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**IMPORTANCE OF HYBRIDIZATION AND ECOLOGICAL DIFFERENTIATION IN
THE SUCCESS OF *LYTHRUM SALICARIA* IN NORTH AMERICA**

By

Jaimie Melissa Houghton-Thompson

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ABSTRACT

IMPORTANCE OF HYBRIDIZATION AND ECOLOGICAL DIFFERENTIATION IN THE SUCCESS OF *LYTHRUM SALICARIA* IN NORTH AMERICA

By

Jaimie Melissa Houghton-Thompson

Lythrum salicaria is much more invasive in North America than its native Europe (Batra *et al.* 1986). One possibility for how it became more invasive is that it hybridized with a close relative in North America, such as *L. alatum*, and gained genes that made it better adapted to its new environment. This hypothesis was first tested by searching for morphological evidence of hybridization. By examining a variety of purple loosestrife populations across the northeastern United States, several traits were found that are not present in European populations of purple loosestrife but are found in North American winged loosestrife. These unique morphs found in North American purple loosestrife suggest hybridization between the two species. In support of this, we identified intermediate sized *L. salicaria* where the two species grow sympatrically and could have hybridized.

Amplified fragment length polymorphism (AFLP) markers were then used to further analyze the relationship between *L. salicaria* and *L. alatum*, and search for evidence of hybridization. Twenty-seven North American and eleven European populations of *L. salicaria* and *L. alatum* were screened with 5 primer pairs, and then eight Michigan populations (selected for allopatry or sympatry between the two species) were screened with an additional 18 primer pairs. When the resulting patterns of molecular diversity were examined, North American *L. salicaria* clustered more closely

to European *L. salicaria* than to North American *L. alatum*, and no evidence of inter-species hybridization was found. However, a considerable amount of differentiation was observed among the North American *L. salicaria*. This differentiation may be the real reason why *L. salicaria* became so successful in North America.

I dedicate this work to my parents, William and Jean Houghton, who have always believed I could do anything I set my mind to, and my fiancé, Matthew Thompson, who was always there to listen when I thought I couldn't go on.

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CHAPTER 1

THE IMPLICATIONS OF POLYPLOIDY IN *LYTHRUM SALICARIA*

Introduction

It has been suggested that some exotic species are preadapted to become invasive if they are released from natural predators in their new environment (Blossey *et al.*, 1992). As a result, the control of invasives has moved more and more into the arena of biological control, operating under the assumption that if a critical natural control agent can be found, the fecundity of the invasive species, and their ability to invade, will be severely hampered if not eliminated. Recent studies, however, ask if invasives are truly 'born' and become invasive in a new environment simply because of the lack of natural enemies, or if they are 'made' and their invasive ability evolves after colonization (Ellstrand and Schierenbeck, 2000).

There is support for both of these hypotheses. Some invasive species have been controlled by the introduction of a biological control agent (Dodd, 1959; Huffaker and Kennett, 1959), suggesting that the release from biotic pressure was the main factor allowing invasion to occur. However, only a small fraction of species, when taken out of their natural environment and introduced to a new ecosystem, do develop invasiveness, and even for species that do become invasive, there is often a considerable lag time between their first introduction and their becoming invasive (Ewel *et al.*, 1999). If release from biotic pressure was the only prerequisite to invasiveness, the spread of the species should occur quickly after the release from predators. Ellstrand and Schierenbeck

(2000) have proposed that the reason for this delay may often be that interspecific hybridization with native species must occur, allowing invasiveness to evolve after the introduction of new genes. Abbott (1992) observed that interspecific hybridization involving a non-native and a native or non-native species has led to the development of numerous new sexually reproducing species. The models of Ellstrand and Schierenbeck extend this idea to include previously-isolated, allopatric populations of sexually reproducing species as well.

However, species can also evolve invasiveness without an influx of genes via hybridization. Considerable ecological differentiation has been observed in many native species, and it is certainly conceivable that such differentiation could occur in an introduced species if its founders carried sufficient genetic variability. Polyploidy may be an excellent way for this variability to be stored. Several aspects of polyploidy contribute to their success as a whole, including their polyphyletic origin, which incorporates high levels of genetic diversity into the species from recurrent formation from multiple parent populations (Soltis and Soltis, 2000). As a result, polyploids have higher levels of heterozygosity than their diploid progenitors and less inbreeding depression. The genetic variability found in polyploids can be further assorted through genomic rearrangement, and in the case of autopolyploids, tetrasomic inheritance.

An underlying theme in our laboratory is the investigation of how polyploidy, especially autopolyploidy, plays a role in a species' survival and evolutionary history. In my project, that question was addressed by testing for interactions between an introduced, invasive autopolyploid, *Lythrum salicaria* (purple loosestrife) and a closely related native diploid species to explore if introgression has occurred, which could explain the

increased invasiveness of the introduced autopolyploid. The alternative hypothesis was that genetic variability already present in *L. salicaria* reassorted and allowed previously unrevealed characteristics to be expressed, expanding its ability to adapt and invade. *L. salicaria* (purple loosestrife) is a highly invasive, exotic, autotetraploid plant that has been the focus of recent attention because of its ability to become the dominant species in wetlands, replacing many native species (Cutright, 1986; Batra *et al.*, 1986).

Lythrum in North America

Purple loosestrife is a tall, perennial, autotetraploid plant found in wetland habitats. It is native to Eurasia, but was introduced into North America in the early 1800's (Pursh, 1814) both accidentally through ship ballast and purposely through seed sales (Mack, 1991). It is considered invasive and a noxious weed in several states due to its ability to eliminate pre-existing native species in wetlands (Cutright, 1986). Over the last century, its range has spread westward from New England and it has now successfully established itself as far west as the Pacific Northwest (Stuckey, 1980). Although it is aggressive in North America, it is not in its native Eurasia (Batra *et al.*, 1986).

Purple loosestrife was initially introduced to North America in the early 1800's, specifically in New England, but it was not recognized as invasive until the 1930's, when it began to form monospecific stands in the floodplain pastures of the St. Lawrence River in Quebec (Louis-Marie, 1944). Since that time, it has followed a distinct pattern of invasion across the US. Typically, it remains unobtrusive for a long period (at least 20

years) followed by a brief period (of less than 3 years) in which it becomes dominant in many parts of that region (Stuckey, 1980). One possibility why purple loosestrife is much more dominant in North America than Eurasia may be that favorable genes have introgressed from a close relative native in North America. If this is the case, the most likely candidate is *Lythrum alatum* (winged loosestrife), a widespread diploid species that is closely related to purple loosestrife, not found in Eurasia, and has habitat overlap at its outer edges with purple loosestrife, but is typically found in drier areas. There are 11 known species of *Lythrum* native to North America, but *L. alatum* is the most widespread. Winged loosestrife is a shorter, less showy species than purple loosestrife (Blackwell, 1970), and grows in wet meadows as a sub-dominant (Cody, 1978).

There is evidence that the genomes of winged and purple loosestrife are compatible, as some cultivars of purple loosestrife were generated by breeders by crossing the two species (Anderson and Ascher, 1993). Most of these cultivars are self-sterile, but recent work has shown that these cultivars are fully fertile when crossed with the wild species of purple loosestrife (Lindgren and Clay, 1993; Anderson and Ascher, 1993; Ottenbreit and Staniforth, 1994). Purple loosestrife is self incompatible and heterostylous (Darwin, 1877) and as a result the cultivars are self-sterile.

While no direct evidence of hybridization in the wild has been documented between winged and purple loosestrife, bees and butterflies have been observed to move between these species when they grow sympatrically (Levin, 1970), and hybridization could occur between the two species via unreduced gametes (Anderson and Ascher, 1993). Morphological characters thought to be unique to winged loosestrife have also been observed in purple loosestrife populations (Anderson and Ascher, 1994) and the

hybrids produced by breeders have been shown to be highly fertile tetraploids, which can readily backcross to the tetraploid purple loosestrife.

Type of polyploidy in *Lythrum*

There are two major types of polyploids: autopolyploids and allopolyploids. Autopolyploidy arises when only one type of chromosome set is doubled, while allopolyploidy occurs when two nonhomologous genomes are united. Most polyploids are thought to originate through unreduced gametes (Harlan and deWet, 1975). Until recently (Soltis and Soltis, 1993, 1995), autopolyploidy was considered to be much rarer in natural populations than allopolyploidy. The traditional view of autopolyploidy was that it is generally maladaptive: that there would be multivalent pairing of chromosomes and this would lead to reduced pollen and seed fertility because of unbalanced chromosome segregation at meiosis (Stebbins, 1950, 1971). Successful establishment of an autopolyploid was therefore seen as unlikely, particularly since their adaptations were predicted to be very similar to their parent species (Levin, 1983; Soltis and Rieseberg, 1986). These assumptions have now been tested in a wide range of polyploid species using molecular markers, and segregation ratios have indicated the reverse – autopolyploids are often highly fertile. In many autopolyploids, multiple homologues actually pair in random assortments of bivalents rather than in multivalents, and as a result pairing relationships are normal (Soltis and Rieseberg, 1986; Krebs and Hancock, 1989; Samuel *et al.* 1990; Qu *et al.*, 1998). Autopolyploidy is much more common than

previously thought because many autopolyploids were misidentified as allopolyploids, based on bivalent pairing, but this bivalent pairing is random with tetrasomic inheritance.

Molecular studies of autopolyploid species have revealed three important characters that may confer an evolutionary advantage over their diploid progenitors: enzyme multiplicity, increased heterozygosity, and increased allelic diversity (Soltis and Soltis, 1993). This increased biochemical diversity may confer a fitness advantage to polyploids (Levin, 1983), especially autotetraploids because of the tetrasomic inheritance they exhibit. Fertility is high in many autopolyploid species because the multiple homologues pair in random assortments of bivalents rather than in multivalents (Qu *et al.*, 1998). A number of studies comparing genetic variation (as a function of polymorphic loci and mean observed heterozygosity) have shown autotetraploids to be more genetically variable than related diploid species (Shore, 1991; Soltis and Soltis, 1989; Lumaet, 1986).

Autopolyploidy could contribute greatly to the success of an introduced autotetraploid species if its diploid progenitor (or close relative) is located in the area of introduction. An alien autotetraploid species can cross with a closely related diploid species with little chromosomal divergence if the diploid produces unreduced gametes, since the unification of the reduced gamete from the tetraploid with the unreduced one from the diploid would result in tetraploid F_1 progeny with normal tetrasomic pairing association, as shown in Figure 1B. Through backcrossing, advantageous genes from the diploid genome could become incorporated into the tetraploid genome. Over time, this could allow genes to be injected into the tetraploid genome that allow the alien species to further adapt to the new environment.

Alternatively, autopolyploidy in *L. salicaria* may have allowed unique adaptations to arise via reassortment once exposed to a new environment. The increased level of heterozygosity generally observed in polyploids may have given some traits the plasticity needed to adapt to a new habitat. Heterozygosity is fixed in allopolyploids, due to the fact that the two ancestral genomes do not pair with each other in the polyploid. In autopolyploidy, with pairing freely allowed among all chromosome sets, reassortment into new ecotypes suited for a wider range of habitats is possible. Genetic variability already present in *L. salicaria* may have reassorted without hybridization into new morphologies and adaptive types. Several species of *Lythrum* in Europe are diploid, and some carry similar traits to *L. alatum* (Tutin *et al.*, 1968). Some of these species could be the natural progenitors of *L. salicaria*, although this possibility has not been tested. If these species are the original progenitors to the tetraploid *L. salicaria*, seemingly new traits in *L. salicaria* could actually have lain hidden in the ancient polyploid genome and reassorted when *L. salicaria* encountered new habitats in North America. The presence of new habitats in North America may have presented the opportunity for these genes to reassort and become phenotypically expressed.

Researchers have referred to *L. salicaria* as an allotetraploid because it is thought to be an interspecific hybrid (Strefeler *et al.* 1996), but at the chromosome level, it probably acts as an autopolyploid. This is supported by the discovery that the inheritance patterns of style length are tetrasomic (Fisher, 1949). Breeding evidence that purple and winged loosestrife can intercross, and the fact that these cultivar hybrids can backcross to purple loosestrife (Ottenbreit and Staniforth, 1994), are also compelling evidence that purple loosestrife is an autopolyploid. If, chromosomally, it acted as an allopolyploid,

the two sets of chromosomes in the purple loosestrife gametes would be incompatible and would cause significant fertility reductions in an interspecific cross (Figure 2). The most conclusive evidence that the hybrid acts as an autotetraploid comes from crossing studies where tetraploid purple loosestrife was hybridized with diploid winged loosestrife, and the resulting tetraploid F₁ hybrids produced via unreduced gametes (in the diploid) were completely fertile (Ottenbreit and Staniforth, 1994). A known purple and winged loosestrife hybrid, the cultivar Morden Gleam, was crossed with purple loosestrife and no significant reduction in the fertility of progeny was found when compared to crosses among purple loosestrife. Therefore, several lines of evidence support the hypothesis that *L. salicaria* is an autopolyploid.

The F₁ hybrid between purple and winged loosestrife must be an autopolyploid rather than an allopolyploid, as the unification of a purple loosestrife gamete with an unreduced winged loosestrife gamete in the case of allopolyploidy would result in an unfertile hybrid that had two chromosome sets from the winged gamete that could freely pair, but two sets of chromosomes from the purple gamete that could not pair with each other. Depending on chromosomal divergence between winged and purple loosestrife, either one or none of the purple loosestrife chromosomes could pair with the winged chromosomes (Figure 1). This would result in reduced fertility of the hybrid due to meiotic irregularities, leading to the production of numerous partially aneuploid and/or triploid gametes with poor viability, and backcross hybrids would likely have low vigor and meiotic irregularities. The only way for a hybrid between purple and winged loosestrife to be fully fertile when backcrossed to a purple loosestrife plant is if all four chromosomes of each set in the hybrid can interchangeably pair with each other at

meiosis (Figure 2). This would result in tetrasomic inheritance (four alleles at a locus) (Fisher, 1944, 1949). In the case of an autopolyploid, there would be little chromosomal divergence between winged and purple loosestrife, and the chromosomes could pair in a balanced fashion at meiosis resulting in high fecundity. If there is as little chromosomal divergence between the two species, as illustrated by this example, it is not hard to imagine a pathway for the movement of information from one genome to the other.

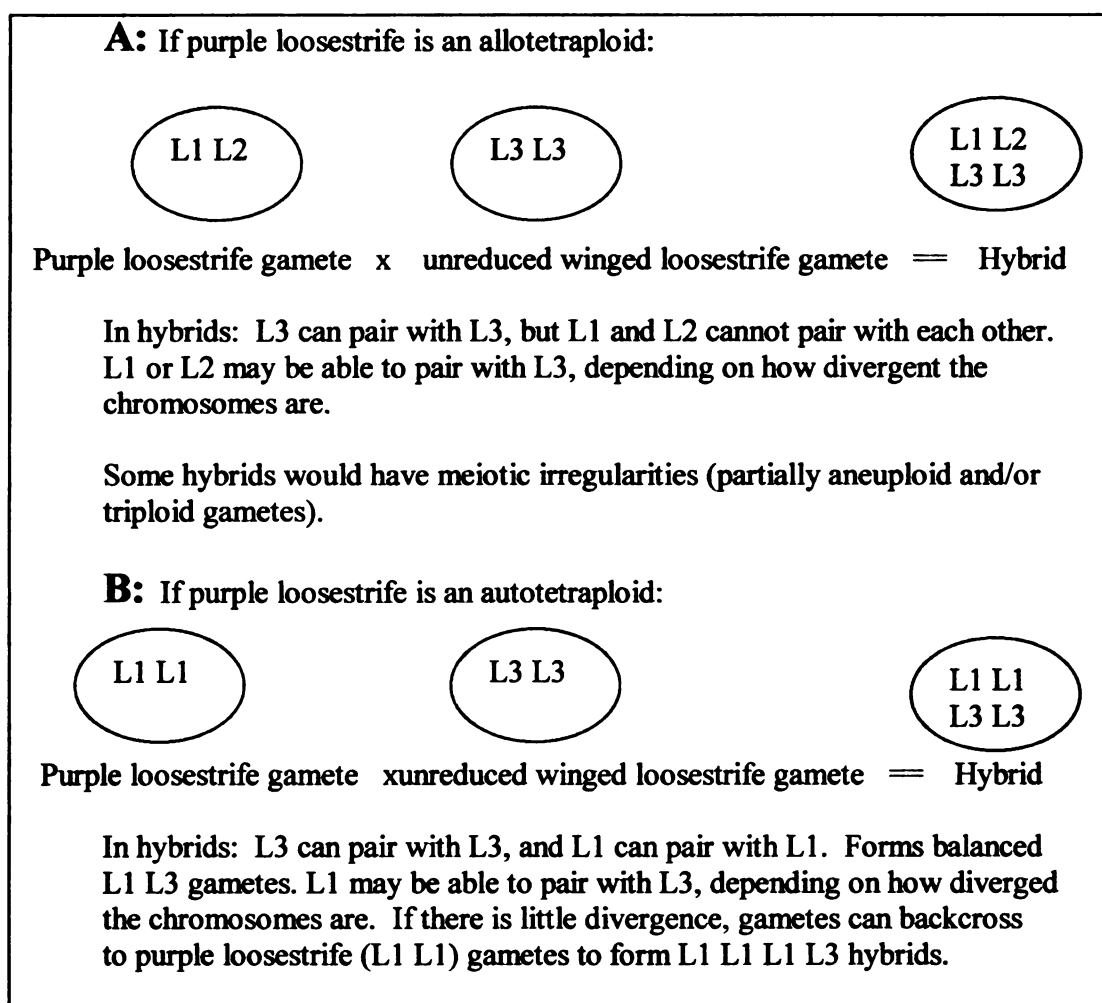


Figure 1: Crossing evidence for type of tetraploidy of purple loosestrife. A is the result of crosses if purple loosestrife is an allotetraploid, B is the case when it is an autotetraploid. Experimental findings of full fertility of hybrids indicates that purple loosestrife is an autopolyploid.

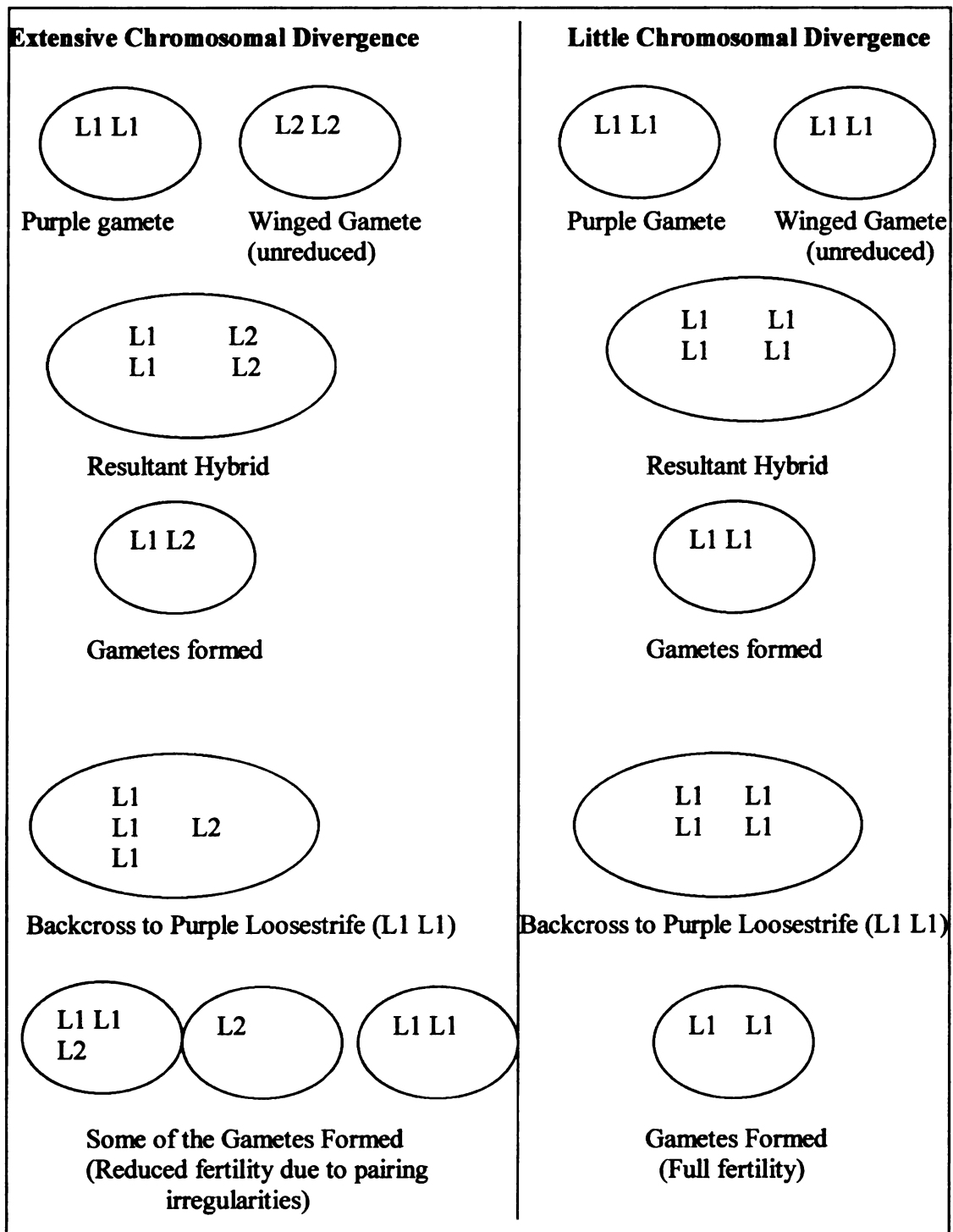


Figure 2: Inheritance patterns in purple x winged loosestrife hybrids where there is little or extensive chromosomal divergence. With little chromosomal divergence, the hybrid exhibits tetrasomic inheritance and backcrosses easily to purple loosestrife. With extensive divergence, meiotic irregularities develop and backcrossing is difficult.

Role of hybridization and polyploidy in *Lythrum*

In recent years, the role of interspecific hybridization in plant evolution has received renewed attention. The importance of interspecific hybridization was long suspected utilizing morphological markers (Anderson, 1949; Riley, 1938; Heiser, 1947 and 1949), and with the advent of molecular markers, considerable direct evidence has accumulated that genes have been exchanged between closely related species of the same ploidy (Arnold et al., 1990, 1992 and 1993; Rieseberg and Warner, 1987; Rieseberg et al., 1988; Rieseberg and Ellstrand, 1993). In some cases, substantial genomic reorganizations have occurred after hybridization resulting in speciation (Arnold, 1993; Rieseberg et al., 1993; Rieseberg, 1995).

Recently, a number of plant families were identified as “hotspots” for hybridization (Ellstrand *et al.*, 1996). Although Lythraceae, the family that includes *Lythrum*, was not included in this discussion, its closest relative, Onagraceae, was, and this family was identified as one of the families with an unusually high level of natural hybridization. *Lythrum* also fits all three criteria that were identified to increase the likelihood of hybridization: perennial habit, outcrossing breeding system, and reproductive modes that can stabilize hybridity (e.g. clonal reproduction).

Our original hypothesis was that there was hybridization occurring between winged and purple loosestrife as it moved across North America, via unreduced gametes, that allowed genes from the winged loosestrife genome to be incorporated into purple loosestrife populations. These incorporated genes then allowed purple loosestrife to more rapidly adapt to North American habitats. We based this hypothesis on previous

morphological (Anderson and Ascher, 1994) and isozyme (Strefeler *et al.*, 1996a, 1996b) studies conducted in Minnesota that indicated that there had been movement of traits from winged loosestrife into purple loosestrife populations. The alternative hypothesis was that the appearance of new traits and adaptation to new habitats of purple loosestrife in North America was due to genetic reassortment in the polyploid, unveiling previously untapped invasive potential. My project addressed these hypotheses in three ways. First, by characterizing both purple loosestrife and winged loosestrife on both a regional level and species level, using genetic [amplified fragment length polymorphism (AFLP)] and morphological markers, and searching for evidence of either movement of genetic material from winged to purple loosestrife or extensive differentiation without hybridization. Second, by observing putative hybrid populations in a controlled environment and determining if the morphological patterns observed in the field have a genetic basis. Third, by obtaining plant material from European populations and discovering whether North American populations of purple loosestrife have diverged from European ones.

This project allowed us to study whether autopolyploidy and interspecific hybridization can play a critical evolutionary role in a species' adaptation to a new environment. To date, ecotypic variation in an autopolyploid species that arose through sexual interaction with a diploid carrying the same genome has not been documented, and levels of genetic differentiation in autopolyploids have only rarely been described, with or without interspecific hybridization. Showing that polyploidy and natural hybridization can be a dynamic process in evolutionary change, especially as a mechanism for

increasing invasiveness, would further our understanding of how plants evolve invasive properties such as weediness, and can help in developing strategies to control them.

Understanding the mechanism of invasion of a polyploid alien species can also be critical to determining methods of biological control, particularly when natural hybridization could lead to the transfer of resistance. Many studies have been performed to determine methods for biological control of purple loosestrife, specifically looking at herbivores that feed on purple loosestrife in its native Eurasia (Blossey, 1995). Based on host specificity, three herbivore species have been chosen: *Galerucella californiensis* (L.) and *G. pusilla* (Duft.), leaf feeding beetles, and *Hylobius transversovittatus* Goeze, a root feeding weevil (Kok *et al.* 1992a, b). In no-choice tests, the leaf feeding beetles were found to oviposition as well as feed on winged loosestrife, although in choice tests, purple loosestrife was preferred (Kok *et al.*, 1992). If hybridization has taken place between purple and winged loosestrife, that could have a significant effect on the susceptibility of both purple and winged loosestrife to Eurasian predators. Two effects are possible: 1) the hybrid could be less susceptible to Eurasian predators than purple loosestrife is in Eurasia, or 2) the hybrid could provide an evolutionary “bridge” for the Eurasian predators to adapt to feeding on winged loosestrife. Documentation of hybridization occurring in the wild between these species will allow environmental managers to make more informed decisions about biological control agents. If purple loosestrife in North America becomes more resistant to Eurasian predators than the Eurasian purple loosestrife, much time and money could be wasted breeding and releasing insects that will have little or no effect. Additionally, if the Eurasian predators are more effective predators of F₁ hybrids, our native species may be put at risk.

CHAPTER 2

MORPHOLOGICAL DIVERSITY IN NORTH AMERICAN *LYTHRUM*

Introduction

Lythrum salicaria, purple loosestrife, was introduced to North America from Eurasia in the early 1800's and now ranges from New England to the Pacific Northwest. Its closest relative is *L. alatum*, more commonly known as winged loosestrife, a shorter, less showy diploid species that is native to North America and is not present in Eurasia (Blackwell, 1970). Winged loosestrife grows in wet meadows as a sub-dominant (Cody, 1978), and its preferred habitat overlaps extensively with purple loosestrife.

Purple and winged loosestrife can intercross, and they probably share a common genome. Purple loosestrife has been identified as an autotetraploid by previous researchers (Fisher, 1949) by determining that inheritance patterns of style length were tetrasomic. Purple and winged loosestrife have been successfully hybridized by breeders, and these cultivar hybrids can be successfully backcrossed to purple loosestrife (Ottenbreit and Staniforth, 1994). Winged loosestrife is a diploid species, therefore the most likely way for these two species to intercross is through an unreduced gamete in winged loosestrife fertilizing a natural diploid gamete in purple loosestrife. The resultant hybrid would be tetraploid, and could backcross to purple loosestrife. Unreduced gametes have been shown to be important in the evolution of numerous autopolyploid species including blueberry (Qu *et al.*, 1998), potato (Lam, 1974) and alfalfa (Veronesi *et al.*, 1986).

There are several taxonomic characters that are found in winged loosestrife that are not present in European populations of purple loosestrife (Graham, 1975; Table 1). All of these morphological characters have been anecdotally observed in purple loosestrife populations in Minnesota (Anderson and Aster, 1994), but similar searches have not been made across the entire range of purple loosestrife. The research described herein sought to determine whether winged loosestrife traits occur elsewhere in purple loosestrife populations across the northeastern United States, following its original invasion pattern. We found that winged loosestrife traits do appear in purple loosestrife all across its North American range, and that some purple loosestrife populations which are sympatric with winged loosestrife have heights and leaf lengths intermediate to most other winged and purple populations. These observations are consistent with the possibility that purple and winged loosestrife have hybridized in North America, although the possibility can not be excluded that purple loosestrife has evolved traits similar to those found in winged loosestrife without hybridization.

Table 1: Taxonomic characteristics that separate North American winged loosestrife (*Lythrum alatum*) and Eurasian purple loosestrife (*Lythrum salicaria*) (Graham, 1975).

<i>Lythrum alatum</i>	<i>Lythrum salicaria</i>
1 to 2 flowers per leaf axil	4 or more flowers per leaf axil
leaves alternately placed	leaves placed opposite or whorled
glabrous, oblong calyx	Pubescent calyx
distylous	Tristylous
dwarf, less than 3 feet tall	4 feet tall or more
Oblong-Ovate to Linear-Lanceolate Leaves	Lanceolate leaves

Materials and Methods

Population surveys

Lythrum salicaria and *L. alatum* populations were surveyed after flowering in the months of July and August. Michigan populations were surveyed in August, 1997, Ohio populations in July, 1998, and Massachusetts and Wisconsin populations in July, 1999. Distribution of collection sites by map location are outlined in Figure 3, and details for each population (region, species, name, abbreviation) are found in Table 2. Population size estimates and short descriptions of the habitats of each population are listed in Table 3. The main difference in population habitats was water depth. Populations ranged from permanent flooding at depths greater than 30 cm, to no history of flooding whatsoever, with all available water determined by rainfall.

L. salicaria clones were surveyed in a transect across the width of each population, at a distance of ten meters from each other, to minimize the possibility of repeat sampling of the same clone. In general, individual clones were distinct, forming colonies of 20 - 25 individual shoots. An attempt was made to sample at least 50 clones from each population, although some populations were too limited to collect this many samples without duplication. *L. alatum* clones were also surveyed 10 meters apart in the larger populations, but in the smaller populations, plants were examined as close as 1 m, as *L. alatum* plants do not exhibit the same degree of vegetative underground growth as *L. salicaria*., so distinguishing between different plants was much easier. In the large populations, 50 clones were surveyed at regular intervals, but in the small populations, all

distinct clones were surveyed. Total numbers of clones surveyed in both *L. salicaria* and *L. alatum* populations are listed in Table 3.

A total of six taxonomic traits (Table 1) were surveyed in each clone. Flower number was counted in each leaf axil along a random shoot in each clone and the average number of flowers per leaf axil was recorded. Placement of leaves along the stem was also noted as either alternate, opposite, or whorled. The calyx was rated as either glabrous or pubescent, by examining all calyxes along another random shoot in each clone. Style length was recorded on 10 randomly selected flowers on each clone to determine if there was deviation from distyly in *Lythrum alatum* or tristily in *Lythrum salicaria*. The height of the tallest shoot of each clone was also measured. Leaf length and width of two to three randomly selected leaves on each plant was measured for use in determining leaf shape, via ratios (length / width).

Table 2: Location and species found at each study site.

State	Species	Site	Abbr.	Latitude	Longitude
Michigan	<i>L.salicaria</i>	Lake Lansing	LLA	42:44:59 N	084:24:02 W
Michigan	<i>L.salicaria</i>	Crow Island St. Game Area	CIA	43:28:11 N	083:54:14 W
Michigan	<i>L.salicaria</i>	Shiawassee R. St. Game Area	SRA	43:23:13 N	083:57:58 W
Michigan	<i>L.salicaria</i>	Quanicassee Wildlife Area B	QWB	43:35:00 N	083:40:51 W
Michigan	<i>L.salicaria</i>	Quanicassee Wildlife Area A	QWA	43:35:00 N	083:40:51 W
Michigan	<i>L.salicaria</i>	Sheep Farm B	SFB	43:39:13 N	083:27:58 W
Michigan	<i>L.salicaria</i>	Harsen's Island	HIS	42:35:22 N	082:35:19 W
Michigan	<i>L.alatum</i>	Sheep Farm A	SFA	43:39:13 N	083:27:58 W
Michigan	<i>L.alatum</i>	Algonac State Park	ASP	42:37:06 N	082:31:52 W
Michigan	<i>L.alatum</i>	Wildfowl Bay	WFB	43:53:00 N	083:22:00 W
Michigan	<i>L.alatum</i>	Rose Island Railroad	RIR	43:46:58 N	083:25:53 W
Michigan	<i>L.alatum</i>	Harsen's Island	HIW	42:35:22 N	082:35:19 W
Wisconsin	<i>L.salicaria</i>	Wee Know School	WKS	43:06:18 N	088:20:31 W
Wisconsin	<i>L.salicaria</i>	Bark River	BRI	43:04:50 N	088:15:40 W
Wisconsin	<i>L.salicaria</i>	Duck Creek	DCR	44:33:43 N	088:04:09 W
Wisconsin	<i>L.salicaria</i>	Senior Citizen Center	SCC	42:54:38 N	087:51:38 W
Wisconsin	<i>L.salicaria</i>	Tichigan Lake	TLP	42:49:44 N	088:11:51 W
Wisconsin	<i>L.alatum</i>	Janesville	JAN	42:40:58 N	089:01:07 W
Wisconsin	<i>L.alatum</i>	Herbarium Preserve	HPR	43:04:53 N	088:54:42 W
Wisconsin	<i>L.alatum</i>	Tichigan Lake	TLW	42:49:44 N	088:11:51 W
Wisconsin	<i>L.alatum</i>	Nature Conservancy	NCO	42:30:44 N	087:48:33 W
Ohio	<i>L.salicaria</i>	Ottawa Natl Wildlife Refuge	ONP	41:36:56 N	083:12:58 W
Ohio	<i>L.salicaria</i>	Kildeer	KIL	41:02:39 N	083:39:00 W
Ohio	<i>L.alatum</i>	Kitty Todd A	KTA	41:34:46 N	083:37:02 W
Ohio	<i>L.alatum</i>	Kitty Todd B	KTB	41:34:46 N	083:37:02 W
Ohio	<i>L.alatum</i>	Kildeer	KIW	41:02:39 N	083:39:00 W
Ohio	<i>L.alatum</i>	Ottawa Natl Wildlife Refuge	ONW	41:36:56 N	083:12:58 W
Massachusetts	<i>L.salicaria</i>	Field Farm	FFA	42:42:43 N	073:12:15 W
Massachusetts	<i>L.salicaria</i>	West Pittsfield	WPI	42:25:51 N	073:18:37 W
Massachusetts	<i>L.alatum</i>	Sheffield	SHE	42:06:37 N	073:21:20 W

Table 3: Loosestrife population size and type of environment at each study site.

Site	Species	Pop. Size	Water Depth	Community
Lake Lansing	<i>L. salicaria</i>	>500	Perm. flooding 5cm-30cm	Purple loosestrife, sedge, reed canary grass
Crow Island Game Area	<i>L. salicaria</i>	100-500	Perm. flooding >30cm	Purple loosestrife, cattails
Shiawassee Game Area	<i>L. salicaria</i>	>500	Perm. flooding >30cm	Purple loosestrife, rice-cut grass, arrow arum, reed canary grass
Quanicassee Wildlife B	<i>L. salicaria</i>	50-100	Periodic flooding >30cm	Purple loosestrife, sedge, blue-joint grass, water smartweed
Quanicassee Wildlife A	<i>L. salicaria</i>	>500	Periodic flood 5cm-30cm	Purple loosestrife, sedge, blue-joint grass, water smartweed
Sheep Farm B	<i>L. salicaria</i>	50-100	No flooding	Purple loosestrife, misc. grasses
Harsen's Island	<i>L. salicaria</i>	20-50	Periodic flood but <5cm	Purple and winged loosestrife, cattails, misc. grasses
Sheep Farm A	<i>L. alatum</i>	50-100	No flooding	Winged loosestrife, grasses and shrubs
Algonac State Park	<i>L. alatum</i>	100-500	No flooding	Winged loosestrife, grasses, annual wildflowers
Wildfowl Bay	<i>L. alatum</i>	50-100	Periodic flood but <5cm	Winged loosestrife and tall grasses
Rose Island Railroad	<i>L. alatum</i>	<20	No flooding	Winged loosestrife, trees, large shrubs, grasses
Harsen's Island	<i>L. alatum</i>	20-50	Periodic flood but <5cm	Purple and winged loosestrife, cattails, misc. grasses
Wee Know School	<i>L. salicaria</i>	100-500	No flooding	Purple loosestrife and cattails
Bark River	<i>L. salicaria</i>	50-100	Perm. flooding 5cm-30cm	Purple loosestrife, short grasses
Duck Creek	<i>L. salicaria</i>	50-100	Perm. flooding >30cm	Purple loosestrife, trees, short grasses
Senior Citizen Center	<i>L. salicaria</i>	20-50	No flooding	Purple loosestrife, tall grasses, trees
Tichigan Lake	<i>L. salicaria</i>	50-100	Perm. flooding >30cm	Purple loosestrife, cattails
Janesville	<i>L. alatum</i>	50-100	No flooding	Winged loosestrife, tall grasses, small shrubs
Herbarium Preserve	<i>L. alatum</i>	50-100	No flooding	Winged loosestrife, wildflowers, tall grasses, small shrubs
Tichigan Lake	<i>L. alatum</i>	20-50	No flooding	Winged loosestrife, grasses, and hedges, trees
Nature Conservancy	<i>L. alatum</i>	50-100	No flooding	Winged loosestrife, tall grasses
Ottawa Natl Wildlife Ref.	<i>L. salicaria</i>	100-500	No flooding	Purple and winged loosestrife, tall grasses, short grasses
Kildeer	<i>L. alatum</i>	50-100	No flooding	Purple and winged loosestrife, brambles, tall grasses, some cattail
Kildeer	<i>L. salicaria</i>	<20	No flooding	Purple and winged loosestrife, brambles, tall grasses, some cattail
Kitty Todd	<i>L. alatum</i>	50-100	No flooding	Winged loosestrife, trees, bushes, tall grasses
Ottawa Natl Wildlife Ref.	<i>L. alatum</i>	50-100	No flooding	Purple and winged loosestrife, misc. grasses
Field Farm	<i>L. salicaria</i>	20-50	Periodic flood but <5cm	Purple loosestrife, tall grasses, trees, tall bushes
West Pittsfield	<i>L. salicaria</i>	20-50	Perm. flooding >30cm	Purple loosestrife, cattail
Sheffield	<i>L. alatum</i>	50-100	No flooding	Winged loosestrife, tall grasses

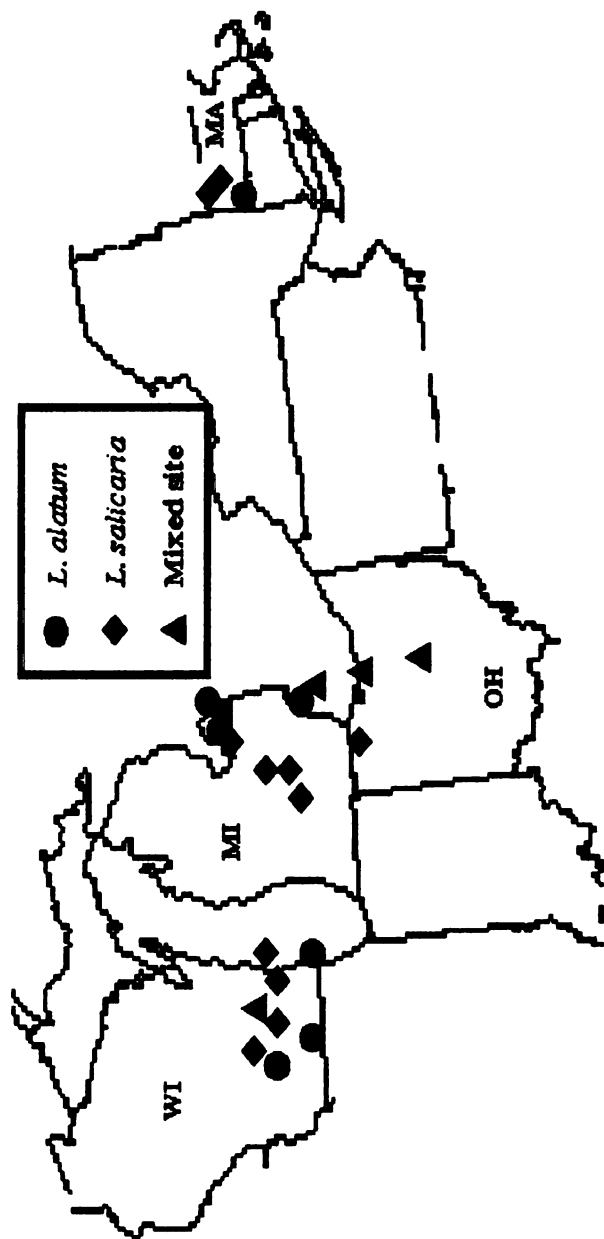


Figure 3: Locations of *Lythrum salicaria*, *L. alatum*, and mixed populations surveyed in this study. *L. salicaria* is denoted with a diamond, *L. alatum* with a circle, and mixed sites with a triangle. States are indicated by abbreviation.

Common garden experiment

Seed was collected from 8 Michigan populations (4 *L. salicaria* and 4 *L. alatum*) in late September of 2000. Four purple loosestrife and four winged loosestrife populations were selected (HIS, SFB, LLA, QWB, HIW, SFA, ASP, and WFB). These populations were chosen to represent two allopatric and two sympatric populations of each species. The sympatric populations of purple loosestrife chosen (HIS and SFB) were previously determined to have intermediate heights and leaf lengths in the field studies. A randomly selected shoot from each of 20 random clones was collected along a transect across each population. Ten flowers were chosen from each shoot, and their seed were spread onto moistened soil in a covered tray and allowed to germinate under artificial light. After germination, seedlings were transplanted to 10 cm. pots and grown in a single greenhouse at Michigan State University, East Lansing MI, under 12 hour day lengths supplemented with artificial light. They were each fertilized with 2 tablespoons Osmocote (The Scotts Company, Marysville, OH) after transplantation and watered daily. Plants were arranged on three benches in a completely randomized block design (bench being the blocking factor), and allowed to grow until flowering (approximately 8 weeks). At flowering, the same traits were measured that varied in the field (height, flower number, style length, and leaf size). Height was recorded for the tallest shoot in each plant. Number of flowers per leaf axil were counted for 10 - 20 axils in each plant and the most common number was recorded. Style was measured on a random shoot and recorded as long, mid, or short. Leaf size was measured on a randomly selected fully expanded leaf of each plant.

Univariate statistics, Analysis of Variance (ANOVA), and Principle Component Analysis on all data were performed using the SAS statistical software package (Cary, NC).

Results

All the *L. alatum* clones had the taxonomic traits considered diagnostic for *L. alatum*. Among the *L. salicaria* clones, the calyx and styly traits were always true for *L. salicaria*; however, the height of *L. salicaria* clones varied widely on a population level, from $x=74.8$ to 173.6 cm. The purple loosestrife populations HIS ($x=107.5$ cm), ONP (109.5 cm), KIL (74.8 cm) and SFB (96.7 cm) had mean heights significantly closer to typical *L. alatum* ($x= 50.4 - 68.1$ cm) than *L. salicaria* (Table 4, Figure 5). All four of these purple loosestrife populations were also sympatric to winged loosestrife. Similarly, leaf length in the *L. salicaria* populations HIS ($x= 26.4$ mm) and SFB (41.4 mm) were also much closer to the typical mean leaf length of *L. alatum* ($x= 8.0 - 22.4$ mm) than *L. salicaria* ($x= 26.4 - 78.9$ mm) (Figures 6 and 7). Length/width ratios were significantly smaller in *L. alatum* ($x= 3.33 - 3.93$) than *L. salicaria* ($x= 3.26 - 5.63$), but there were no significant differences among the individual species populations (Figures 8 and 9). A varying percentage of clones in all *L. salicaria* populations exhibited the traits thought to be diagnostic for *L. alatum*, alternate leaf placement along the stem and fewer than four flowers per leaf axil (Table 4). ANOVAS for height, leaf length, leaf width, and leaf length/width ratio are in Tables 5 and 7.

Table 4: Prevalence of *L. alatum* diagnostic traits in populations of *L. salicaria* and *L. alatum* surveyed in North America. For qualitative traits, the percentage of *L. salicaria* carrying *L. alatum* traits is reported. For quantitative traits, the mean and range of each trait are denoted.

Species	State	Pop.	<4 flowers	Alternate Leaf Placement	Height mean	Leaf length Mean	Leaf width mean	Leaf length/width ratio mean
<i>L. salicaria</i>	Michigan	LLA	22%	18%	173.6 (+/- 9.9)	48.0(+/-9.35)	8.8(+/-1.28)	5.47(+/-0.81)
<i>L. salicaria</i>	Michigan	QWA	26%	16%	146.3 (+/-12.0)	75.7(+/-7.56)	16.3(+/-1.78)	4.85(+/-0.74)
<i>L. salicaria</i>	Michigan	QWB	30%	12%	126.3 (+/-15.6)	62.1(+/-9.23)	11.7(+/-1.73)	5.43(+/-0.68)
<i>L. salicaria</i>	Michigan	SFB	9%	7%	96.7(+/-11.9)	41.4(+/-7.25)	9.0(+/-1.36)	4.71(+/-0.60)
<i>L. salicaria</i>	Michigan	CIA	20%	17%	146.4(+/-8.7)	52.6(+/-6.03)	12.1(+/-1.30)	4.49(+/-0.64)
<i>L. salicaria</i>	Michigan	SRA	30%	8%	183(+/-15.2)	78.9(+/-9.10)	14.4(+/-1.54)	5.59(+/-0.55)
<i>L. salicaria</i>	Michigan	HIS	27%	33%	107.5(+/-10.2)	26.4(+/-4.14)	8.2(+/-0.87)	3.26(+/-0.46)
<i>L. salicaria</i>	Ohio	ONP	16%	14%	109.5(+/-11.4)			
<i>L. salicaria</i>	Ohio	KIL	0%	0%	74.8(+/-9.0)			
<i>L. salicaria</i>	Wisconsin	DCR	8%	15%	137.5(+/-17.6)	69.3(+/-4.60)	13.5(+/-1.71)	5.31(+/-0.47)
<i>L. salicaria</i>	Wisconsin	BRI	10%	13%	137.0(+/-16.0)	54.8(+/-4.83)	10.3(+/-1.22)	5.49(+/-0.63)
<i>L. salicaria</i>	Wisconsin	WKS	8%	16%	136.7(+/-15.0)	58.2(+/-5.34)	11.8(+/-1.15)	5.06(+/-0.55)
<i>L. salicaria</i>	Wisconsin	SCC	0%	5%	129.9(+/-16.3)	68.7(+/-6.45)	13.4(+/-2.07)	5.49(+/-0.81)
<i>L. salicaria</i>	Wisconsin	TLP	26%	13%	131.4(+/-19.5)	67.2(+/-6.21)	12.3(+/-1.21)	5.63(+/-0.68)
<i>L. salicaria</i>	Massachusetts	FFA	17%	17%	121.8(+/-10.0)			
<i>L. salicaria</i>	Massachusetts	WPI	7%	36%	128.2(+/-9.5)			
<i>L. alatum</i>	Michigan	ASP	100%	100%	68.1(+/-7.1)	11.4(+/-1.92)	2.9(+/-0.51)	3.97(+/-0.39)
<i>L. alatum</i>	Michigan	HIW	100%	100%	65.0(+/-6.4)	8.0(+/-0.83)	2.4(+/-0.25)	3.40(+/-0.35)
<i>L. alatum</i>	Michigan	RIR	100%	100%	67.0(+/-5.7)	12.6(+/-1.18)	3.3(+/-0.41)	3.93(+/-0.33)
<i>L. alatum</i>	Michigan	SFA	100%	100%	60.4(+/-4.9)	14.0(+/-2.66)	3.6(+/-0.58)	3.84(+/-0.37)
<i>L. alatum</i>	Michigan	WFB	100%	100%	53.8(+/-5.1)	11.0(+/-0.98)	3.0(+/-0.40)	3.88(+/-0.43)
<i>L. alatum</i>	Ohio	KTA	100%	100%	50.4(+/-4.5)			
<i>L. alatum</i>	Ohio	KTB	100%	100%	53.3(+/-5.8)			
<i>L. alatum</i>	Ohio	ONW	100%	100%	64.9(+/-6.6)			
<i>L. alatum</i>	Ohio	KIW	100%	100%	53.8(+/-5.9)			
<i>L. alatum</i>	Wisconsin	NCO	100%	100%	53.8(+/-4.9)	21.0(+/-2.15)	6.0(+/-0.79)	3.59(+/-0.30)
<i>L. alatum</i>	Wisconsin	JAN	100%	100%	55.3(+/-6.8)	22.4(+/-3.07)	6.1(+/-0.76)	3.77(+/-0.46)
<i>L. alatum</i>	Wisconsin	HPR	100%	100%	53.3(+/-6.5)	15.1(+/-1.93)	4.3(+/-0.52)	3.51(+/-0.19)
<i>L. alatum</i>	Wisconsin	TLW	100%	100%	55.7(+/-5.9)	16.5(+/-2.02)	5.0(+/-0.71)	3.33(+/-0.18)
<i>L. alatum</i>	Massachusetts	SHE	100%	100%	51.3(+/-5.1)			

Table 5: ANOVA of plant height in 14 populations of *Lythrum alatum* and 16 populations of *L. salicaria* in North America.

Source	DF	SS	MS	F	P	Var. Comp ¹	% Var. ²
Replication	50	91129.365	1822.587	4.91	<.0001	81.08681	2
Species	1	1009008.099	1009008.099	2718.42	<.0001	3069.6	78
Pop(species)	28	346023.656	12357.988	33.29	<.0001	547.67895	14
Error	915	339624.253	371.174			218.3909	6
Total	994	2344528.422				3916.7567	100

¹Variance in each component

²% of variance in each component

Table 6: ANOVA of leaf length, width, and length/width ratio in 9 populations of *Lythrum alatum* and 12 populations of *L. salicaria* in North America.

Length:

Source	DF	SS	MS	F	P	Var. Comp ¹	% Var. ²
Replication	49	7509	153	1.04	0.4104	-2.33	0.0
Species	1	185761	185761	1267.62	<0.001	978.02	77.0
Pop(Species)	19	83526	4396	29.71	<0.001	161.63	12.7
Error	571	84487	147			132.69	10.4
Total	640	453791				1270.01	100.0

Width:

Source	DF	SS	MS	F	P	Var. Comp ¹	% Var. ²
Replication	49	275	5.6	0.89	0.6794	0.002	0.0
Species	1	5831	5831	927.58	<0.001	30.36	72.7
Pop(Species)	19	2920	153	24.45	<0.001	6.49	15.5
Error	571	3589	6.2			4.91	11.8
Total	640	15529				41.762	100.0

Ratio:

Source	DF	SS	MS	F	P	Var. Comp ¹	% Var. ²
Replication	49	66	1.34	1.01	0.4638	-0.01	0.0
Species	1	182	181	136.16	<0.001	0.9	37.3
Pop(Species)	19	127	6.7	5.01	<0.001	0.12	5.0
Error	571	762	1.34			1.4	58.1
Total	640	1211				2.41	100.0

¹ Variance in each component

²% of variance in each component

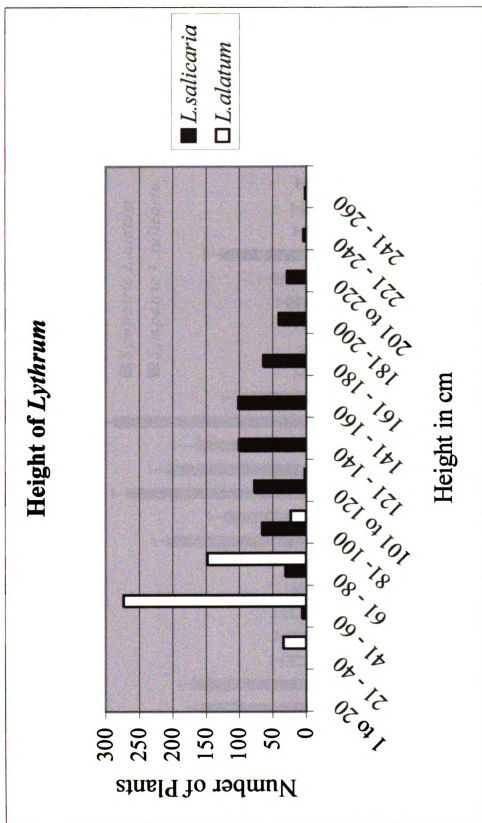


Figure 4: Distribution in height of *Lythrum salicaria* and *L. alatum* from 30 North American populations. *L. salicaria* is denoted in black, *L. alatum* in white.

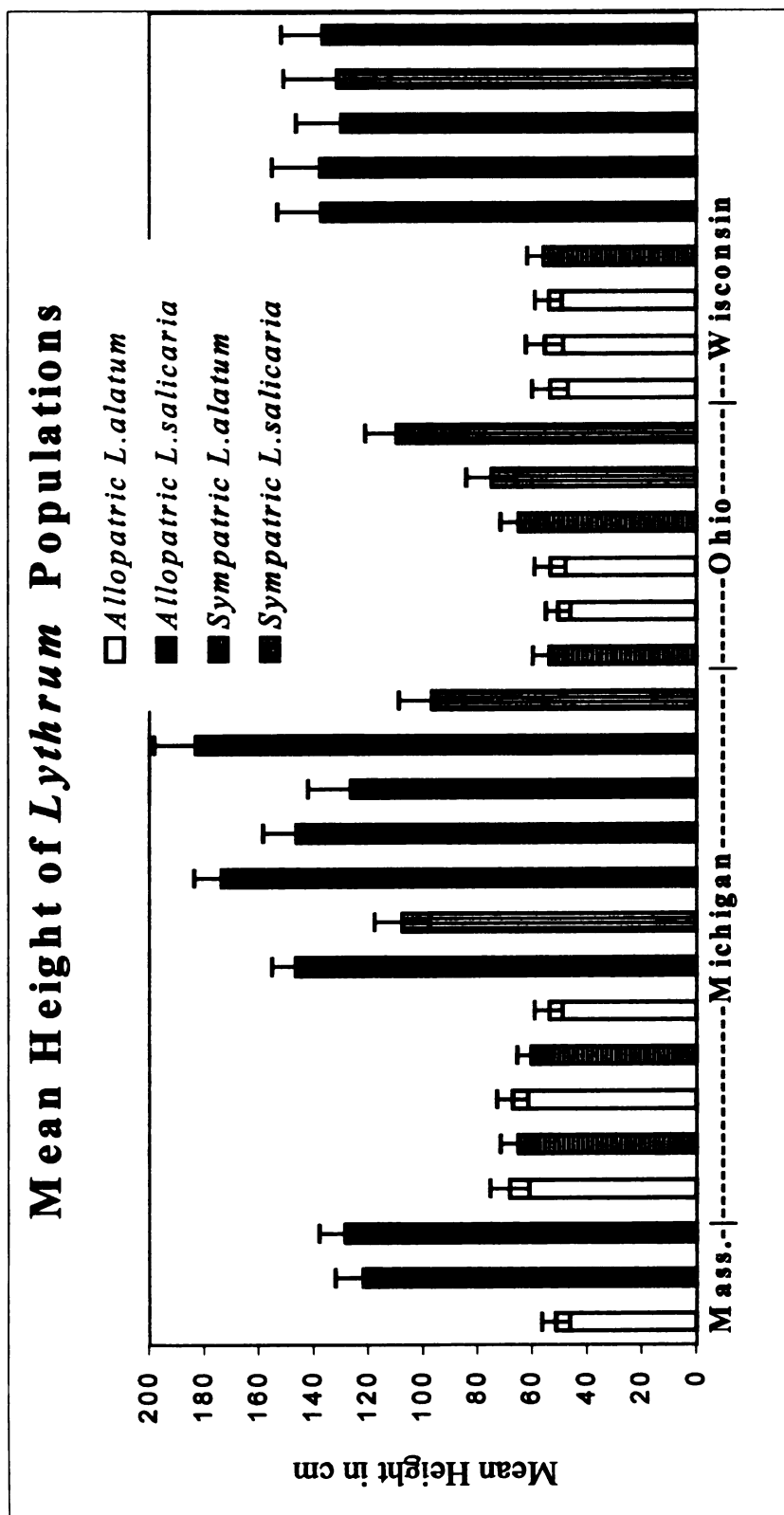


Figure 5: Mean heights of *Lythrum salicaria* and *L. alatum* populations in Massachusetts, Michigan, Ohio, and Wisconsin. Populations from the same state are placed next to each other. *L. salicaria* is denoted in black, *L. alatum* in white, and states are denoted along the x-axis. Striped bars indicated sympatric populations of *L. salicaria* and *L. alatum*.

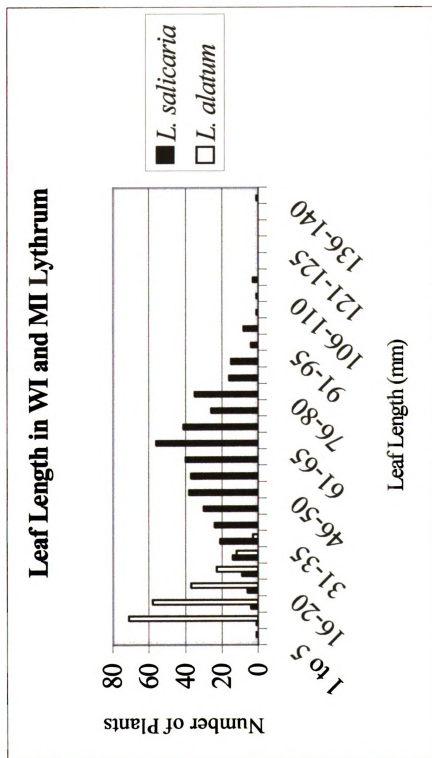


Figure 6: Distribution of leaf length of *Lythrum salicaria* and *L. alatum* from 30 North American populations surveyed. *L. salicaria* is denoted in black, *L. alatum* in white.

Leaf Length in MI and WI Lythrum

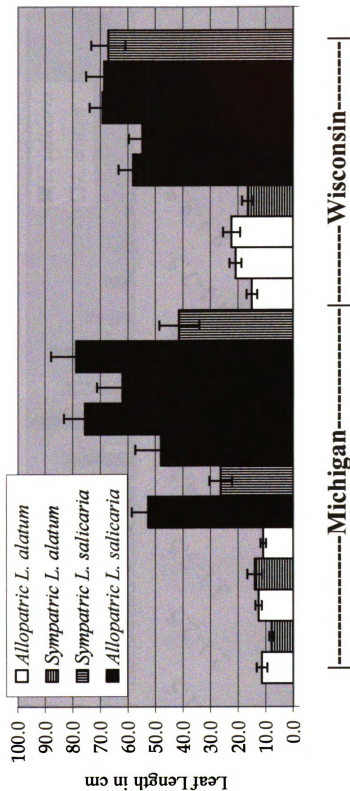


Figure 7: Mean leaf length of *Lythrum salicaria* and *L. alatum* in different populations in Michigan and Wisconsin. Populations from the same state are placed next to each other. *L. salicaria* is denoted in black, *L. alatum* in white, and states are denoted along the x-axis. Striped bars indicate sympatric populations of *L. salicaria* and *L. alatum*.

Leaf Ratio in MI and WI Lythrum

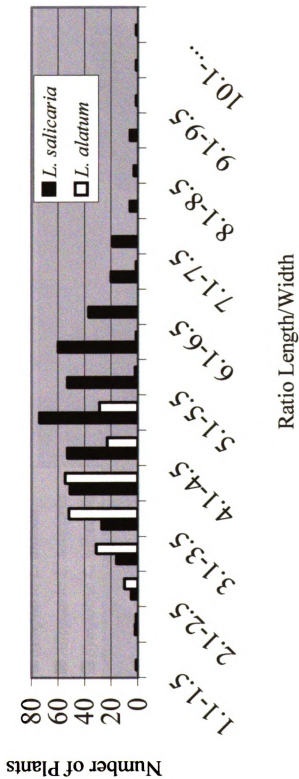


Figure 8: Distribution of leaf ratios (length/width) of *Lythrum salicaria* and *L. alatum* from 21 North American populations surveyed. *L. salicaria* is denoted in black, *L. alatum* in white.

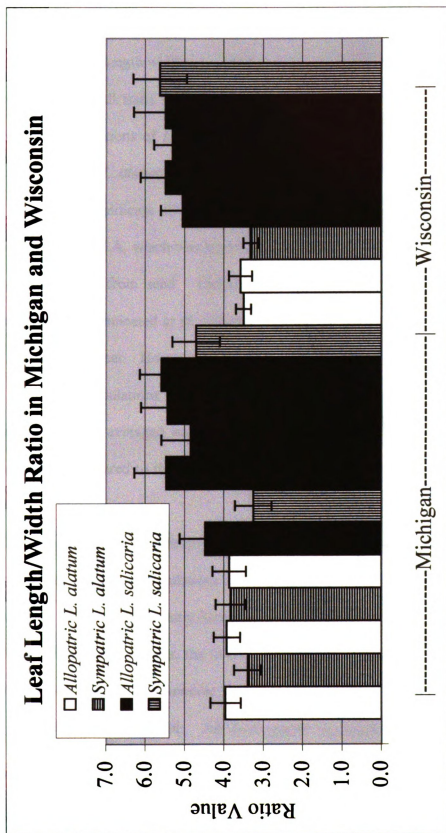


Figure 9: Mean leaf ratios (length/width) of *Lythrum salicaria* and *L. alatum* in 12 Michigan and 9 Wisconsin populations. Populations from the same state are placed next to each other. *L. salicaria* is denoted in black, *L. alatum* in white, and states are denoted along the x-axis. Striped bars indicate sympatric populations of *L. salicaria* and *L. alatum*.

Each of the 'intermediate' populations of purple loosestrife was compared to allopatric purple and winged loosestrife individually for height (Figure 10), leaf length (Figure 11), and leaf length/width ratio (Figure 12). Principle component analysis was then used to look at all traits simultaneously, and the Harsen's Island (HIS) and Sheep Farm B (SFB) populations of *L. salicaria* appeared to be intermediate between all the other *L. salicaria* and *L. alatum* populations (Figure 13).

There were significant differences ($P < 0.05$) in the rate of germination of seeds from the population LLA, which was significantly higher than the other purple loosestrife populations collected from seed. Unfortunately, none of the *L. alatum* populations produced seed that germinated at all. This may be due to a dormancy requirement for *L. alatum* that was not met. However, germination of HIS was still lower than the other purple loosestrife populations collected, as in the field, with some families having germination rates that averaged less than 1 seedling per flower, but this trend was non-significant when compared to the other populations. ANOVA of germination is in Table 7.

HIS and SFB had significantly shorter heights and smaller leaf values than the other two purple loosestrife populations in the greenhouse (Table 8). Although absolute values of traits differed significantly between naturally occurring and greenhouse grown plants from the same populations, the overall trend of relationships between populations for each trait was preserved. However, few *L. salicaria* plants carried the other two *L. alatum* traits found in the field. All four purple loosestrife populations exhibited overwhelming numbers of plants (>95%) with opposite leaf placement vs. either alternate

(the diagnostic for winged loosestrife) or whorled, and four or more flowers per leaf axil compared to fewer than two flowers (the diagnostic for winged loosestrife).

Table 7: ANOVA of seedlings produced per flower in four populations of *Lythrum salicaria* in Michigan. Two of the populations were sympatric to *L. alatum*, two were allopatric. Number of seedlings was determined for 10 flowers each of 10 clones from each population.

Source	DF	SS	MS	F	P
Replication	9	5016	557	1.05	0.4285
Population	3	46308	15436	29.08	<0.001
Error	27	14332	530		
Total	39	65657			

Table 8: Height and leaf characteristics of four populations of *Lythrum salicaria* grown from seed in a common greenhouse at Michigan State University, East Lansing, MI. Two of the populations were sympatric with *L. alatum* [Harsen's Island (HIS) and Sheep Farm B (SFB)], and two were allopatric [Lake Lansing (LLA) and Quanicassee B (QWB)]. Values are given for natural populations and seedlings grown in a greenhouse. Mean values were compared using SAS (Cary, NC). Bold values are significant at P=0.05.

Natural populations:

Greenhouse populations:

Height:

Mean		LLA	QWB	SFB
107.5	HIS	<.0001	0.0118	0.151
173.6	LLA		<.0001	<.0001
126.3	QWB			<.0001
96.7	SFB			

Mean		LLA	QWB	SFB
67.9	HIS	<.0001	<.0001	0.508
87.7	LLA		<.0001	<.0001
77.3	QWB			<.0001
69.2	SFB			

Leaf Length:

Mean		LLA	QWB	SFB
26.4	HIS	<.0001	<.0001	0.0028
48	LLA		0.0003	0.0547
62.1	QWB			<.0001
41.4	SFB			

Mean		LLA	QWB	SFB
69.5	HIS	0.0005	0.0064	0.0185
76.2	LLA		0.3043	<.0001
74.6	QWB			<.0001
65	SFB			

Leaf Width:

Mean		LLA	QWB	SFB
8.2	HIS	0.4939	<.0001	0.3485
8.8	LLA		<.0001	0.7029
11.7	QWB			<.0001
9	SFB			

Mean		LLA	QWB	SFB
21.1	HIS	0.0929	0.0022	0.2828
19.9	LLA		<.0001	0.4563
23.4	QWB			<.0001
20.4	SFB			

Leaf Length/Width Ratio:

Mean		LLA	QWB	SFB
3.3	HIS	<.0001	<.0001	0.0006
5.5	LLA		0.9139	0.0079
5.4	QWB			0.0236
4.7	SFB			

Mean		LLA	QWB	SFB
3.4	HIS	<.0001	0.7588	0.9574
4.1	LLA		<.0001	<.0001
3.3	QWB			0.7596
3.4	SFB			

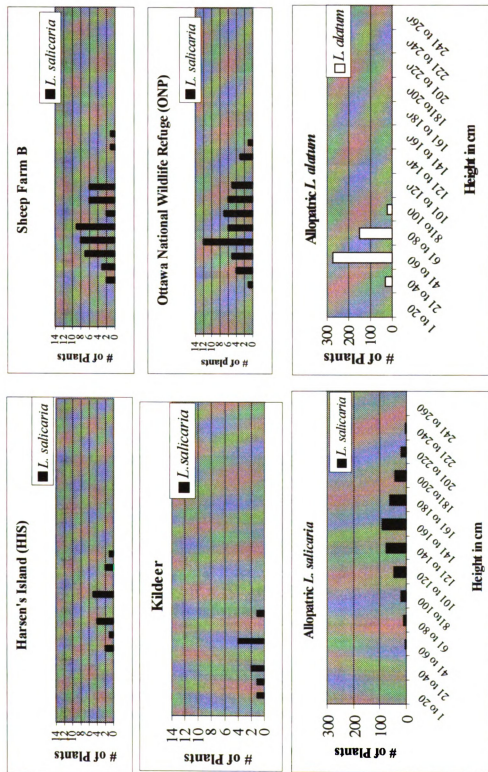


Figure 10: Distribution in height of all allopatric *Lythrum salicaria* and *L. alatum* populations (bottom panels) compared to four sympatric populations of *L. salicaria* (Harsen's Island, Sheep Farm B, Ottawa National Wildlife Refuge, and Kildeer) skewed towards *L. alatum* morphology.

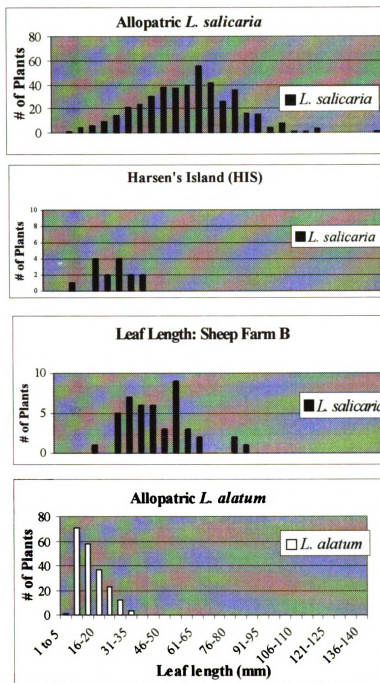


Figure 11: Distribution in leaf length in all allopatric *L. salicaria* and *L. alatum* (top and bottom frames) compared to two sympatric populations of *L. salicaria* (Harsen's Island, Sheep Farm B) skewed towards *L. alatum* morphology.

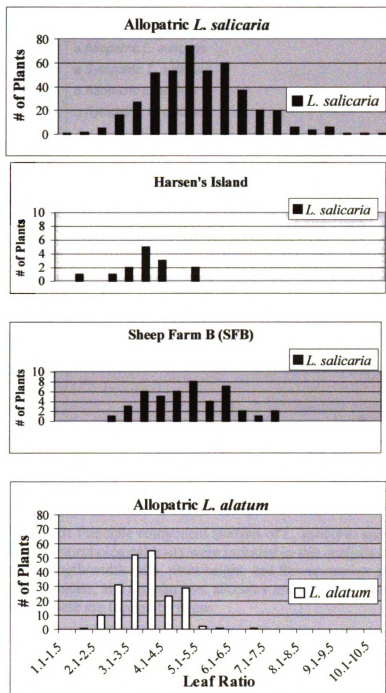


Figure 12: Distribution in leaf length/width ratios in all allopatric *Lythrum salicaria* and *L. alatum* (top and bottom frames) compared to leaf length/width ratios in two sympatric populations of *L. salicaria* (Harsen's Island and Sheep Farm B) skewed towards *L. alatum* morphology.

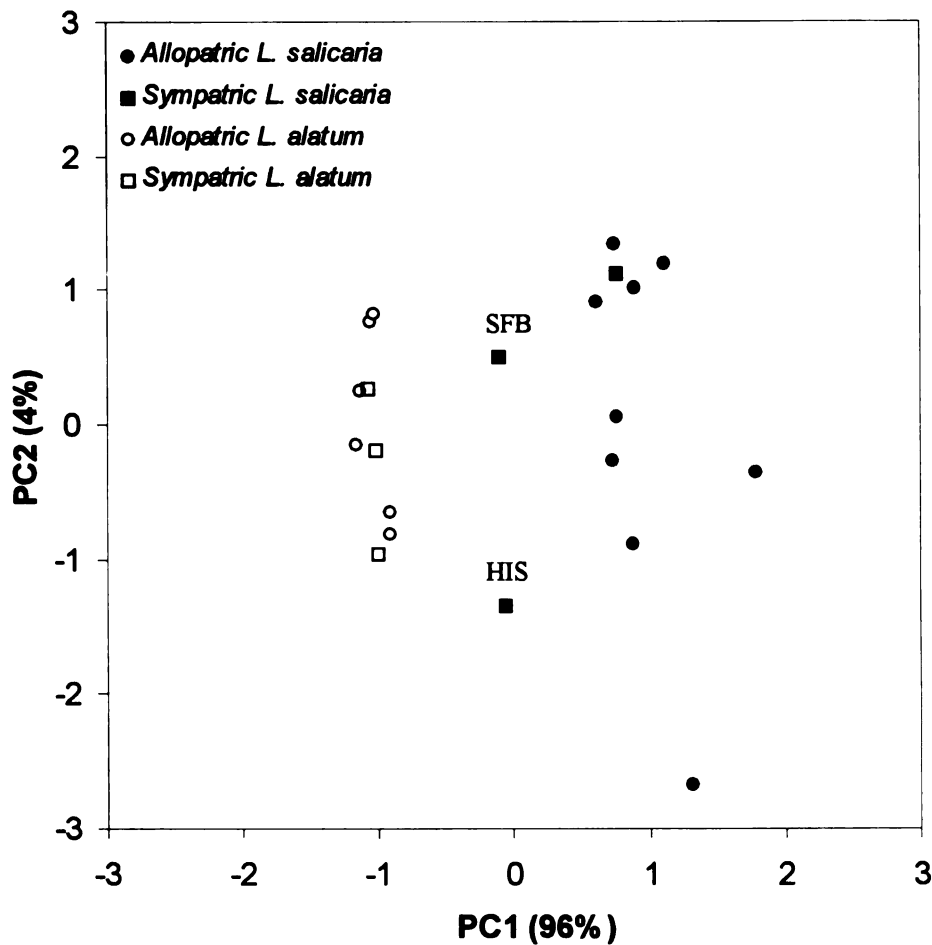


Figure 13: Principle component analysis of *L. salicaria* and *L. alatum* populations. All variable morphological traits were included in this analysis (leaf placement, length, width, length/width ratio, plant height, and flower number per leaf axil). Region of origin, species, and sympatry vs. allopatry are denoted. Two populations which appear intermediate are labeled by name.

Discussion

Most of the North American *L. salicaria* carried at least a few *L. alatum* traits that have not been described in Eurasian *L. salicaria*. This suggests that the two species may have hybridized in North America, supporting the morphological evidence in Minnesota

described by Anderson and Ascher (1994), but on a much larger geographic scale. None of the *L. alatum* populations carried any of the *L. salicaria* traits, which is not surprising, since the theoretical hybrid would be tetraploid and only capable of backcrossing to purple loosestrife populations, unless triploids exist (which is rare in plant species). Screening 20 random winged loosestrife plants, unreduced gametes were produced at a rate of 0.35% (70 unreduced gametes in 20,000 pollen grains screened, data not shown). Because the *L. alatum* traits appear across the entire range of *L. salicaria*, such a hybridization would have had to occur several times throughout the history of the two species, or the hybridization occurred early in the establishment of purple loosestrife and spread across North America.

The possibility of multiple hybridizations is supported by the two *L. salicaria* populations (HIS and SFB) which were intermediate in height and leaf ratio between the typical *L. salicaria* and *L. alatum* populations in both the native field and common greenhouse. This intermediacy may be evidence that they are hybrid swarms, suggesting that interspecific hybridization is ongoing, although it remains possible that purple loosestrife is evolving a more xeric ecotype favoring winged loosestrife habitats. Interestingly, most of the purple loosestrife plants grown in the common greenhouse had the leaf placement and flower numbers typical of purple loosestrife, even though plants of both typical purple and typical winged loosestrife traits were collected in the field for use in this study. These traits must have a strong environmental component.

Purple loosestrife was not considered invasive until well after its establishment in North America, specifically when it began to form monospecific stands in the floodplain pastures of the St. Lawrence River in Quebec in the 1930's (Louis-Marie, 1944). The

subsequent invasions of purple loosestrife have followed a distinct pattern, a few plants appear in a region, the plant stays a very minor member of the community for 20 - 40 years, then in a very short interval (approximately 5 years) becomes the dominant member of the wetland community (Stuckey, 1980). Ellstrand and Schierenbeck (2000) have suggested that some exotic invasive plants only become invasive after hybridization with a native species, and one of the examples they site is *L. salicaria*, using isozyme evidence provided by Strefeler *et al.* (1996). This isozyme evidence was not conclusive, which is why I chose to do further, more in depth studies. My morphological data lends further support to the hybridization hypothesis. The delays in invasion may occur because purple loosestrife must gain and reassort genetic material from winged loosestrife to help it adapt to new North American habitats.

Not knowing the genetic basis for any of the morphological traits studied, it remains possible that invasiveness in purple loosestrife arose through genetic divergence without interspecific hybridization, or new mutations have arisen in purple loosestrife genetic material after its arrival in North America. It is also possible that an ecotype is evolving in purple loosestrife that is better adapted to the drier habitats of winged loosestrife through reassortment of its own genetic variability. In the next chapter, we will use AFLP markers to show that, indeed, ecological differentiation without interspecific hybridization is the most likely basis of *L. salicaria's* ecological success in North America.

Knowing whether hybridization or ecological differentiation has played the primary role in purple loosestrife's success in North America could be critical to determining methods of control. Much interest has been devoted to methods for

biological control of purple loosestrife, specifically searching for herbivores that feed on purple loosestrife in its native Eurasia (Blossey, 1995). Based on host specificity, three herbivore species have been chosen: *Galerucella californiensis* (L.) and *G. pusilla* (Duft.), leaf feeding beetles, and *Hylobius transversovittatus* Goeze, a root feeding weevil (Kok *et al.* 1992a, b). In no-choice tests, the leaf feeding beetles were found to oviposition as well as feed on winged loosestrife, although in choice tests, purple loosestrife was preferred (Kok *et al.*, 1992).

If hybridization has taken place between purple and winged loosestrife, it could have altered the susceptibility of purple loosestrife to Eurasian predators. Two effects are possible. First, the hybrid could be less susceptible to Eurasian predators than purple loosestrife is in Eurasia and pass that resistance on to purple loosestrife populations. Alternatively, the hybrid could provide an evolutionary “bridge” for the Eurasian predators to adapt to feeding on winged loosestrife. Documentation of hybridization occurring in the wild between these species will allow environmental managers to make more informed decisions about biological control agents. If purple loosestrife in North America becomes more resistant to Eurasian predators than the Eurasian purple loosestrife, much time and money could be wasted breeding and releasing insects that will have little or no effect. Additionally, if the Eurasian predators are more effective predators of F₁ hybrids, our native species may be put at risk.

CHAPTER 3
MOLECULAR DIVERSITY IN EUROPEAN AND NORTH AMERICAN
LYTHRUM

Introduction

Lythrum salicaria became an invasive, noxious weed after arriving in North America, but it is not considered invasive in its native environment (Batra *et al.* 1986). One possibility for how it became invasive is that it hybridized with a close relative in North America, such as *L. alatum*, and gained genes that made it better adapted to this new environment. In Chapter 2, I tested this hypothesis by searching for morphological evidence of hybridization. By examining a variety of purple loosestrife populations across the northeastern United States, I found several traits that are not present in European populations of purple loosestrife but are found in North American winged loosestrife. These unique morphs found in North American purple loosestrife suggest hybridization between the two species (Chapter 2; Anderson and Ascher, 1994; Strefeler *et al.*, 1996). In support of this, I identified intermediate sized *L. salicaria* where the two species grow sympatrically and could have hybridized (Chapter 2).

However, it is also possible that the genetic variability already present in *L. salicaria* has reassorted without hybridization into new morphologies and adaptive types. This might have been enhanced by its polyploid nature. There are several aspects of polyploids that can contribute to their success. They can hybridize with their diploid progenitors via unreduced gametes, or form recurrently from multiple parent populations.

Their polyphyletic origin also can lead to the incorporation of high levels of genetic diversity (Soltis and Soltis, 2000). As a result, polyploids have higher levels of heterozygosity than their diploid progenitors and less inbreeding depression. The high level of genetic variability found in polyploids can be further assorted through genomic rearrangement, and in the case of autopolyploids, tetrasomic inheritance. All of these factors are quite applicable to *L. salicaria* and may have contributed its success as it encountered new habitats across North America.

We used amplified fragment length polymorphism (AFLP) markers to further analyze the relationship between *L. salicaria* and *L. alatum*, and search for evidence of hybridization. We screened all of our North American and European populations of *L. salicaria* and *L. alatum* with 5 primer pairs, and then evaluated eight Michigan populations (selected for allopatry or sympatry between the two species) with an additional 18 primer pairs, to determine the genetic relationship between *L. salicaria* and *L. alatum* in North America, and North American *L. salicaria* to European *L. salicaria*. We found that there is no molecular evidence of hybridization in North America between the two species. However, we did find that North American *L. salicaria* has differentiated from European *L. salicaria*, and this differentiation may be the real reason that *L. salicaria* has been so successful in North America.

The utility of AFLP analysis

Molecular markers are useful in determining genetic evolution and changes within an organism. Morphological traits are controlled by a wide range of gene numbers and frequently have large environmental components, making them often misleading in

evolutionary studies. By using molecular markers, the changes across an entire genome can be studied with no extra weight assigned to any single trait or change (Tohme *et al.*, 1996).

Amplified fragment length polymorphism (AFLP) analysis, as developed by Vos *et al.* (1995), has fast become a favored method of DNA marker analysis. This technique combines the reliability of RFLP markers with the time efficiency of PCR-based markers and yields at least 10 times more genetic loci per primer pair than RFLP or RAPD analysis in many crop species (Tohme *et al.*, 1996). The resulting DNA fingerprint yields a large number of genetic markers, and the multiplex ratio (the number of information points analyzed per experiment) is higher than for other commonly used markers (RFLPs, RAPDs, or SSRs) (Powell *et al.*, 1996). AFLPs have been used in many species to study evolution and genetic relationships, with some examples including soybean (Maughan *et al.*, 1996), lettuce (Hill *et al.*, 1996), tea (Paul *et al.*, 1997), tef (Bai *et al.*, 1999), olive (Angiolillo *et al.*, 1999), and cassava (Roa *et al.*, 1997).

AFLP analysis has been used to determine both genetic similarity and average amount of polymorphism in a number of different species groups. Comparing different cultivated and natural olive species, Angiolillo *et al.* (1999) found the average percentage of polymorphism to be between 51% for one primer combination and 83% for another. Vroh *et al.* (1999) compared upland cotton to the wild species and found that genetic similarity between the two groups ranged from 29.5% to 43.2%. Within closely related tef accessions, however, Bai *et al.* (1999) detected a very low level of polymorphism (18%), but still detected differentiation within these closely related individuals. One common denominator for AFLP analysis is its ability to discern relationships in even the

closest of related individuals; this made it ideal for searching for hybridization between *L. salicaria* and *L. alatum*, because the closeness of the relationship between the two species was not previously known, and could be quite high.

Materials and Methods

Plant collection

Plant tissue was collected from each clone of *L. salicaria* and *L. alatum* surveyed in the previous morphological studies. Approximately 10 young green leaves were collected from every clone in early to late June before full growth and flowering occurred. The leaves were placed in a ziploc bag with enough silica gel to completely cover the leaf tissue. Bags were then labeled as to population and individual, sealed, and stored at room temperature for several months until the DNA was extracted. Number of individuals collected from each population corresponds with numbers surveyed in the previous morphological study, outlined in Table 4.

European samples of *L. salicaria* were obtained from Dr. Bernd Blossey at Cornell University. Seed had been collected from 11 European populations and grown in a common garden at Cornell University. These populations originated from Germany, England, Ireland, Austria, and Finland. Four to six leaves were collected from five clones in each European population, covered with silica gel, and shipped to Michigan State University.

Six cultivars of *L. salicaria* were purchased based on availability. Three clones each of Robert, Roseum Superbaum, and Purple Spires were purchased from Wrenwood

of Berkeley Springs (Berkeley Springs, WV). Three clones each of Happy, Morden's Gleam, and Morden's Pink were purchased from Bluestone Perennials (Madison, OH). New leaves were collected from each plant the morning that DNA was to be extracted.

DNA extraction

DNA was extracted following the protocol of Doyle and Doyle (1990) with modification. Three to four leaves from each clone (approximately 1g) were placed in a sterile mortar, liquid nitrogen was added, and the leaves were then ground to a fine powder. CTAB buffer [2% w/v hexadecyltrimethylammonium bromide (CTAB), 1.4 M NaCl, 0.2% v/v 2-mercaptoethanol, 20 mM EDTA, 100 mM Tris-HCl (pH 8.0)] was added 800 uL at a time twice for a total of 1600 uL, and the material was ground into a slurry. The resulting homogenate was poured into two 1.6 mL tubes and gently mixed by inversion. The tubes were placed in a water bath at 60°C for 30 – 60 minutes, with periodic mixing by inversion. The tubes were then removed from the water bath and allowed to return to room temperature. Equal volumes of Chloroform: Isoamyl alcohol (24:1) were added to each tube (approximately 800 uL), and the tubes were inverted to gently mix them. The tubes were centrifuged at 14,000 x g for 5 minutes, and then the top aqueous layer was transferred to a new tube. Equal volume (approximately 800 uL) of ethanol acetate (4 % 3 M NaAc and 96 % EtOH) was added to each tube, gently mixed, then the DNA was allowed to precipitate for 10 to 30 minutes at room temperature. The tubes were centrifuged at 14,000 x g for 5 minutes, and the supernatant was gently poured or pipetted off, saving the pellet. The pellet was washed in 70% EtOH, and then spun for 5 minutes at 14,000 x g. The tube was then left open

overnight in a sterile laminar flow hood, to allow the pellet to dry thoroughly, before being resuspended in 50 uL TE.

DNA preparation for AFLP analysis

Gibco BRL-Life Technology (Rockville, MD) reagents were used for all digestions, ligations, and other AFLP preparations and experiments. First, 200 ng of DNA were digested in 5 uL of 5X Restriction digest buffer, 2 uL of EcoR1/Mse 1 restriction enzyme solution, and an appropriate amount of autoclaved ddH₂O to bring the final volume to 20 uL per reaction. This solution was held at 37°C for 2 hours, then the enzymes were deactivated at 70°C for 15 minutes. Next, ligations were performed by adding 19.2 uL of the Adaptor/Ligation solution (containing Gibco patented extensions to ligate to the sticky ends of our digested DNA) and 0.8 uL of T4 DNA ligase directly to each digestion. This was incubated at 20°C for 3 hours. After ligations, 10 uL of each solution was pipetted into a new tube and diluted 1:10 with 90 uL of 0.1 M Tris/EDTA (TE). The remaining ligation solution was stored at -20°C. The diluted ligation solution was then used for the pre-amplification reaction.

For each preamplification reaction, 20.0 uL of Pre Amp Primer Mix I, 2.5 uL 10X PCR Buffer, 1.0 uL 50 mM MgCl₂, 0.5 uL Taq DNA polymerase, and 0.5 uL of the diluted template DNA from the ligation reaction were mixed together in a tube, and then held in a Perkin Elmer 9600 PCR machine for 20 cycles of the following program: 94°C for 30 seconds, 56°C for 1 minute, and 72°C for 1 minute. The program finished with 72°C for 10 minutes and then a 4°C soak. 5 uL of the preamplified reaction was then run on a 2% Tris, Boric Acid, and EDTA (TBE) gel, and photographed to score for relative

DNA concentration. The brightness of the DNA smear was used to determine the final dilution of the sample. Bright, clearly visualized pre-amplified reactions were diluted 1:20; while faint, identifiable but not clearly visualized pre-amplified reactions were diluted 1:10, and absent smears were left undiluted. These diluted samples were then stored at -20°C pending AFLP amplification reactions.

AFLP reactions

Initial screens included 10 individuals from each of the North American purple and winged populations, as well as 5 individuals from each European population and 3 samples of each cultivar. The 320 samples were divided into four sets of 80, with two positive controls (*Arabidopsis* and Tomato DNA) and two negative controls (ddH₂O) included. Five primer pairs were selected to use in this study, which are listed in Table 9. Each reaction consisted of 0.5 uL EcoR1 primer, 4.5 uL Mse1 primer and dNTP mixture, 2.5 uL 10X PCR buffer, 0.8 uL 50 mM MgCl₂, 0.5 uL Taq polymerase, 13.7 uL ddH₂O, and 2.5 uL diluted template DNA for a total reaction volume of 25 uL. Reactions were performed in a Perkin Elmer 9600 PCR thermocycler set with the following parameters: 35 cycles of 94°C for 30 seconds, 56°C for 30 seconds, 72°C for 1 minute, then 72°C for 10 minutes and a 4°C soak. The reaction solutions were then stored at -20°C until being run on a gel.

Eight populations of *Lythrum* in Michigan were selected for further analysis: 1) four populations of *L. salicaria*, two sympatric with *L. alatum* and two allopatric, and 2) four populations of *L. alatum*, two sympatric with *L. salicaria* and two allopatric. The sympatric populations of *L. salicaria* were chosen because they were shorter and had

smaller leaves than the other *L. salicaria* populations, even in the greenhouse. Five samples from each of the species populations were analyzed with 18 primer pairs (Table 10) following the above protocol.

Polyacrylamide gel electrophoresis

A 5% acrylamide gel (12% acrylamide:bis, 1X TBE, 40X Urea w/v) was poured into a BioRad Sequi-Gen GT Sequencing Cell (Hercules, CA) and polymerized by adding 700 μ L 10X ammonium persulfate and 200 μ L TEMED per 150 mL acrylamide solution. Formamide buffer containing xyleneol and bromphenol indicators were added to the DNA reaction solutions, and the samples were denatured by heating to 96°C for 5 minutes, then stored on ice to preserve denaturation while loading into the gel. The gel was loaded through a 96 well 0.4 mm comb, with Gibco 10 bp and 25 bp ladders on both ends. The gel was run at 85 W with a variable voltage (1500 – 1700 average) for approximately 3 hours (when the xyleneol color marker was approximately 10 cm from the bottom of the gel). One glass plate was then removed from the gel, and the gel was fixed, stained and developed on the other glass plate.

Silver staining technique

Fix/Stop (10% acetic acid), staining (1% silver nitrate and 0.6% formaldehyde), and developing (0.03g/mL sodium carbonate, 0.6% formaldehyde, and 0.002 mg/ml sodium thiosulfate) solutions were prepared from the protocol in the Promega kit (Madison, WI). The gel was placed in the fix/stop solution for 20 minutes (until the dye band disappeared), and washed three times in ddH₂O for 2 minutes each. The gel was

then shaken in the staining solution for 1 hour, washed in ddH₂O for 10 seconds, and placed in ice cold developing solution for 5 minutes, until bands appeared and were clearly identifiable, but background staining was still low. The reaction was then stopped by the addition of the fix/stop solution, the gel was shaken for 5 minutes, and rinsed in ddH₂O for 5 minutes. The gel was air dried overnight.

Exposure of APC Film

The gel was placed face up on a light box. Under red light, the film was aligned on the gel. The white light box was then turned on for 50 – 180 seconds, depending the darkness of the gel bands and background staining. The film was developed using the Kodak X-omat processor (Rochester, NY).

Scoring the gel

The gel was placed on a white light box and bands were scored for presence or absence in each lane. Results were compiled for every band that was clearly scorable, based on intensity of the band and smearing of the gel samples. This process was repeated for every primer combination run in both experiments.

Statistical Analyses

SAS (Cary, NC) and NTSYSpc (Setauket, NY) were used for all statistical analyses, including Cluster Analysis and Principle Component Analysis. The first three principle components were then represented graphically (Figures 14 and 16). Cluster analysis was performed with UPGMA similarity coefficients.

Results

Study with all populations

Five primer pairs were used to study 10 individuals from each population, as well as five individuals from each European population and three samples from each cultivar. Sixty-four total fragments were observed, with 17 of those unique to *L. salicaria* and 12 unique to *L. alatum*. Of the remaining 35 fragments, 23 were monomorphic in all species, and 12 were polymorphic. However, no fragment appeared in both North American *L. salicaria* and *L. alatum* that was not also present in some proportion of the European *L. salicaria* (Table 9).

Three distinct groups were observed in the principle component analysis (Figure 14): *L. salicaria* from North America, *L. salicaria* from Europe, and *L. alatum*. The cultivars formed a distinct subgroup within the European *L. salicaria*. This result was borne out by cluster analysis (Figure 15), where two major clusters were evident, one of *L. salicaria* and one of *L. alatum*. The native North American *L. salicaria* cluster monophyletically, forming a distinct cluster separate from the European *L. salicaria* and the cultivars of *L. salicaria*, suggesting that the evolutionary relationships between the North American *L. salicaria* are more recent than to the European *L. salicaria*. For the most part, individuals from the same North American populations clustered together. There was a clinal trend within the North American *L. salicaria*, with many of the populations from the same region clustering together; however, this relationship was violated with the Ohio and Massachusetts populations.

Table 9: Summary of AFLP variation in 15 populations of *Lythrum salicaria* and 12 populations of *L. alatum* in North America, 11 European populations of *L. salicaria*, and 6 cultivars. Included are number of fragments for each primer, with numbers unique to each species and number shared between species.

Primer Comb.	# Frag. Unique to		# Frag. in both	Total # Frag.
	<i>L. salicaria</i>	<i>L. alatum</i>		
M-CAG/E-ACT	0	1	6	7
M-CAG/E-AGG	5	2	14	21
M-CAG/E-AAG	2	3	6	11
M-CAG/E-ACG	5	1	6	12
M-CAG/E-ACC	5	5	3	13
Total	17	12	35	64

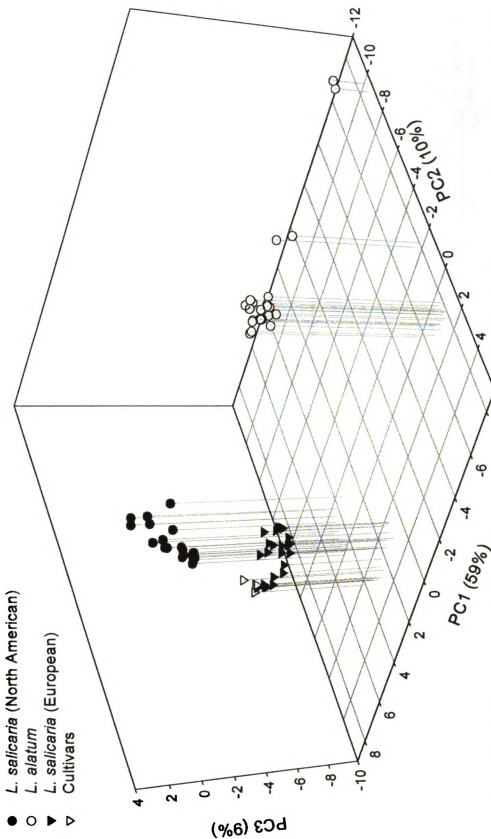


Figure 14: Principle Component Analysis for all populations included in study. North American *L. salicaria* is indicated by filled circles, European *L. salicaria* by filled triangles, cultivars of *L. salicaria* by open triangles, and *L. alatum* by open circles. Percentage that each principle component contributed to overall variation is indicated in parentheses on the appropriate axis.

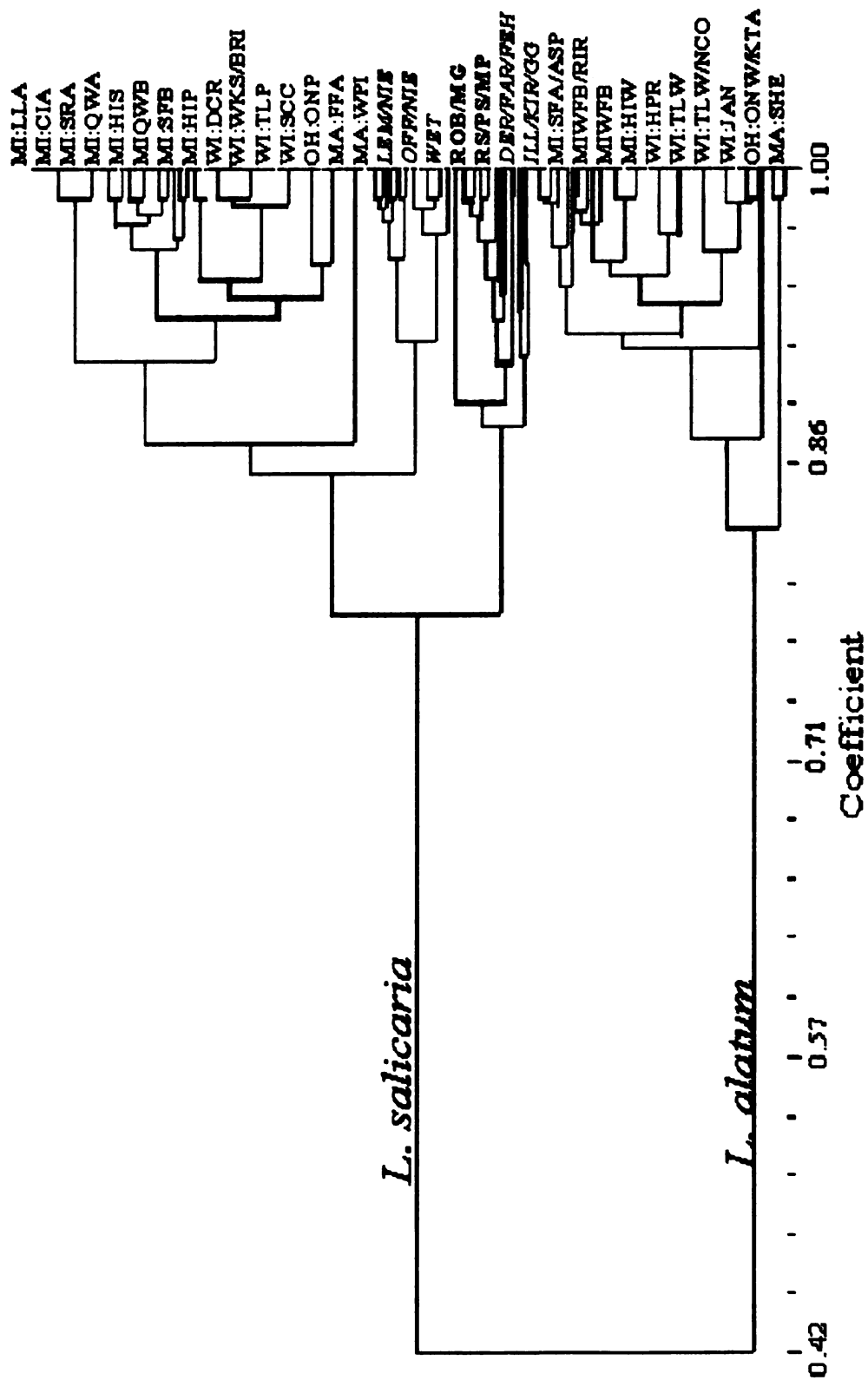


Figure 15: Cluster analysis of *L. salicaria* and *L. alatum*, including all populations studied in analysis. The top cluster includes all *L. salicaria*, and the lower cluster includes all *L. alatum*. The top cluster can be further broken into North American *L. salicaria*, cultivars, and European *L. salicaria*. Abbreviation for state of origin is listed before each North American population name.

Further study of Michigan populations

Eighteen additional primer pairs were selected to study five individuals from each of eight Michigan populations. Using the additional eighteen primer pairs, 216 total fragments were observed, with 76 of those unique to *L. salicaria* and 86 unique to *L. alatum*. Of the remaining 54 fragments, all were monomorphic in both species (Table 10).

Both principle component analysis and cluster analysis split the populations into two separate groups based solely on species (Figures 16 and 17). The sympatric populations of *L. salicaria* that appeared morphologically intermediate to *L. alatum* did not appear intermediate using the molecular markers. They clustered within *L. salicaria*, separate from *L. alatum*. Individuals from the same population always clustered together. An example of a gel run in this experiment is in Figure 18.

Table 10: Summary of AFLP variation in four populations of *Lythrum salicaria* and four populations of *L. alatum* in Michigan. Included are number of fragments for each primer, with numbers unique to each species and number shared between species.

Primer Combination	# Frag. Unique to:		# Frag. In both	Total # Frag.
	<i>L. salicaria</i>	<i>L. alatum</i>		
M-CAC/E-AAG	2	1	2	5
M-CAC/E-AAC	1	4	2	7
M-CAA/E-AAG	3	2	4	9
M-CAA/E-AAC	1	1	0	2
M-CAC/E-AGG	2	3	1	6
M-CAT/E-AAG	4	1	3	8
M-CTC/E-ACT	3	4	8	15
M-CTC/E-ACC	3	6	2	11
M-CTC/E-AGC	9	10	1	20
M-CTG/E-ACC	9	10	3	22
M-CTG/E-AGC	3	12	5	20
M-CTT/E-AGC	21	16	16	52
M-CTA/E-AGG	1	0	0	1
M-CTA/E-ACT	1	1	0	2
M-CAT/E-AAC	6	1	1	8
M-CAT/E-AGG	1	2	2	5
M-CTA/E-AAG	5	6	2	13
M-CTA/E-AAC	1	6	2	9
M-CAG/E-ACT	1	2	4	7
M-CAG/E-AGG	5	3	13	21
M-CAG/E-AAG	4	4	3	11
M-CAG/E-ACG	6	1	5	12
M-CAG/E-ACC	5	5	3	13
Total	97	101	83	281

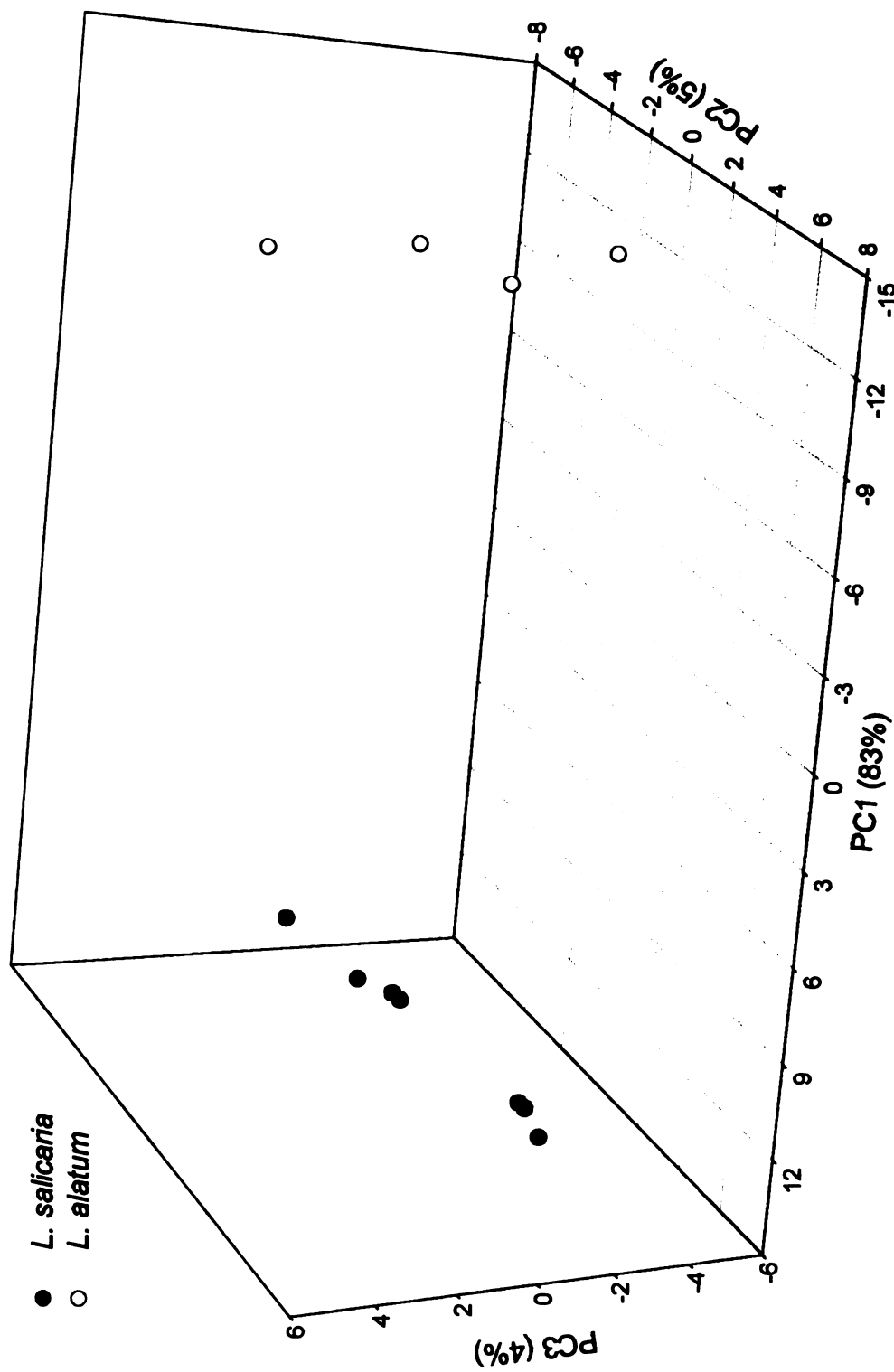


Figure 16: Principle component analysis of the 8 Michigan populations more extensively studied. *L. salicaria* populations are on the left, *L. alatum* populations on the right. Percentage that each principle component contributed to overall variation is indicated in parentheses on the appropriate axis.

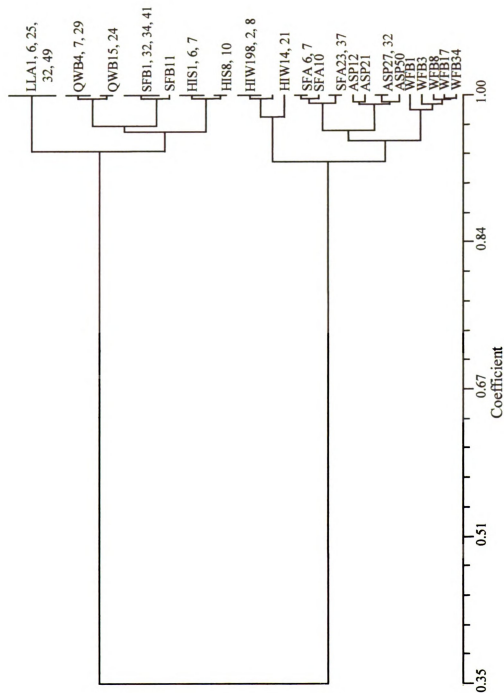


Figure 17: Cluster analysis of the eight Michigan populations of *L. salicaria* and *L. alatum* more intensively studied. The top cluster includes all *L. salicaria* populations, the lower cluster includes all *L. alatum* populations.

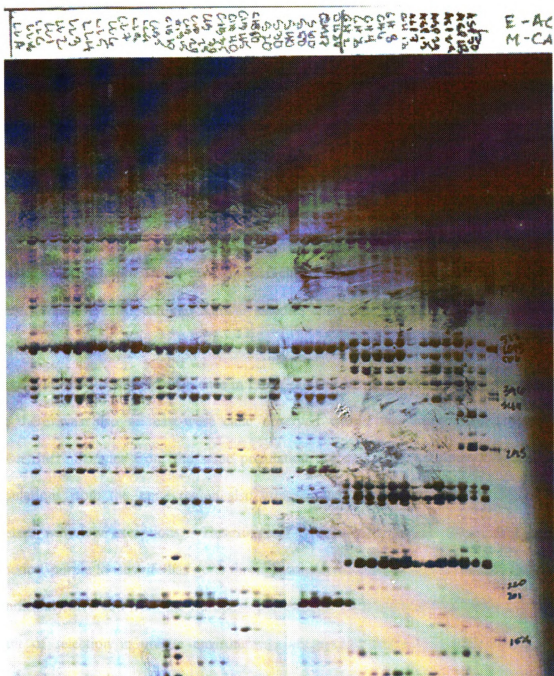


Figure 18: An example of a polyacrylamide gel run in the Michigan experiment. Purple loosestrife is on the left, winged loosestrife is on the right. Some markers are present in purple loosestrife and not winged loosestrife, and vice versa, and some markers are present in both species.

Discussion

Unlike the morphological results, the molecular data provide no evidence of hybridization between the two *Lythrum* species. The North American purple loosestrife have clearly diverged from the native species in Europe, but there is no evidence that a hybridization between purple and winged loosestrife in North America contributed to that differentiation. When patterns of molecular diversity were examined, North American purple loosestrife formed its own distinct cluster within the larger purple loosestrife cluster, with the European and cultivar samples clustering outside the North American purple loosestrife cluster.

Not only was there difference in European and North American purple loosestrife, but there was also an east-west cline observed within the North American purple loosestrife. Samples from the same population, by and large, grouped together, and populations from the same region also grouped into the same subgroups within the clusters with few exceptions. This suggests that over the course of invading North America, purple loosestrife has gone through considerable evolutionary change, and have significantly diverged from the European populations. It is not known if this is solely the result of selection in unique environments or partially due to founder effects of a restricted sample.

Character state analysis of all molecular markers revealed one marker from the global experiment that could be consistent with hybridization, for it was present in some of the North American purple loosestrife, and some, but not all, of the winged loosestrife, and none of the European purple loosestrife (AGG325c). However, even that pattern is

suspect when determining hybridization, for it is present in Massachusetts purple loosestrife, but not in Massachusetts winged loosestrife. Two markers are present in all winged loosestrife and a few North American purple loosestrife (AGG300, AAG475), but are also present in some of the European purple loosestrife, arguing against a hybrid origin of these markers in purple loosestrife, and nine markers are present in all winged loosestrife but none of the purple loosestrife, which argues against a hybridization event.

All the commercially available cultivars were also examined. Included in this group were Morden's Gleam and Morden's Pink, which are claimed to be first or second generation hybrids of *L. salicaria* x *L. alatum*. In both the principle component analysis and cluster analysis, all of the cultivars, including the two putative hybrids, clustered more closely to European purple loosestrife than North American purple loosestrife or winged loosestrife. This suggests that these cultivars have not extensively hybridized with North American purple loosestrife, and the integrity of the cultivars in nursery stock remains. It also suggests that the reported hybrid ancestry of the Morden cultivars is unlikely. Visually, we could see no morphological differences between the Morden cultivars and wild purple loosestrife populations.

In an often cited paper, Strefeler (1996) examined isozyme diversity in 11 Minnesota populations of purple loosestrife, one New Jersey purple loosestrife population, and three Minnesota winged loosestrife populations, and concluded that winged and purple loosestrife could have hybridized in North America. These conclusions were based on the fact that *L. salicaria* and *L. alatum* shared many allozymes; however, no species specific winged loosestrife allozymes were found to have moved into purple loosestrife individuals, and their cluster analysis clearly distinguished

the *L. salicaria* from *L. alatum* individuals. It is our contention that their evidence does not support the conclusion that the two species have hybridized.

Strefler et al. (1996) suggested that further analysis was needed with a larger number of winged loosestrife populations to "adequately define the role of *L. alatum* in the evolution of weedy purple loosestrife". Our molecular analysis did just this, as it surveyed 13 populations of purple loosestrife and 12 populations of winged loosestrife, spread over four states, as well as eleven populations of European purple loosestrife and six purple loosestrife cultivars. The first molecular experiment included 64 total bands, 51 of which were polymorphic, and 14 of which were specific to winged loosestrife. The second experiment, with a smaller number of populations (four of each species) included an additional 216 bands, 162 of which were polymorphic, and 86 which were specific to winged loosestrife. Within this broad, comprehensive survey, there was no evidence of hybridization between these two species. So, if there has been no hybridization between purple and winged loosestrife, why does purple loosestrife in North America exhibit traits not previously seen in Europe? Some of the traits previously observed in the purple loosestrife in Minnesota (Anderson and Aster, 1994) specifically glabrous vs. pubescent calyx and distyly vs. tristily, were not observed in our survey. Also, within the common greenhouse experiment, some of the variable traits (leaf placement and flowers per leaf axil) almost completely disappeared, which indicates that there must be an environmental component to these traits. There were 'intermediate' populations of purple loosestrife for height, flower and leaf traits in our screening across the northeastern geographic range on the morphological level, but these populations did not show intermediacy in their molecular markers. It may be that purple loosestrife is

evolving a shorter genotype that is adapted to the more xeric habitats favored by winged loosestrife in North America.

Polyploidy in *L. salicaria* may have played a role in its ability to adapt to new habitats in North America. The increased level of heterozygosity generally observed in polyploids may have preadapted *L. salicaria* with sufficient plasticity to fill many habitats. This may explain why purple loosestrife is found in a much wider range of habitats, specifically water availability, in North America as compared to its native Eurasia. Being autopolyploid, it also has the potential to carry high levels of genomic diversity, which could be reassorted through tetrasomic inheritance. Several species of *Lythrum* in Europe are diploid and some exhibit similar traits to *L. alatum*. Some of these species could be the natural progenitors of *L. salicaria*. For example, *L. borystenicum* is a dwarf species with either alternate or opposite leaf placement, and *L. portula*'s flowers are placed solitarily in leaf axils (Tutin *et al.*, 1968). If some of these species are the original progenitors to the tetraploid *L. salicaria*, these seemingly new traits could actually be hidden as recessives in the ancient polyploid genome and reassorted when *L. salicaria* encountered new habitats in North America.

In conclusion, while purple loosestrife did not appear to hybridize with winged loosestrife in North America, it does seem that there has been significant evolution within purple loosestrife since its original arrival in North America. This evolution has allowed purple loosestrife in North America to invade new habitats previously inaccessible to the species, and may very well have contributed to the overall success as an invader of alien habitats.

APPENDIX

Table 11: AFLP data for global experiment. MseI primer for all combinations is CAG, EcoRI primer is indicated. Molecular weight of fragment is indicated after primer name. Presence of fragment is indicated with 1, absence with 0. North American purple loosestrife is bold, European purple loosestrife is italicized, and cultivars are underlined.

[illegible]

[illegible]

Table 11 continued[illegible]

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Table 11 continued

	C o n t a i n e r																													
	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	
	C	C	C	C	C	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	A	A	A
	T	T	T	T	T	T	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
	5	4	3	3	2	2	1	5	4	4	4	4	4	4	3	3	3	3	2	2	2	2	2	2	1	1	1	1	7	6
	0	5	7	5	7	4	9	0	8	8	8	8	5	5	0	0	5	2	2	2	0	9	8	7	5	2	9	8	5	4
	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	5	5	5	0	0	0	0	0	0	5	5	0	0	0	0
									a	b	c	d	a	b	a	b													0	0
HPR9	1	1	1	0	1	0	1	1	0	0	0	0	0	0	1	1	1	0	1	1	1	0	0	1	1	1	1	1	0	0
HPR12	1	1	1	0	1	0	1	1	0	0	0	0	0	0	1	1	1	0	1	1	1	0	0	1	1	1	1	1	0	0
HPR15	1	1	1	0	1	0	1	1	0	0	0	0	0	0	1	1	1	0	1	1	1	0	0	1	1	1	1	1	0	0
HPR3	1	1	1	0	1	0	1	1	0	0	0	0	0	0	1	1	1	0	1	1	1	0	0	1	1	1	1	1	0	0
HPR11	1	1	1	0	1	0	1	1	0	0	0	0	0	0	1	1	1	0	1	1	1	0	0	1	1	1	1	1	0	0
HPR16	1	1	1	0	1	0	1	1	0	0	0	0	0	0	1	1	1	0	1	1	1	0	0	1	1	1	1	1	0	0
HPR17	1	1	1	0	1	0	1	1	0	0	0	0	0	0	1	1	1	0	1	1	1	0	0	1	1	1	1	1	0	0
HPR20	1	1	1	0	1	0	1	1	0	0	0	0	0	0	1	1	1	0	1	1	1	0	0	1	1	1	1	1	0	0
TLW1	1	1	1	0	1	1	1	1	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0
TLW3	1	1	1	0	1	1	1	1	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0
TLW5	1	1	1	0	1	1	1	1	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0
TLW7	1	1	1	0	1	1	1	1	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0
TLW9	1	1	1	0	1	1	1	1	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0
TLW11	1	1	1	0	1	1	1	1	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0
TLW13	1	1	1	0	1	1	1	1	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0
TLW15	1	1	1	0	1	1	1	1	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0
TLW16	1	1	1	0	1	1	1	1	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0
TLW17	1	1	1	0	1	1	1	1	0	0	0	0	0	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0
NCO1	1	1	1	0	1	1	1	1	1	0	0	0	0	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0
NCO5	1	1	1	0	1	1	1	1	1	0	0	0	0	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0
NCO8	1	1	1	0	1	1	1	1	1	0	0	0	0	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0
NCO15	1	1	1	0	1	1	1	1	1	0	0	0	0	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0
NCO20	1	1	1	0	1	1	1	1	1	0	0	0	0	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0
NCO27	1	1	1	0	1	1	1	1	1	0	0	0	0	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0
NCO36	1	1	1	0	1	1	1	1	1	0	0	0	0	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0
NCO40	1	1	1	0	1	1	1	1	1	0	0	0	0	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0
NCO45	1	1	1	0	1	1	1	1	1	0	0	0	0	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0
NCO51	1	1	1	0	1	1	1	1	1	0	0	0	0	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0
ONP1	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	0	1	1	0	1	1	1	1	1	1	1	0
ONP7	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	0	1	1	0	1	1	1	1	1	1	1	0
ONP9	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	0	1	1	0	1	1	1	1	1	1	1	0
ONP16	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	0	1	1	0	1	1	1	1	1	1	1	0
ONP17	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	0	1	1	0	1	1	1	1	1	1	1	0
ONP21	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	0	1	1	0	1	1	1	1	1	1	1	0
ONP27	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	0	1	1	0	1	1	1	1	1	1	1	0
ONP29	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	0	1	1	0	1	1	1	1	1	1	1	0
ONP36	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	0	1	1	0	1	1	1	1	1	1	1	0
ONP42	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	0	1	1	0	1	1	1	1	1	1	1	0
ONW3	1	1	1	0	1	0	1	0	1	0	1	0	1	0	1	1	0	1	1	1	0	1	1	1	1	1	1	1	0	0
ONW17	1	1	1	0	1	0	1	0	1	0	1	0	1	0	1	1	0	1	1	1	0	1	1	1	1	1	1	1	0	0
ONW22	1	1	1	0	1	0	1	0	1	0	1	0	1	0	1	1	0	1	1	1	0	1	1	1	1	1	1	1	0	0
ONW24	1	1	1	0	1	0	1	0	1	0	1	0	1	0	1	1	0	1	1	1	0	1	1	1	1	1	1	1	0	0
ONW27	1	1	1	0	1	0	1	0	1	0	1	0	1	0	1	1	0	1	1	1	0	1	1	1	1	1	1	1	0	0
ONW31	1	1	1	0	1	0	1	0	1	0	1	0	1	0	1	1	0	1	1	1	0	1	1	1	1	1	1	1	0	0
ONW42	1	1	1	0	1	0	1	0	1	0	1	0	1	0	1	1	0	1	1	1	0	1	1	1	1	1	1	1	0	0
ONW45	1	1	1	0	1	0	1	0	1	0	1	0	1	0	1	1	0	1	1	1	0	1	1	1	1	1	1	1	0	0

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	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	A	A	A	T	T	T	T	T	T	T	T	T	T	T	T	T	A	A	A	A	A
	G	G	G	G	G	G	G	G	G	G	G	G	G	C	C	A	A	A	A	A	A	A	A	A	G	G	G	G	G	G	A	A	A	A	A		
	C	C	C	C	C	C	C	C	C	C	C	C	G	T	T	C	C	C	C	C	C	C	G	G	G	G	G	G	G	G	G	G	G	G	G		
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	3	3	3	2	2	2	1	1	1	0	0	0	3	0	8	2	1	9	7	2	7	7	6	0	0	2	3	7	5	3	3	0					
	8	5	2	8	5	0	7	5	0	5	3	0	0	0	0	0	0	0	0	5	5	5	0	0	0	0	5	0	5	0	5	0	5	0	5		
LLA1	1	1	0	0	0	1	1	1	1	0	1	0	1	1	0	1	0	1	1	1	1	1	1	1	1	0	1	1	0	0	0	0	0	0			
LLA6	1	1	0	0	0	1	1	1	1	0	1	0	1	1	0	1	0	1	1	1	1	1	1	1	1	0	1	1	0	0	0	0	0	0			
LLA25	1	1	0	0	0	1	1	1	1	0	1	0	1	1	0	1	0	1	1	1	1	1	1	1	1	0	1	1	0	0	0	0	0	0			
LLA32	1	1	0	0	0	1	1	1	1	0	1	0	1	1	0	1	0	1	1	1	1	1	1	1	1	0	1	1	0	0	0	0	0	0			
LLA49	1	1	0	0	0	1	1	1	1	0	1	0	1	1	0	1	0	1	1	1	1	1	1	1	1	0	1	1	0	0	0	0	0	0			
QWB4	1	1	0	0	0	1	1	1	1	0	1	0	1	1	0	1	0	1	1	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0			
QWB7	1	1	0	0	0	1	1	1	1	0	1	0	1	1	0	1	0	1	1	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0			
QWB15	1	1	0	0	0	1	1	1	1	0	1	0	1	1	0	1	0	1	1	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0			
QWB24	1	1	0	0	0	1	1	1	1	0	1	0	1	1	0	1	0	1	1	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0			
QWB29	1	1	0	0	0	1	1	1	1	0	1	0	1	1	0	1	0	1	1	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0			
SFB1	1	1	0	0	0	1	1	1	1	0	1	0	1	1	0	1	0	1	1	1	1	1	1	1	1	0	0	1	1	0	0	0	1	0			
SFB11	1	1	0	0	0	1	1	1	1	0	1	0	1	1	0	1	0	1	1	1	1	1	1	1	1	0	0	1	1	0	0	0	1	0			
SFB32	1	1	0	0	0	1	1	1	1	0	1	0	1	1	0	1																					

Table 12 continued[illegible]

[illegible]

Table 12 continued

	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	
	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	
	G	G	G	G	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	
	2	1	1	1	4	3	3	2	2	2	1	1	1	9	9	9	9	9	9	
	2	8	8	8	7	3	2	2	2	2	6	2	0	0	0	0	0	0	0	
	0	5	5	5	5	0	0	5	5	5	0	0	0	a	b	c	d			
	a b c				a a b c				a											
					b				b											
LLA1	1	1	1	1	1	0	0	0	1	0	1	1	1	0	0	1	1			
LLA6	1	1	1	1	1	0	0	0	1	0	1	1	1	0	0	1	1			
LLA25	1	1	1	1	1	0	0	0	1	0	1	1	1	0	0	1	1			
LLA32	1	1	1	1	1	0	0	0	1	0	1	1	1	0	0	1	1			
LLA49	1	1	1	1	1	0	0	0	1	0	1	1	1	0	0	1	1			
QWB4	1	0	1	1	0	1	0	0	1	0	1	1	1	0	0	1	1			
QWB7	1	0	1	1	0	1	0	0	1	0	1	1	1	0	0	1	1			
QWB15	1	0	1	1	0	1	0	0	1	0	1	1	1	0	0	1	1			
QWB24	1	0	1	1	0	1	0	0	1	0	1	1	1	0	0	1	1			
QWB29	1	0	1	1	0	1	0	0	1	0	1	1	1	0	0	1	1			
SFB1	1	0	1	1	1	1	0	0	1	0	1	1	1	0	0	1	1			
SFB11	1	0	1	1	1	1	0	0	1	0	1	1	1	0	0	1	1			
SFB32	1	0	1	1	1	1	0	0	1	0	1	1	1	0	0	1	1			
SFB34	1	0	1	1	1	1	0	0	1	0	1	1	1	0	0	1	1			
SFB41	1	0	1	1	1	1	0	0	1	0	1	1	1	0	0	1	1			
HIS1	1	1	1	1	1	1	0	0	1	0	1	1	1	0	0	1	1			
HIS6	1	1	1	1	1	1	0	0	1	0	1	1	1	0	0	1	1			
HIS7	1	1	1	1	1	1	0	0	1	0	1	1	1	0	0	1	1			
HIS8	1	1	1	1	1	1	0	0	1	0	1	1	1	0	0	1	1			
HIS10	1	1	1	1	1	1	0	0	1	0	1	1	1	0	0	1	1			
HIW198	0	1	1	0	0	1	1	1	0	1	0	0	0	1	1	1	1			
HIW2	0	1	1	0	0	1	1	1	0	1	0	0	0	1	1	1	1			
HIW8	0	1	1	0	0	1	1	1	0	1	0	0	0	1	1	1	1			
HIW14	0	1	1	0	0	1	1	1	0	1	0	0	0	1	1	1	1			
HIW21	0	1	1	0	0	0	1	1	0	1	0	0	0	1	1	1	1			
SFA6	0	1	1	0	0	1	1	1	0	1	0	0	0	1	1	1	1			
SFA7	0	1	1	0	0	1	1	1	0	1	0	0	0	1	1	1	1			
SFA10	0	1	1	0	0	1	1	1	0	1	0	0	0	1	1	1	1			
SFA23	0	1	1	0	0	1	1	1	0	1	0	0	0	1	1	1	1			
SFA37	0	1	1	0	0	1	1	1	0	1	0	0	0	1	1	1	1			
ASP12	0	1	1	0	0	1	1	1	0	1	0	0	0	1	1	1	1			
ASP21	0	1	1	0	0	1	1	1	0	1	0	0	0	1	1	1	1			
ASP27	0	1	1	0	0	1	1	1	0	1	0	0	0	1	1	1	1			
ASP32	0	1	1	0	0	1	1	1	0	1	0	0	0	1	1	1	1			
ASP50	0	1	1	0	0	1	1	1	0	1	0	0	0	1	1	1	1			
WFB1	0	1	1	0	0	1	1	1	0	1	0	0	0	1	1	1	1			
WFB3	0	1	1	0	0	1	1	1	0	1	0	0	0	1	1	1	1			
WFB8	0	1	1	0	0	1	1	1	0	1	0	0	0	1	1	1	1			
WFB17	0	1	1	0	0	1	1	1	0	1	0	0	0	1	1	1	1			
WFB34	0	1	1	0	0	1	1	1	0	1	0	0	0	1	1	1	1			

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