	1.35	0.17	1	3.74
33	3	1.36	0.85	0.01	0	2.22
34	3	1.69	0.52	0.10	0	2.31

35	3	2.64	1.22	0.32	0	4.18
36	3	6.10	3.15	0.73	0	9.98
37	3	2.35	1.25	0.06	0	3.66
38	3	2.33	0.13	0.01	1	2.47
39	3	2.13	0.94	0.19	1	3.26
40	3	0.46	0.13	0.01	0	0.60
41	3	0.12	0.04	0.00	0	0.16
42	3	2.47	1.08	0.45	1	4.00
43	3	0.81	0.35	0.00	1	1.16
44	3	1.50	0.61	0.13	1	2.24
45	3	1.90	0.77	0.04	0	2.71
46	4	1.31	0.52	0.01	0	1.84
47	4	3.12	1.28	0.07	1	4.47
48	4	1.59	1.06	0.00	6	2.65
49	4	2.53	1.36	0.50	3	4.39
50	4	0.61	0.20	0.00	0	0.81
51	4	0.57	0.18	0.03	1	0.78
52	4	1.77	0.97	0.14	0	2.88
53	4	0.98	0.56	0.07	0	1.61
54	4	0.85	0.29	0.01	1	1.15
55	4	0.84	0.29	0.01	0	1.14
56	4	2.13	0.87	0.11	0	3.11
57	4	1.45	0.73	0.12	1	2.30

58	4	2.06	0.92	0.13	0	3.11
59	4	1.31	0.65	0.34	0	2.30
60	4	0.24	0.10	0.00	0	0.34

Table 4: Descriptive statistics for experiment #2

	Treatments			
	Homogeneous		heterogenous	
Parameters	Mean	SD	Mean	SD
Total Biomass	2.69	1.85	2.59	1.85
Leaf Mass	1.75	1.15	1.70	1.13
Stem Mass	0.70	0.48	0.77	0.61
Adventitious Root Mass	0.24	0.30	0.13	0.18
Flower Count	0.63	0.72	0.67	1.21
Stem Mass per Biomass	0.25	0.05	0.29	0.06
Adventitious Root Mass per Biomass	0.063	0.058	0.037	0.038
Inflorescence Count per Biomass	0.47	0.73	0.29	0.50