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Examining the Invasion of Bush Honeysuckle Through a Phylogenetic Analysis

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Examining the Invasion of Bush Honeysuckle Through a Phylogenetic Analysis

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By

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Table of Contents

Abstract

The mechanisms underlying the continual spread of invasive plants within their respective non-native ranges is a major focal point to invasion research. Many theories have been proposed to understand these invasions, each with different implications for the predicted range for invasive plants. *Lonicera maackii* provides an exceptional opportunity to examine the effectiveness of these theories to explain the trends of invasive plants. *Lonicera maackii* is native to eastern Asia, but has invaded much of the eastern United States, presenting a severe threat to the health of forest and other natural areas. Recent analysis of the climatic envelopes uncovered a significant separation between the environmental conditions of the native east Asian *L. maackii* points and the invasive North American points. This separation was consistent with the predicted versus actual probable occurrence maps of North America and east Asia. Here I conduct a targeted phylogenetic analysis of *L. maackii* populations to investigate the natural and invasive history of the plant through DNA capture and analysis from herbarium samples. Leaf tissues from 95 individuals collected from major herbaria around the country were used to develop an intraspecific phylogeny to provide an evolutionary context for the success of *L. maackii* in its invasive range. Specifically, my work will be instrumental in pinpointing exactly the sequence of events that led to introduction and subsequent spread throughout the United States.

Introduction

Biological invasions are a powerful force of change whereby species spread beyond their native geographic ranges. For many plant species, ranges are determined by several biogeographic factors such as mountains, oceans, and landmasses (Lowry & Lester, 2006). Nonetheless, human activity has allowed plants to overcome these barriers resulting in invasive plant species spreading rapidly into non-native ranges. This expansion has the potential to result in destructive economic and ecological impacts (Catford et al., 2009). The meaning of an introduction in a biodiversity context, essentially states that the plant or its propagule has been transported by humans across a major geographical barrier. Naturalization occurs when both biotic and abiotic barriers to survival as well as barriers to regular reproduction barriers are overcome. Invasion is the concept that requires introduced species to produce reproductive offspring in areas distant from the sites of introduction (Richardson et al., 2000).

Research on invasive plants has proposed several explanations pertaining to the excessive success of plants outside their native range. For example, the Novel Weapon hypothesis proposes that certain invasive plant species gain advantages over native plants by obtaining unique biochemical characteristics that damage the prosperity of the nearby plants. The Empty Niche hypothesis suggests that more species can exists in a particular ecosystem or habitat than at that exact point in time (Shea & Chesson 2002). *Lonicera maackii*, also known as "bush honeysuckle" and "Amur Honeysuckle," presents an exceptional model to evaluate the various hypotheses regarding the spread of species into novel environments (McNeish & McEwan 2016). *L. maackii* is a great model of long-distance dispersal and abundant propagule production in invasive plants. These highly successful invasive shrubs have large fruiting events and produce seeds that can germinate in various light conditions, temperature stratification, and soil

conditions. Likewise, *L. maackii* has a competitive growth pattern which is a critical phenotypic characteristic that helps assist its growth in new habitats (McNeish & McEwan 2016).

The *L. maackii* dramatically modifies substrate, resources, and ecosystem processes which can result in significant changes to plant and animal communities in that habitat. Empirical evidence supports that *L. maackii* invasion has dramatic negative effects on native plants in North America (McNeish & McEwan, 2016). Forests with this invasive shrub exhibit less herb fecundity, fitness, and growth (Gould & Gorchov, 2000). Likewise, the effects of invasive plants on animal communities cannot go unnoticed. Pyšek et al. (2012) conducted an analysis that found nearly 70% of studies reported non-native invasive species vegetation negatively affected animal communities.

Our current understanding of the *L. maackii* invasion supports both the Novel Weapon and Empty Niche hypotheses. For example, there is supporting evidence for *L. maackii* producing compounds that biochemically prevent growth in neighboring plants (Medina-Villar et al., 2020). *L. maackii* can occupy a vacant niche space in a non-native community (Smith 2014). Recent conducted research focusing on geographic range modeling in *L. maackii* and was also found to be consistent with the hypotheses mentioned previously (Palmer 2021). As shown in Figure 1, a major climatic shift occurred between the native range of *L. maackii* (East Asia) and its non-native range (eastern North America).

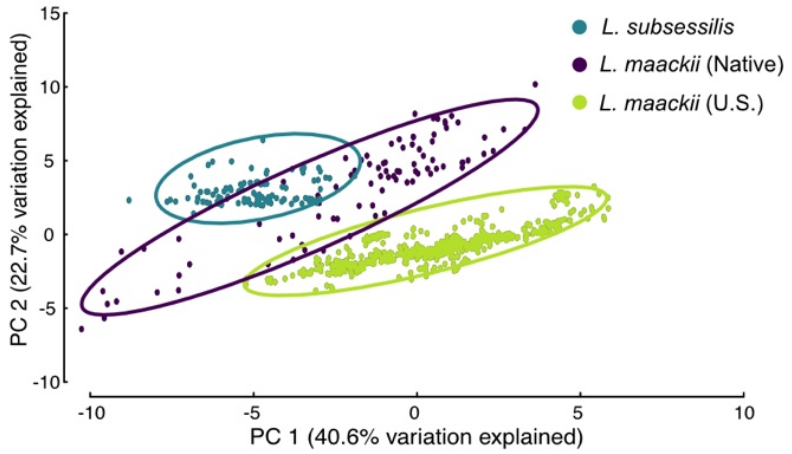

Figure 1 Principal component analysis of climatic variables for native Chinese and invasive U.S. *Lonicera maackii* and its sister species *L. subsessilis*. Palmer (2021)

While native *L. maackii* populations occupy a climatic niche that is more closely related to *L. subsessilis*, the sister species to *L. maackii*, than the invasive *L. maackii* populations of the US, suggests a rapid and drastic shift in the climates inhabited by *L. maackii* in North America. However, we cannot rule out the possibility that the founding populations come from the margins of its native range. As shown in Figure 2, when projecting the probable occurrence of the native range onto North America, the model shows a dramatic deviation between the observed range of *L. maackii* and the predicted range. Figure 2A is the native east Asian points of *L. maackii*, while Figure 2B shows the projected climate region the invasive shrub is expected to be found. However, Figure 2C shows where *L. maackii* is found in North America while Figure 2D projects that same climatic region onto east Asia. Further research of the history of this invasive species could give insight as to why these extreme differences in climate conditions is observed.

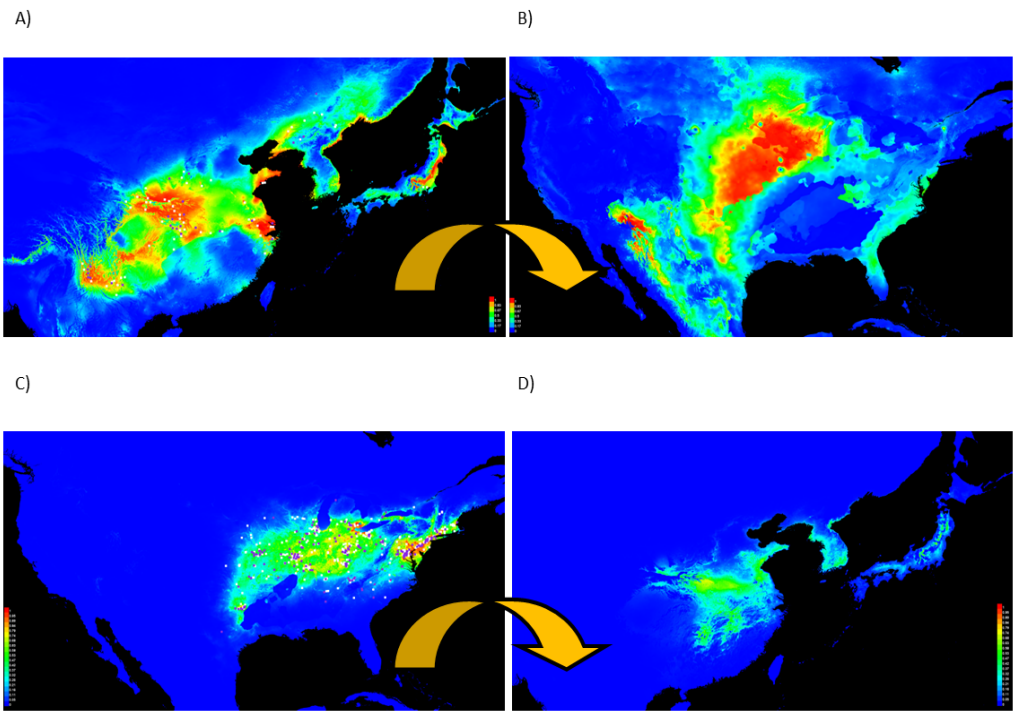

Figure 2: Maxent probable occurrence maps of *L. maackii* for A) native Chinese points and B) model projected onto North America. These models were also drawn from *L..maackii* from C) North America and D) projected onto China. Red coloration denotes higher probability of occurrence with blue coloration denoting a lower probability of occurrence.

Here I conduct an intraspecific phylogenetic analysis of *L. maackii* populations to explore its invasive history through DNA capture and analysis from herbarium samples. To determine the origins of the initial introduction of *L. maackii* into the U.S., a phylogeny was constructed, which describes the relationship among species that refers to the evolutionary history and development of those populations. There are three possible scenarios for how *L. maackii* was introduced and subsequently spread across much of the eastern half of the U.S. (Figure 2): 1) the invasive populations are the result of a single introduction of *L. maackii* from an extreme part of the range; 2) there were multiple introductions consist of several occasions of *L. maackii*; and 3) that the invasive *L. maackii* was incorrectly described and is related from a source species not yet identified.

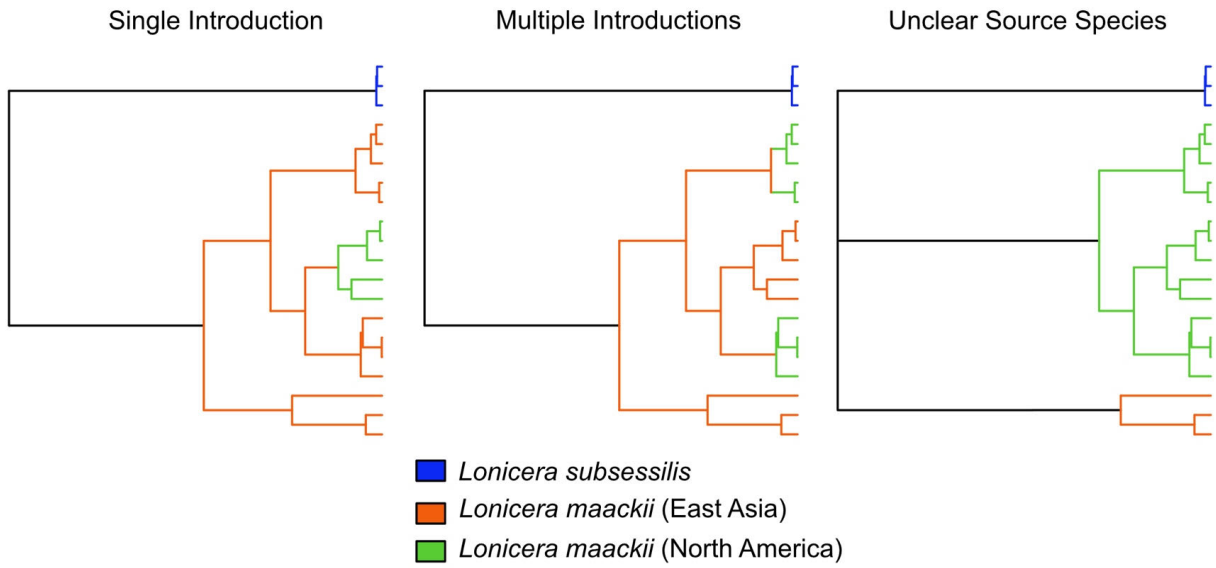

Figure 3 Potential mechanisms underlying how *L. maackii* was introduced into its non-native range, eastern North America, from its origin in eastern Asia.

This research can provide context for the climatic envelope supported in previous research. The phylogeny conducted will draw connections between the Chinese places of origin and the introduced populations in eastern North America. Likewise, this research could provide an evolutionary context for understanding the mechanisms underlying the spread of *L. maackii* throughout eastern North America. The results of this research will also provide data for the ongoing debate regarding invasion theory.

Materials and Methods

I. Leaf Samples and DNA Extractions

Leaf tissues were obtained from 95 leaf tissue collected from various specimens collected in western and northeastern China, Japan, Russian Far East, and eastern North America. These samples were collected by traveling to several distinguished herbaria, including the New York Botanical Garden, the Harvard Herbaria, and the Missouri Botanical Garden. Likewise,

specimens were also taken from the University of Arkansas Herbarium. Leaf material from the closest relatives of *L. maackii*, *L. ruprechtiana* and *L. subsessilis,* were also collected and included in the phylogenetic analysis. Including these sister taxa as an "outgroup" is a standard practice in phylogenetics and helped root the tree, which pulls down the most ancestral *L. maackii* populations to better evaluate when *L. maackii* moved into eastern North America.

The protocol used for DNA extraction closely reflects the protocol described in Brewer et al. (2019). A standard CTAB buffer with 10% PVP40 was used to remove any secondary compounds specific to *L. maackii.* This buffer was followed by a treatment with RNase to remove RNA from the solution. After many purification steps, the DNA is precipitated and rinsed in ethanol multiple times before it sits overnight. A Nanodrop is used to assess the purity of the sample and Qubit is used to measure the concentrations of DNA from each sample. The DNA then runs through an agarose gel to determine the size of the DNA and assess degradation (see Fig. 3A). The extraction protocol was developed and verified using herbarium samples collected from the University of Arkansas Herbarium.

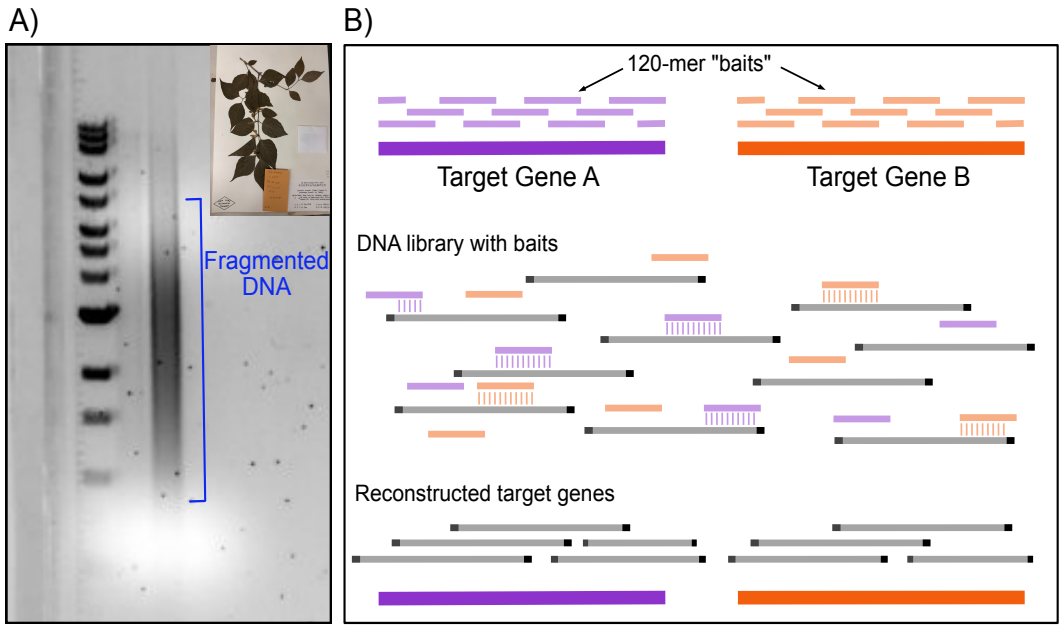

Figure 4 A) A typical agarose gel run from DNA extracted from an herbarium specimen. The "smear" is indicative of degraded DNA. B) A schematic of the "Hyb-seq" sequencing approach for generating sequence libraries from the degraded DNA. Small RNA "baits" target specific fragments of a target gene, which get amplified and then reconstructed to form a complete sequence. Modified from Johnson et al (2019).

One major challenge regarding sequencing DNA from herbarium specimens is that they are significantly degraded causes target genes for sequencing to exist as short fragments (Brewer et al. 2019). Due to this, standard Sanger DNA sequencing methods are ineffective or demand an extraordinary amount of effort to amplify or clone them. To assemble libraries (i.e. samples that are prepared for sequencing) from herbarium DNA extractions, recent advantages in target enrichment sequence technology which uses short RNA probes to hybridize and capture the fragments of target genes (this technology is often referred to as "Hyb-seq") were used. The product of this technology are fragments of target genes that can easily be joined together to arrange the entire target gene (see Fig. 4B).

II. DNA Amplification and Sequencing

DNA was isolated using the CTAB method. Extraction of the DNA was then followed by precipitation. The following day, the DNA quality and quantity was checked using: nanodrop to

assess sample purity, qubit to measure sample quantity, and agarose gel to visualize size of genomic DNA and any degradation. Templates of the nrDNA internal transcribed spacer region (ITS) were prepared using the primers ITS5 (5′-GGA AGG AGA AGT CGT AAC AAG G-3′; Suh et al. 1993) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′; White et al. 1990). The ITS region is an ideal marker for deciphering relationships among closely related species because it is easy to detect even from small amount of DNA and contains a high degree of sequence variation.

Polymerase chain-reaction (PCR) amplifications followed procedures described by Zimmer et al. (2002), with some modifications to increase success with degraded tissue. PCR amplification system is as follows: 3.0µl genomic DNA, 5.0µl Taq DNA Polymerase, 1.0µl Primer ITS4 (10µM), 1.0µl Primer ITS5 (10µM), 1.0µl DMSO, 2.0µl BSA, and 10.0µl ddH20, with a total volume 25.0µl per reaction. PCR reaction conditions for the thermocycler are denaturing at 94°C for 1 minute; after which is the cycling procedure: denaturing at 94°C for 1 minute, annealing at 48°C for 1 minute, extending at 72°C for 1 minute, a total of 50 cycles; then extending at 72°C for 5 minutes; hold at 4°C for preservation.

The PCR products were electrophoresed using a 2.0% agarose gel in 1X TAE buffer, stained with SybrSafe gel stain to confirm product and estimate concentration. The PCR products were then cleaned using a modified Exo-SAP protocol. The Exo-SAP cleaning is as follows: 0.25µl Exonuclease I, 0.50µl Shrimp Alkaline Phosphatase, 2.25µl ddH2O, and 25µl PCR product. The conditions for Exo-SAP in the thermocycler are 37°C for 30 minutes, 80°C for 15 minutes, hold at 12°C for preservation. Samples were then prepped for sequencing via Eurofins Genomics. Each sample submitted included 3µl PCR product, 1µl primer (either forward or reverse, not both), and 6µl ddH2O.

III. Phylogenetic Analysis

To determine the origins of the initial introduction of *L. maackii* into the U.S., a phylogeny was constructed, which describes the relationship among species that refers to the evolutionary history and development of those populations. Phylogenetic analysis of *L. maackii* can provide an understanding for the context for climate modeling results from Andrew Palmer's undergraduate thesis. The resulting phylogeny will elucidate whether *L. maackii* populations in North America originated from a single introduction or multiple introductions. The phylogeny was inferred using the R package *phangorn* (Schliep 2011) from an alignment of our sequences from MAFFT (Katoh and Standley 2013).

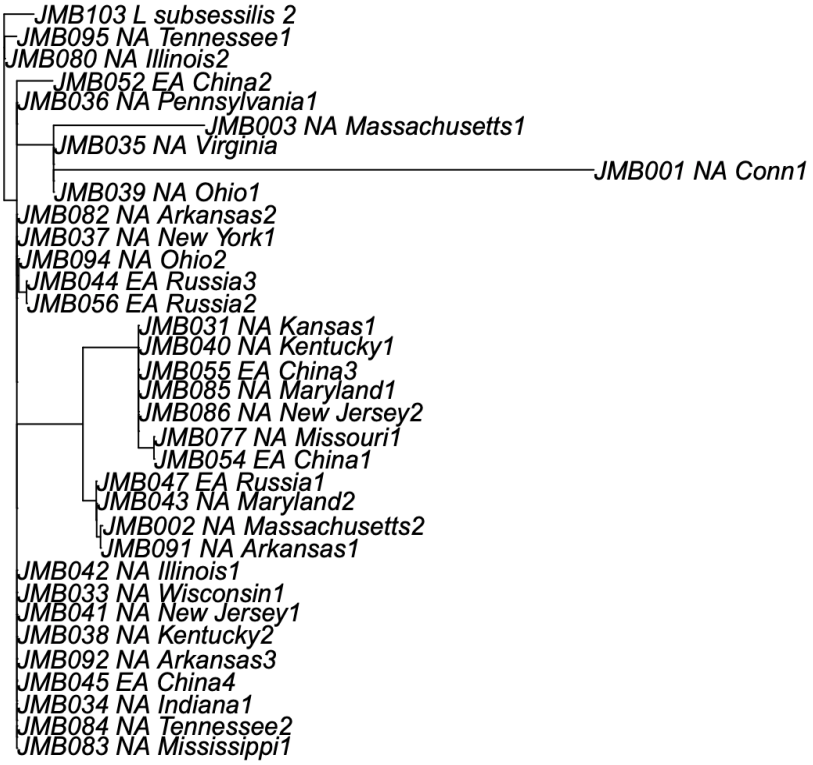

Figure 5 The phylogenetic tree constructed from herbarium samples of *L. maackii*.

Results and Discussion

Across the 95 leaf samples taken from herbarium specimens, we were able to not only amplify DNA for 35 of these specimens, but we were also able to sequence a common marker for phylogenetic analysis. It is notable that herbarium specimens date as far back as 1966, and the methods used here were able to amplify usable DNA. This is a clear demonstration of the utility of historical collections, such as herbarium specimens, for addressing biogeographical and historical questions.

With regards to the east Asian origin of the North American populations of *L. maackii*, our phylogenetic analysis recovered a rather muddled picture. There seems to be no obvious grouping of east Asian clades and North American clades, with specimens from each region being scattered throughout the phylogeny. As of now, these results support the unclear source species hypothesis described above. We suspect that these results are due to generally low variation exhibited by individuals in their ITS region. This suggests that even faster evolving markers may be necessary to properly decipher relationships among *L. maackii* populations.

Future steps will attempt to gather more genetic data from across populations to increase variability in the sample. The ITS sequences are considerably conserved among the set of samples we sequenced, meaning it was difficult to parse out relationships with single-nucleotide polymorphisms, or identify any patterns. With ddRAD, large stretches DNA sequence from each individual will be collected. This could potentially provide a greater chance to capture useful variation when it comes to testing hypotheses regarding the origin of the invasion.

Conclusions

The implications of this research can have a significant impact across the field of invasive biology and land management techniques. The powerful invasion of *L. maackii* dramatically

modifies substrate, resources, and ecosystem processes which can result in significant changes to plant and animal communities in that habitat. Climate conditions were unlikely to present a significant constraint against the range expansion and invasion of *L. maackii* in novel environments. Understanding the invasion of *L. maackii* in North America could assist in developing broad ideas for land management strategies and conservation efforts which focus on eliminating and slowing the spread of invasive species into fragile habitats. Overall, a better understanding of invasive plant species in general it may be possible to predict further invasions and range expansions of ecologically damaging species like *L. maackii.*

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