2009

Understanding the Genetic Consequences of Rapid Range Expansion: A Case Study Using the Invasive Microstegium vimineum Trin. (Poaceae)

Stephen Baker
Virginia Commonwealth University

College of Humanities and Science
Virginia Commonwealth University

This is to certify that the thesis prepared by Stephen Andrew Baker entitled UNDERSTANDING THE GENETIC CONSEQUENCES OF RAPID RANGE EXPANSION: A CASE STUDY USING THE INVASIVE MICROSTEGIUM VIMINEUM TRIN. (POACEAE) has been approved by his committee as satisfactory completion of the thesis requirement for the degree of Masters of Science in Biology

---

Dr. Rodney J. Dyer Ph.D, Thesis Director, Department of Biology, College of Humanities and Sciences

---

Dr. Edward L. Boone Ph.D, Committee Member, Department of Statistical Sciences, College of Humanities and Sciences

---

Dr. Bonnie L. Brown Ph.D, Committee Member, Department of Biology, College of Humanities and Sciences

---

Dr. Donald R. Young Ph.D, Committee Member & Department Chair of Biology, College of Humanities and Sciences

---

Dr. Fred M. Hawkridge Ph.D, Interim Dean of the College of Humanities and Sciences

---

Dr. F. Douglas Boudinot, Dean of the Graduate School

July 24, 2009

UNDERSTANDING THE GENETIC CONSEQUENCES OF RAPID RANGE EXPANSION: A CASE STUDY USING THE INVASIVE MICROSTEGIUM VIMINEUM TRIN. (POACEAE)

A thesis submitted in partial fulfillment of the requirements for the degree of M.S. Biology at Virginia Commonwealth University.

by

STEPHEN ANDREW BAKER
A.S. Science, J. Sargeant Reynolds Community College, 2006
B.S. Biology, Virginia Commonwealth University, 2006

Director: DR. RODNEY J. DYER
ASSISTANT PROFESSOR, DEPARTMENT OF BIOLOGY

Virginia Commonwealth University
Richmond, Virginia
July, 2009

Acknowledgements

I want to thank my committee members: Dr. Rodney Dyer, Dr. Edward Boone, Dr. Bonnie Brown, Dr. Gregory Plunkett and Dr. Young for their help, guidance and support throughout this process. I also want to thank all the members of Dyer lab for their help sampling, ideas, and guidance and support especially Vikki Gardiakos, Dan Carr, Candace Dillon and Dr. Ryan Garrick. I especially want to thank my family and friends for all their support during my time at VCU as well as all the professors for the knowledge they have shared over the years. Thank you to Karen Pullen for help sampling. I want to thank the Virginia Department of Conservation and Recreation and the Virginia Department of Game and Inland Fisheries for permission to sample from state parks and wildlife management areas. Lastly, I want to thank the VCU Rice Center for Environmental Life Science for financial support.

Table of Contents

Page

Table of Contents (Continued)

List of Tables

Page

List of Figures

Page

Abstract

Understanding the Genetic Consequences of Rapid Range Expansion: A Case Study Using the Invasive *Microstegium vimineum* Trin. (Poaceae)

By Stephen Andrew Baker

A thesis submitted in partial fulfillment of the requirements for the degree of Masters of Science at Virginia Commonwealth University.

Virginia Commonwealth University, 2009

Thesis Director: Dr. Rodney J. Dyer
Assistant Professor, Department of Biology

Global temperature changes are predicted to influence the distributions of plants and can have significant consequences for population genetic structure. Both the nature of these consequences and the processes that shape them are of interest for both conservation genetics and the development of realistic management programs. Rapid range expansion occurs on short temporal scales not conducive to conventional phylogeographical analyses. This paper presents the findings from a population genetic study of the invasive grass *Microstegium vimineum* Trin. A. Camus throughout the James River Basin of Virginia. Genotypic analysis using Amplified Fragment Length Polymorphism (AFLP) molecular

markers were used to test for evidence of rapid range expansion and the effects associated with colonization and spread of *Microstegium vimineum*. Within the James River Basin three genetically distinct clusters were identified that were not clearly associated with natural geographic boundaries and recent founder events were also inferred. The James River Basin also appears to act as a corridor for long-distance dispersal events. These findings contribute to our knowledge of the genetic consequences of rapid range expansion for invasive species, and more importantly, native species. Contrary to several studies, the present research also indicates that long-distance dispersal is not rare and can be a major contributor to the genetic structure following range expansion.

Introduction

The distribution of genetic variation in plant species is influenced by recent range disturbances (i.e. expansion or shifting) caused by large-scale climatic variation (Taberlet *et al*. 1998, Hewitt 2000). Changes in global climate facilitate shifts in the current distribution of resident species and rapid range expansions into new habitat. The genetic consequences of these range disturbances are varied and depend on both the rate and method by which populations expand or shift into new habitats. Range expansion itself has previously been studied on temporal scales suitable primarily for phylogeography (e.g., Nason *et al*. 2002, Avise 2004, McLachlan *et al*. 2005, Garrick *et al*. 2009), but this only provides insights into previous processes that have shaped the genetic distribution we see today. Invasive species present an opportunity to study rapid range expansion on more contemporary time scales. Many of the same factors and processes (i.e., multiple introductions, long-distance dispersal, genetic drift, mating-system and founder effects) that occur during range disturbances for native plant species are also experienced by invasive plant species as they colonize novel habitats.

Founder effects are of particular interest because colonization is often characterized by introduction into novel habitats by small numbers of individuals from which expansion occurs both demographically and spatially. Founder effects occur when a very few

individuals establish a new population that has only a subset of the total genetic variation of the parent population (Mayr 1963). These newly colonized populations are often characterized by low genetic diversity, as compared to that present in the native range, due in part to the restricted number of individuals that initially established the population (see Barrett *et al*. 2008). The reduction in allelic diversity is expected to be inversely proportional to the allelic frequency, with rare alleles absent from newly colonized populations. This reduction in genetic diversity created by founding events has a profound effect on genetic structure among populations as they expand from their point of initial introduction or shift in response to changing ecological conditions (e.g., Ward 2006). For example, a one-dimensional geographic expansion into previously uninhabited areas with successive founder events with concomitant reductions in gene flow, due to increasing physical distance, results in a stepping-stone pattern of decreasing genetic diversity (van de Wouw *et al*. 2008). This low genetic diversity expected in newly founded populations is not permanent, subsequent immigration (e.g., secondary colonization) or gene flow into the site will tend to diversify within-population structure (Amsellem *et al*. 2000 and Pappert *et al*. 2000).

The distribution of genetic variation produced from a single introduction with continuous expansion and low to moderate levels of gene flow is predicted to result in a pattern of isolation-by-distance (IBD; i.e., genetic differentiation is an increasing function of physical distance, see Wright 1943). The consequences of these multiple introductions depends upon the source of immigrants. Multiple introductions from the same source will

generally increase genetic diversity of the newly established sites, making them appear to be more similar to the source population. Under this scenario, the resulting structure of newly colonized populations would appear to be either founded by a larger number of individuals than it actually was, or to have substantially higher rates of gene flow with parental populations than they actually do. Conversely, if the introductions are from alternate sources then individuals from these admixed populations may appear to be intermediate in diversity between the source populations. For example, Pappert *et al*. (2000) suggests that multiple introductions of kudzu (*Pueraria lobata*) across the Southeastern United States was a driving factor in the observed high levels of genetic diversity among populations.

Although multiple introductions from alternate sources may increase within population diversity, among-population genetic differentiation will tend to be reduced. This is due to more admixed populations being created and reducing the among-population genetic variance. This homogenization due to multiple founding populations can also easily be confused with high levels of ongoing gene flow so it is important to consider several lines of independent evidence when reconstructing demographic histories. The general expectation of reduced diversity within newly colonized populations can provide insights into the directionality of range expansion. Subsequent colonizations from the leading edge of the range expansion will result in a serial pattern of diversity loss across the landscape. For example, Garrick *et al*. (2009) found decreasing nuclear diversity in two separate lineages of *Euphorbia lomelii* along a latitudinal axis where diversity

decreased from north to south in the Baja California peninsula indicating southward expansion along the peninsula. Another obvious indication of the direction of range expansion is serial nesting of populations in a bifurcating tree where recently colonized populations form the more derived populations and the ancestral populations are the source of expansion (e.g., Nason *et al*. 2002).

Another factor that may dampen apparent patterns of isolation-by-distance across a landscape for a species expanding its range is discontinuous spread. If expansion is not continuous across the landscape, but characterized by episodic long-distance jumps, the spatial distribution of genetic structure may be coarsely granular and not oriented along apparent spatial axes (Baker 1974, Hengeveld 1989, Novak & Mack 2001, Suarez *et al*. 2001, Williams *et al*. 2007). Long-distance jumps followed by subsequent local diffusion increases the apparent heterogeneity of genetic variation across a species' range until the main expanding front of the species' range makes secondary contact (e.g. Austerlitz *et al*. 1997).

In addition to purely demographic processes, species mating systems may also influence the distribution of genetic variation within and among populations. In general, plants that reproduce primarily by self-fertilization typically have the lowest overall genetic diversity compared to mixed mating or obligate out-crossing species (Hamrick & Godt 1996). However, species that self- fertilize are more effective colonizers because populations can be founded by one individual or a small number of individuals (van

Kleunen *et al*. 2007). Conversely, obligate out-crossing plants (e.g., those that cannot produce self-fertilized seeds) typically maintain the highest levels of genetic diversity but require larger colonizing populations to maintain viable breeding populations during the initial invasion phase. Species with mixed-mating systems produce both self-fertilized and out-crossed offspring and are typically intermediate in maintaining genetic diversity (Hamrick & Godt 1996). As a result, species with similar demographic histories may maintain different levels of genetic diversity depending upon how the mating system influences the genetic structure of offspring during each reproductive event.

Microstegium vimineum Trin. Poaceae, commonly referred to as Chinese packing-grass is an annual, C4, shade-tolerant, mixed-mating system grass with gravity dispersed seeds that is native to Asia and was introduced into Tennessee, USA around 1919 (Fairbrothers & Gray 1972). This species is thought to have been introduced relatively few times due to no agricultural importance and its introduction is most likely the product of its use as a packing material before the invent of synthetic packaging materials. Since its introduction, *M. vimineum* has spread to the eastern United States south of New York and east of the Mississippi River. The ability to flourish in a variety of light conditions due to phenotypic plasticity (Cheplick 2006) as well as the ability to produce both self-fertilized and out-crossed seeds every generation makes *Microstegium vimineum* an especially invasive species. It has also been found to prevent forest re-generation as well as shade out native under-story plants and following a disturbance (Oswalt *et al*. 2007, Archibald-Shaw 2009). Gene flow within and among populations of *M. vimineum* is facilitated by wind for

pollination and by gravity for seeds, although there is anecdotal evidence that they can also be transported via runoff (Baker, pers. obs.). Seeds of *M. vimineum* may also remain in the seed bank for up to three (Barden 1987) years making eradication difficult and increasing the chances of anthropogenic introduction into previously un-colonized areas via soil movement. Due to the ability to rapidly colonize and proliferate in novel areas and how fast it has spread since introduction, *M. vimineum* is an ideal candidate to study the genetic consequences of rapid range expansion.

The objectives of this study were to examine the spatial distribution of genetic variation in *Microstegium vimineum* within the James River Basin (JRB) of Virginia. The use of the JRB as the research area is motivated by the methods which seeds are dispersed in order to form new populations. The specific questions addressed in this research were: 1) Is there significant genetic variation among the sampled populations in the JRB? A recent and relatively rapid expansion from the initial introduction may result in a very low level of overall genetic diversity. 2) Are populations in the JRB, all of which are relatively recent in origin, characteristic of a single or multiple introductions? Homogeneous structure at individual sites would suggest a single or few initial colonists followed by localized expansion whereas high levels of diversity within colonized sites would be indicative of multiple colonists or secondary colonization. 3) Across the JRB, are there general trends in the distribution of genetic structure that indicate the directionality of range expansion? The initial introduction into the United States was to the west of the study area so there is a general expectation that expansion has occurred from west to east.

Conversely, the JRB could have been colonized from several different lineages, thereby dampening apparent spatial expansion across the JRB.

Methods

Taxon Sampling

Microstegium vimineum was sampled from within the JRB, a 27,000km^2 watershed extending from the Alleghany Mountains in the western part of the Commonwealth of Virginia eastward to the Chesapeake Bay along the eastern coast. Sites outside of the basin were also chosen for sampling and were used as out-groups for later analyses. Sites (herein referred to as populations) for sample collection consisted of Virginia Commonwealth University's Rice Center for Environmental Science, state parks, wildlife management areas as well as roadsides. Sampling was performed on both sides of the James River to determine if there was evidence of the river acting as a barrier or corridor for range expansion. Each population was geo-referenced and complete individuals were haphazardly collected from within each location at intervals of two feet. Individuals were stored in plastic bags at -80°C until DNA extraction.

DNA Extraction

Genomic DNA was extracted using a DNeasy 96 Plant Kit by Qiagen (Valencia, CA, USA) using the protocol prescribed for frozen leaf tissue. Extracted DNA was purified via

ethanol precipitation by adding 67µL of 7.5M ammonium acetate to the genomic DNA and 400µL of 100% ethyl alcohol. The solution was chilled at -20°C for 20 min and then centrifuged at 2,400g in a Sigma-Aldrich 4-15°C centrifuge (St. Louis, MO, USA) for 10 min. The supernatant was discarded and 1mL of 70% ethyl alcohol was added to the precipitate and briefly centrifuged. The supernatant was discarded and the precipitate was allowed to air dry under a biological safety cabinet overnight. DNA was re-suspended in 50µL of ddH_2O and the concentration was determined using the Thermo Scientific Nanodrop 1000 (Waltham, MA, USA) and standardized with ddH_2O to a final concentration of 5ng/µL and stored at -20°C.

Genetic Markers & Genotyping

Purified genomic DNA was digested using *EcoRI* and *MseI* (New England Biolabs, Ipswich, MA, USA) for 3 h at 37°C using a master mix of 6.7µL of ddH_2O, 2µL of 10x *EcoRI* buffer (New England Biolabs, Ipswich, MA, USA), 0.2µL 100x BSA, 1µL *MseI* (10,000 U/mL) and 0.1µL *EcoRI* (100,000 U/mL) to 10µL genomic DNA per reaction. Following digestion, restriction enzymes were denatured for 20 min at 65°C.

Adapters for *EcoRI* and *MseI* were prepared by heating 100µL each of the respective forward and reverse adapters at 95°C for 5 min and allowed to cool slowly to room temperature. PCR adapters were ligated to the restriction sites of the digested DNA

using a ligation master mix consisting of 8.5µL ddH_2O, 4µL 10x ligase buffer (Bioline USA, Boston, MA, USA), 1.5µL 50µM *EcoRI* Adapter, 11.5µL 50µM *MseI* Adapter, 4µL 10x ATP (Bioline USA, Boston, MA, USA) and 0.5µL T4 DNA ligase (Bioline USA, Boston, MA, USA) per reaction. Digested DNA was ligated for 16 h at 16°C and then diluted 1:15 with ddH_2O.

Pre-selective amplification was performed by adding 2µL of ligation product to a master mix of 1.4µL ddH_2O, 5µL GoTaq® Green Master Mix (Promega, Madison, WI, USA), 0.8µL 5µM *Eco*+A primer, 0.8µL 5µM *Mse*+C (Table 1) primer per reaction. The pre-selective amplification had the following PCR profile: 75°C for 2 min, then 20 cycles of 94°C for 50 sec, 56°C for 1 min, 72°C for 2 min, with a final step of 60°C for 30 min. Amplified products were then diluted 1:5 with ddH_2O.

Four primer combinations were used for selective amplification *Eco-AGC*(5'-FAM)/*Mse-CAA*, *Eco-ACG*(TET)/*Mse-CAA*, *Eco-ACA*(5'-FAM)/*Mse-CAC* and *Eco-AAC*(TET)/*Mse-CAC* (Table 1). Selective amplification was performed by combining 4µL pre-selective amplification product, 4µL ddH_2O, 0.8µL 5µM *Eco*+3 primer fluorescently labeled with 5' FAM dye, 0.8µL 5µM *Eco*+3 primer fluorescently labeled with TET dye, 0.4µL *Mse*+3 primer and 10µL GoTaq® Green Master Mix (Promega, Madison, WI, USA). Selective amplification was carried out with an initial step of 94°C for 2 min, then a 19 cycle touchdown of 94°C for 30s, 65°C for 30s, reduced by one degree every cycle, and then 72°C for 2 min. Following the touchdown, 35 cycles of 94°C for 30s, 56°C for 30s,

72°C for 2 min with a final step of 60°C for 30 min. The four primer pairs were initially screened for reproducible polymorphic loci by amplifying eight individuals from four populations spread across the entire JRB.

Capillary gel electrophoresis was conducted on a MegaBACE 1000 (Amersham Biosciences Pittsburgh, PA, USA) with MegaBACE Instrument Control Software v2.5. Samples were prepared as follows: 0.5μL of a 1:1 dilution of ddH_2O to final amplified products, 8.65μL 0.1% Tween20, 0.35μL ET-Rox 550bp DNA size standard (Amersham Biosciences). Samples were run with the following conditions: injection voltage; 3kV, injection time; 130s, run voltage; 10kV, run time; 100 min, dyes; GT dye set 1) (ROX, FAM, TET and HEX).

Selective amplification products were sized using a ROX dye-labeled 550 base pair sizing standard. Electropherograms were analyzed with Fragment Profiler v1.2 (Amersham Biosciences). AFLP loci were determined by selecting peaks with an intensity of 200 relative fluorescent units (rfu) or higher in at least one individual. AFLP repeatability was assessed using the dual-tube method described in Bonin *et al*. (2004). Capillary electrophoresis was performed on samples from the same reaction three times to ensure consistency of genotype assignment and each genotype was verified by visual inspection in Fragment Profiler. AFLP genotypes were scored "1" for presence and "0" for absence of an allele for all loci with the lowest peak intensity at which an allele was distinguished from background noise was 70 rfu. Samples with genotype failures were re-amplified

from genomic DNA and were electrophoresed on 1.5% agarose gels at 80V for 3 h to verify the presence of AFLP products before capillary gel electrophoresis was re-run.

Within-Population Genetic Diversity

The amount of within-population genetic diversity was estimated from several complementary summary statistics. First, genetic diversity within populations was measured by percent polymorphic loci (*P*) and Shannon's information index (*I*; Shannon 1948) for each population using GENALEX 6.1 (Peakall & Smouse 2006). These two summarizing parameters provided measures of diversity that were used as indicators of demographic processes. Low levels of diversity were indicators of recent founder events or isolated populations and high levels of diversity were indicative of high levels of gene flow or secondary colonization. Next, statistical differentiation of within-population genetic diversity was determined by testing for the presence of heteroscedasticity (Dyer *in review*) using GENETICSTUDIO (Dyer 2009). This analysis tests the equality of within-population genetic diversity using a multi-locus approach.

Among-Population Differentiation

Spatial discontinuity of genetic structure among populations was tested using STRUCTURE 2.2 (Pritchard *et al*. 2000). STRUCTURE was run both with out-groups to determine if expansion has been continuous from outside of the basin as well as without out-groups to ascertain if the JRB has an effect on gene flow. STRUCTURE parameters were: 100,000 iteration burn-in, 1,000,000 iterations run length, all other parameters set to default for K=1-20 clusters with each K run twice. Discontinuity determined by STRUCTURE were used to determine if geographic features (e.g., James River) function as either barriers to range expansion or ongoing sources of vicariance reducing or preventing gene flow.

Next, genetic distance among populations was calculated using GENALEX for each pair-wise population using Nei's genetic distance (Nei 1978) and subsequently used to construct a neighbor-joining tree using PHYLIP 3.66 (Felsenstein 1993). Both an un-rooted neighbor-joining tree and a rooted tree using populations collected outside the JRB were constructed. Clades in the neighbor-joining tree were compared to clusters inferred from STRUCTURE for congruence.

Finally, a hierarchical analysis of molecular variance (AMOVA, Excoffier *et al*. 1992) was used to analyze among-strata genetic differentiation on populations as well as clusters determined by STRUCTURE. For each pair-wise population and cluster, regional and population differentiation (Φ_{ST}, Φ_{RT} and Φ_{SR}) were calculated using GENETICSTUDIO and used as indicators of among-population, among-cluster and among-population within

cluster differentiation. Individual clusters were also tested for population differentiation by AMOVA in order to estimate the amount of gene flow. Pair-wise Φ_{ST} values for populations were calculated using GENETICSTUDIO and were used as a measure of differentiation between populations in conjunction with pair-wise genetic distances. Hierarchical AMOVA was performed with and without out-groups to determine if the genetic differentiation seen was influenced by populations outside of the basin.

Range Expansion

To examine localized gene flow that may be occurring after colonization, spatial auto-correlation was tested using GENALEX to resolve the distance at which population correlation became insignificant. These distances are informative for determining if there is spatial genetic structure indicative of gene flow between neighbor populations in addition to any patterns of overall long-distance dispersal. All populations within the JRB were analyzed for spatial auto-correlation and spatial auto-correlation was also tested for populations within their respective cluster to determine the distances of gene flow and the possibility of long-distance dispersal. Isolation-by-distance (IBD) was assessed to determine if genetic dissimilarity seen among populations is a function of the spatial distances between them. Significance for IBD was determined by Mantel tests (Mantel 1967) using Nei's genetic distance and geographic distance.

Each cluster of populations and all separate populations were tested to determine whether expansion has been continuous across the basin in a stepping-stone pattern or if it has been partitioned by multiple introductions or long-distance dispersal. If a significant correlation between genetic distance and geographic distance was detected, a best-fit regression test was performed for diversity parameters against longitude and latitude to determine directionality of range expansion. Also, because the JRB is not oriented strictly in a west-east direction, a principal coordinate rotation of the spatial coordinates was also performed. These rotated coordinates were used to determine if the clusters identified by STRUCTURE were spatially distributed along an axis in the JRB. The expectation was that range expansion has occurred in a west to east direction corresponding with a one-dimensional expansion from the initial origin of invasion in Tennessee, USA.

The degree to which spatial structure is influencing the distribution of among-population genetic variance was determined by a Step-wise analysis of molecular variance (STAMOVA; Dyer *et al*. 2004). Here the effects of spatial location were removed as a covariate prior to estimating the degree of among population differentiation, $\Phi_{ST|Spatial}$. Differences between the uncorrected, Φ_{ST}, and the one corrected for spatial location were estimated using GENETICSTUDIO (Dyer 2009). The effect of spatial location on genetic variation was also tested for each cluster identified by STRUCTURE.

Lastly, a Population Graph was created to describe the manner in which genetic variation is distributed amongst populations (see Dyer 2009). The resulting network

topology was used to infer which populations may have in the past or are still experiencing gene flow and for assessing congruence with clusters identified by STRUCTURE. The genetic distance between populations in the network were then regressed on a physical separation (using a Mantel approach; see Garrick 2009) to estimate isolation-by-graph-distance (IBGD), which is similar to the IBD estimates above, but potentially more informative as it takes into consideration the genetic covariance among all populations conditional on their covariance with all other populations, whereas IBD takes populations in a pair-wise fashion ignoring the covariance of the remaining populations. The analysis of IBGD also provides an indication of which subset of edges may be spatially extended or compressed given the genetic covariance among populations pairs. Extended edges (e.g., pairs of populations that are further apart spatially than expected given their genetic covariance; Garrick *et al*. 2009) are consistent with a scenario of long-distance dispersal and/or high rates of gene flow. Conversely, compressed edges (e.g., pairs of populations that are spatially more proximate than expected by their genetic covariance) are representative of conditions where intervening landscape features may be preventing the dispersal of genes.

Results

Taxon Sampling

Within the JRB, 24 populations were found (Table 2; Figure 1) from which 370 individuals were collected. From outside of the basin 74 individuals were collected from three sites outside of the JRB. Two of the out-group populations (CNA and MNSP) were north of the central basin (approximately 60km and 90km respectively). Another out-group population (PUL) was collected from a region 45km southwest of the western-most reach of the JRB. Of all 27 populations, 11 were from state parks, seven were along roadsides, six were from wildlife management areas, one from a county park, one from the VCU Rice Center, and one from a national wildlife refuge.

Genetic Markers & Genotyping

From the 444 individual specimens collected, all loci were successfully amplified repeatably for 359 individuals (80%). Using the two primer combinations (*Eco-AGC/Mse-CAA* and *Eco-ACG/Mse-CAA*, Table 2) 52 loci were polymorphic across all populations and 36 loci (69%) were reproducible. The primer pair *Eco-AGC/Mse-CAA* yielded 11 polymorphic loci between the ranges of 120-160 base pairs, the *Eco-ACG/Mse-CAA*

primer pair yielded 25 polymorphic loci ranging from 109 to 472 base pairs. A total of 8122 bands were scored from the 36 loci of both primer combinations. All loci were polymorphic across all populations with the exception of one private allele found only at out-group site CNA (n=22).

Within-Population Genetic Diversity

The overall mean percent polymorphic loci (P) was 47.94% $\pm$ 3.06% SE, but there was a wide range of values for percent polymorphic loci with the highest percentage at site PSP (P=77.78%) and the lowest at BCWMA (P=19.44%; Table 3). The mean diversity measured by Shannon's I was 0.264 $\pm$ 0.010SE, with the highest observed at TLSP (I=0.380) and the lowest at BCWMA (I=0.089; Table 3). Across all populations, there were significant differences in within population diversity (e.g., heteroscedasticity; p<0.005, F=21.694; Table 4). Tukey's HSD post-hoc test on within-population genetic diversity showed that not all populations significantly differed in diversity (Table 5). Of particular interest was BCWMA where diversity was similar to that observed in AC, HI, BV, HLSP, BBNWR and JRWMA. PSP was significantly more diverse than all populations except AMELC, CHP, NQP and PUL.

Among-Population Differentiation

Fifteen clusters were inferred using STRUCTURE (p=0.0320) when all populations, including the out-groups (CNA, MNSP, PUL) were included in the data set. However, when the out-groups were removed and only the sites within the JRB were considered, STRUCTURE suggested only three clusters (p=0.0421, Figure 2). Within the confines of the JRB the three clusters grouped into geographically proximate regions and were grouped as: (1) West region consisting of 12 populations: PWMA, BCSP, HLSP, AC, BV, BCWMA, AMC, DSP, FFWMA, JRWMA, JRSP and HI with the eastern most population being HI 120km east of the nearest west region population. (2) The central region consisted of only two populations: TLSP and SCSP separated by 16km, and (3) An eastern region that consisted of 10 populations: PSP, NQP, YRSP, YHS, CHP, AMELC, BBNWR, CC, CWMA, RICE with the western most population being AMELC, 27km west of the nearest east region population and 34km away from the nearest west region population. Two populations, HI in the western region and PSP in the eastern region, showed a high degree of admixture. This admixture resulted in HI being most likely placed in the western region but with non-insignificant components of the eastern region and PSP being placed in the eastern region but with components of the western and central regions. The remaining populations did not have appreciable levels of admixture between the three putative regional groups.

Nei's genetic distances (Table 6) ranged from 0.013 to 0.437 and were greater between populations from different regions than between populations from within the same region (mean within: West=0.07, Central=0.08, East=0.07; mean among: West-Central=0.30, West-East=0.24, Central-East=0.23). The neighbor-joining tree (Figure 3) contains three general clades matching the three regions inferred by STRUCTURE. The neighbor-joining tree did not exhibit a pattern of serial nestedness as would be predicted under a one-dimensional stepping-stone model of range expansion. Instead, populations that were spatially proximate were not necessarily nearest neighbors on the tree.

Among-population genetic structure within the JRB was significant at all spatial scales (Table 7). Using the hierarchical clustering of populations suggested by STRUCTURE, significant differences were found in the genetic structure among regions (Φ_{RT}=0.4270; p<0.005), among populations within regions (Φ_{SR}=0.2016, p<0.005), and among all populations (Φ_{ST}=0.5426, p<0.005). When out-groups were considered in the hierarchical AMOVA, the results were not dissimilar (Φ_{ST}=0.5445, Φ_{RT}=0.4265, Φ_{SR}=0.2057) suggesting that the ancestry of out-group populations is not significantly different than that for populations sampled within the JRB. Taken as a pair-wise analysis independent of the regional affiliations, all pairs of populations were significantly differentiated (Φ_{ST} ranged from 0.215-0.827). Within the west and east regions there was also significant structure among populations $\Phi_{ST\,|\,West}$=0.1463 and $\Phi_{ST\,|East}$=0.2225.

Range Expansion

Localized spatial genetic structure, as measured by spatial auto-correlation, was found throughout the data. Across the JRB there was significant spatial auto-correlation among populations up to a distance of 40km (Figure 4). Within region auto-correlated patterns were also observed but with smaller distance classes. The west region exhibited significant spatial auto-correlation among pairs of populations separated by a distance of 10km ($p<0.05$, Figure 5) and a similar, albeit less intense pattern of spatial auto-correlation existed among the populations sampled from the eastern region, where spatial auto-correlation was found up to a pair-wise distance among populations of 5km ($p<0.001$, Figure 6). Auto-correlation was not estimated in the putative central region due to the small number of populations ($K=2$).

The hypothesis of isolation-by-distance, which would be consistent with a history of range expansion, was present for all populations in the JRB when examining the correlation between pair-wise geographic distance and pair-wise genetic distance, as estimated by Nei's genetic distance (Mantel $Z=167.55$, $p=0.001$, $\rho=0.4226$). When considering populations within regional designations, there were conflicting results. There was a significant correlation between genetic distance and geographic distance in the west region (Mantel $Z=11.00$, $p<0.05$, $\rho=0.6286$); however, there was not a significant correlation in the east region (Mantel $Z=4.75$, $p=0.290$, $\rho=0.0781$).

When examining patterns of diversity along spatial gradients (rather than as a function of pair-wise spatial separation) there was no relationship between longitude and Shannon's Diversity Index *I* (F=1.8842, p=0.1821) or *P* (F=2.8695, p=0.1027) for all populations within the JRB. Similarly, there was no relationship between longitude and *I* or *P* in the west region (p=0.3569 and p=0.3015) or in the east region (p=0.9922 and p=0.927). Not surprisingly given the orientation of the JRB and the locations of sampled populations, there were also no relationships between latitude and *I* or *P* at the level of all populations (p=0.0719, F=3.5751 and p=0.1660, F=2.0531, respectively), or among populations in the eastern region (p=0.4349, F=0.6757 and p=0.5010, F=0.4967, respectively). However, there was a significant relationship in the west region between latitude and genetic diversity for both Shannon's *I* and *P* (p=0.0269, F=6.7139, R^2=0.3419, Figure 7 and p=0.0232, F=7.168, R^2=0.3593, Figure 8). Taking latitude and longitude together as a principal coordinate rotation of the spatial coordinates did not reveal any general trends in diversity for *I* or *P* along the entire JRB (p=0.1422 and p=0.2677), within the western region (p=0.1634 and p=0.1158), or within the eastern region (p=0.9439 and p=0.9841).

Given the potential for spatial influences on observed genetic structure, the StAMOVA model was used to determine what proportions of the observed genetic structure (Φ_{ST}) could be explained by spatial structure. Removing the effects of spatial coordinates as a covariate reduced the observed differentiation among populations in the JRB by 9% (Φ_{ST}=0.5445 vs. $\Phi_{ST|Spatial}$=0.4392 ; Table 8). The west and east regions had

much lower levels of variation attributed to spatial structure with 0.27% and 0.33% contributing to increases in genetic structure within the regional partitions, which is consistent with the results from the STRUCTURE and correlation analyses.

The distribution of genetic covariance among populations as depicted in the Population Graph revealed a topology that was elongated along the longitudinal axis and exhibited a compacted topology within putatively identified regions (Figure 9). This topology is consistent with increased gene flow within but not among regions. The mean number of edges, where an edge denotes a pair of populations that have a significant genetic covariance was four (Dyer 2007). A single population (PUL) had only one edge (a pendent) and two populations had six connections (YHS, BV) suggesting a high degree of genetic covariance with spatially proximate populations. The west and east regions identified by STRUCTURE were connected through only two populations (PSP, HI). The relationship between edge lengths, a measure of genetic covariance, and spatial separation among-populations identified extended edges between DSP-CNA, AC-PWMA, AC-HI, BV-HI and CC-BBNWR, which would be consistent with the hypothesis of a long-distance dispersal (Figure 9). Conversely, compressed edges between MNSP-CNA, AMELC-CC, AMELC-PSP and TLSP-SCSP (Figure 9) were found to be connecting populations that were more spatially proximate than expected given the genetic covariance indicating locations of potential intervening vicariance.

Discussion

Fine-scale consequences of range expansion

Fine-scale patterns of genetic diversity produced from rapid range expansion are predicted to be influenced by founder events, bottlenecks and gene flow. The levels of genetic diversity within the JRB vary with some populations being genetically depauperate while others were surprisingly diverse. The overall mean *P* for all populations was 47.94% $\pm$ 3.06 SE (Table 3) which is higher than expected based on introduction history and mating system. The level of diversity found was closer to that of plants that are obligate out-crossers (Hamrick *et al*. 1979) suggesting that although *M. viminem* has the ability to self-fertilize, there is substantial reproduction by out-crossing within the JRB. Recent founder events, suggested by populations with low genetic diversity, are also present within the JRB.

Within the basin there are three populations that exhibit clear indications of recent founder events. In the east region, CWMA exhibited low overall genetic diversity (*I*=0.176, *P*=27.78%, Table 3) and significant genetic covariance with four spatially proximate populations, all of which were located within 30km. This pattern is consistent with either a history of colonization followed by localized dispersal and/or gene flow, or it may be an artifact of relatively recent colonization of the sites by individuals from the

same origin. Both of these scenarios are consistent with the degree of short distance spatial auto-correlation observed in the eastern region (5km; Figure 6). It appears that CWMA also had significantly less within-population multivariate diversity as determined by the heteroscedasticity than one of the neighboring populations (NQP), the remaining three were more diverse suggesting that although CWMA is the product of a recent founder event, there might be other less recent founder events that also have occurred in the east region as well (Table 5).

Another population, BCWMA, located in the west region also exhibited a pattern of genetic structure that is consistent with a recent founder event only with less diversity and admixture than CWMA. The level of diversity found at BCWMA was the lowest of all populations examined with only seven polymorphic loci (19%), that is consistent with a founding by a few individuals. Even with this low level of within-population diversity, it has significant genetic covariance with three geographically proximate populations (AMC, PWMA, AC; Population Graph, Figure 9). Similar to the scenario for CWMA, this among-population covariance could be the result of gene flow from the initial local introduction as spatial auto-correlation in this region was measured up to 10km (Figure 5). It is also possible, though less likely, that this population is the product of successive long-distance dispersal events. There is an extended edge, suggesting long-distance dispersal, on the Population Graph from AC to PWMA (Figure 9), and a subsequent (though not extended) connection between PWMA and BCWMA. This observation indicates that BCWMA could be the result of a local dispersal event following a long-distance

colonization. It is notable that BCWMA has a very low level of within-population genetic diversity as well as lower genetic distance when compared to populations 50-130km away (e.g., AC, AMC and PWMA) rather than with those more spatially proximate. Amsellem *et al*. (2000) found a similar pattern for populations of *Rubus alceifolius* on islands in the Indian Ocean that were founded by a population in Madagascar that was in turn founded by populations in the native range. Interestingly, the closest population to BCWMA, TLSP, is only 15km away, yet there is no direct connection or strong signal of admixture indicating that like CWMA, this is a recent founder event and secondary contact after introduction has not occurred.

Fine-scale genetic analysis also yielded an interesting result from one population located in the east region of the JRB. The population HI, located approximately 120km east of the nearest population belonging to the west region, shows evidence of a founder event originating in the western region. This population had moderately low levels of diversity (Table 3) but showed high levels of admixture in STRUCTURE between the east and west clusters (Figure 2). This admixture of clusters and the presence of an extended edge between two populations in the west region on the Population Graph are indicative of gene flow, yet these populations are separated by over 250km (Figure 9). A possible explanation for this admixture and similarity with two western populations is that there has been a long-distance dispersal from the west region into the east and secondary contact has occurred following this event. The long-distance dispersal and subsequent secondary contact by HI has implications for range expansion of these two clusters.

Large-scale consequences of range expansion

The genetic structure following a rapid range expansion of an invasive species is influenced by multiple introductions from the native range (Pappert *et al*. 2000), long-distance dispersal and gene flow (Wilson *et al*. 2009). Multiple introductions may leave behind patterns similar to long-distance dispersal by native species from populations at the core of their range that are higher in diversity. However for comparison to native species, long-distance dispersal of invasive species does not necessarily have to be from a core or more diverse population. Within the JRB, three separate clusters were inferred from STRUCTURE (Figure 2) suggesting some biologically meaningful barriers to gene flow between groups of populations. However, between these regions there is no obvious geographic barrier such as a river or mountain.

The regional partitioning seen in *M. vimineum* is more likely due to a zone of secondary contact. All of the extended edges in the Population Graph (Figure 9) are aligned on the axis of the JRB. Although these connections alone do not imply directionality (e.g., the edges are based upon covariance that is symmetric between populations), it is consistent with a historical demography where the ancestors of the populations in the western region were colonizing the JRB and a separate colonization process was occurring from the south and entered the JRB on its eastern end. Two lines of reason support the contention that the east region is a product of dispersal from outside of the basin followed by expansion within the basin. First, there cannot be expansion from

the east due to an ocean and second, expansion from the west would have resulted in STRUCTURE identifying populations in the east region as belonging to the western cluster. Although the exact geographic origin of introduction or founding population(s) cannot be identified from the present data there is some support that PUL, a population outside of the basin in southwestern Virginia over 200km away, is part of a cluster that is similar to the eastern cluster. PUL is in the clade with the east region in the NJ tree (Figure 3) and clusters with the east in the STRUCTURE results. The results of STRUCTURE, the NJ tree, and the Population Graph (Figures 2, 3, and 9) are consistent with this notion of secondary contact between both western and eastern regions in the central JRB. Clustering in these groups was strong for all populations except for HI, which is of apparent admixture decent that was geographically located in the east region but belonging to the west region. Genetic distances and pair-wise Φ_{ST} within regions were smaller than genetic distances and Φ_{ST} between populations in different regions except for HI, further supporting the clustering indicated by STRUCTURE and the NJ tree (Table 6). This result of strong clustering of populations into regions is surprising in that range expansion along the JRB was presumed to be continuous rather than being the result of separate introductions.

If the JRB has a recent history of two or more introductions, it is surprising that these lineages have not experienced extensive gene flow that would homogenize the variation among them. Perhaps the invasion is still very young and has yet to experience high rates of gene flow across this contact zone. Anecdotally, it has been observed that the invasion of *Microstegium vimineum* into the VCU Rice Center, a population from which

specific monitoring of this particular species has been conducted over the past four years, has expanded significantly in abundance. This population is located in the central JRB and its recent expansion into most of the secondary forest understory is consistent with the hypothesis that the invasion is relatively recent as it is still displacing local species.

Further support that range expansion in the east region has not been uni-directional or continuous is the lack of significant isolation-by-distance and multiple founder events evident from low diversity in a couple of populations (Table 3). Slatkin (1993) postulated that populations that do not exhibit isolation-by-distance when their dispersal methods would contribute to this pattern suggests a recent introduction. The lack of isolation-by-distance can be due to either multiple long-distance dispersal events or one introduction followed by multiple shorter 'jumps' contributing to a diffuse spread of *M. vimineum*. There was also low among-population differentiation for all populations in the east region (Φ_{ST}=0.2225) with only 0.33% of genetic variation observed due to spatial variation ($\Phi_{ST|Spatial\ East}$=0.2192). Although range expansion in the east region has not been uni-directional or continuous, there are signs of secondary contact with the west region.

In the west region, the pattern of range expansion is influenced by the same processes that are exhibited by the east region (i.e., founder events, high connectivity between populations and potential 'jumps'); however, spatial auto-correlation was significant up to a distance of 10km (Figure 5) indicating that gene flow may be occurring

at a larger distance than in the east. Also, expansion is more continuous than diffuse as evident by significant isolation-by-distance. BCWMA, a population on the eastern front of the west region is the least diverse due to a founder event as discussed earlier. BCWMA is not the only population to show signs of founder events. HI, a population located geographically in the east region was identified as belonging to the western cluster by STRUCTURE (Figure 2). Unlike BCWMA, there is no evidence for nested founder events but instead from one long-distance dispersal event from either AC or BV as indicated by the presence of the two extended edges on the Population Graph (Figure 9).

The genetic consequences of gene flow over greater distances were apparent in two ways. First, in the west region there is evidence of a long-distance dispersal event to HI, PWMA and CNA (outside of the basin) increasing the distance at which gene flow occurs. Second, analysis of molecular variance exhibited less population differentiation than the east region (Φ_{ST}=0.1463 vs. Φ_{ST}=0.2225) that could eventually lead to homogenization of the gene pool in the west region.

Although there are a few similarities between the east and west regions for the pattern of range expansion there is also a stark difference. In the east, range expansion was not continuous or uni-directional, the opposite seems to be the explanation for the west region. Expansion in the west region has been continuous as evident by positive correlation between genetic and geographic distances indicating the presence of isolation-by-distance. Also, continuous expansion in the west region has been in a north to south

direction along the JRB as evident by a positive correlation for decreasing *I* and *P* from north to south (Figures 7 & 8).

It is also possible that due to the directionality of expansion of the west cluster and lack of direction in the east cluster has led to this pattern of asymmetric gene flow. The admixture seen in populations along the border of the east and west regions (i.e., PSP and PWMA, Figure 2) is similar to that found by Caetano *et al*. (2008) where secondary contact in a clustered region between two divergent lineages of *Astronium urundeuva* was interpreted as supporting the notion of secondary contact. The patterns of range expansion exhibited by the east and west regions are good indicators of the genetic consequences of range expansion on two temporal scales. The west region is an example of an older lineage in the JRB undergoing continuous expansion along a geographic corridor and acting as source for diversity to other regions. The east region on the other hand, is a prime example of a newly founded lineage undergoing demographic processes associated with the colonization of a novel area.

The central region consists of only two populations but there is evidence that this lineage is more extensive outside of the basin. These two populations found in the central area of the JRB were found along with another population north of the basin that were all clustered together by STRUCTURE as well as in the NJ tree. These three populations exhibit lower genetic distances and population differentiation among themselves than with other populations in the other two regions. In the Population Graph, compressed edges

connecting these populations further supports the notion that the central region may be a third lineage invading the JRB. However, outside of the clustering and long-distance dispersal events, inferring expansion of this lineage without adequate sampling outside of the JRB would be speculation.

Conclusion

The genetic consequences of rapid range expansion are varied and a model to understand how these processes interact and the genetic structure produced on the landscape level is essential. In the scenario presented here, the variation observed within *M. vimineum*, was consistent with a history of founder effects, varying rates and distances of gene flow, long-distance dispersal and multiple introductions. Founder effects within the JRB created populations that may remain genetically depauperate if gene flow is not increased. For most species, low levels of genetic diversity can have long-term evolutionary consequences for these populations and possibly even the entire species.

Small, genetically similar populations are at a higher risk of genetic drift acting to fix allele frequencies in populations that can have either beneficial or detrimental consequences. If newly founded populations have levels of diversity similar to those found in this study for loci under selective pressure then subsequent inbreeding could result in a beneficial purging of deleterious alleles (e.g., Parisod & Bonvin 2008). Alternatively, low levels of genetic diversity for loci under selective pressure could result in an inability to adapt to changing conditions. However, because *M. vimineum* has evolved the ability to produce both selfed and out-crossed seeds, this low level of diversity may not be as detrimental as it would for other, predominantly out-crossing species. Indeed, at present, even with these low levels of within-population genetic diversity, this

species appears to be very successful in out-competing and marginalizing native understory species.

Each of these processes (e.g., founder events, multiple introductions, secondary contact and long-distance dispersal) has different consequences as demonstrated by *Microstegium vimineum* in the JRB and it is these consequences that are of interest for the conservation of native species. As global temperatures change, plant populations will shift their ranges poleward and upward in elevation (Parmesan 2006) and to determine how these native plants are affected, we can use the information from *M. vimineum* and how populations of this grass has behaved as it has expanded its range. As native plants expand the overall genetic structure that is produced is one that is influenced by the landscape as demonstrated by the longer distances of gene flow within the JRB for *M. vimineum*.

Another pattern demonstrated is that of patchiness of genetic identity where similar genotypes are distributed across the landscape in patches or clusters. This patchiness in conjunction with relatively low rates of gene flow and few dispersal events leads to isolation, vicariance and the continuation of patchiness of similar genotypes. Ibrahim *et al.* (1996) showed that species with leptokurtic dispersal processes such as gravity dispersed seeds, leads to patchiness or clustering of populations with similar allelic frequencies. This patchiness can persist for long periods of time (over 600 generations). Whereas high rates of gene flow may serve to increase the rate of homogenization to eliminate the pattern of

patchiness created. As a result, it is likely that the course granularity of genetic structure observed in this species may be present in future populations for many generations.

Although this study has examined the genetic consequences of rapid range expansion for an invading species, these results are applicable to native species undergoing range expansion in response to global temperature change. However, there is still more research that can be conducted to make more accurate and realistic conservation and management plans. Ward (2006) stresses the importance of research along many scales in order to fully understand the dynamics of a species and the population genetics associated with it. This recommended course will help us to understand the consequences of range expansion across different spatial scales. A finer scale approach over multiple years would benefit our understanding in how low within-population genetic diversity following a founder event is ameliorated over time and at what rate with respect to mating system.

Large-scale dynamics at the global level that encompasses the leading and lagging zones of a range expansion would also be important to understand. Does the lagging zone of range expansion experience similar effects or does adaptation play a role in the survival leaving only those individuals that are suited for the changing environment in existence and if so would gene flow between these populations play a role in the remnant populations? Study into other plant species would also be a way to further our knowledge of the genetic consequences of rapid range expansion. *Microstegium vimineum* is an annual grass with a mixed-mating system that is suitable for understanding how other

plants with similar characteristics will behave but it is not suitable for plants with other mating systems. Research on the genetic consequences of range expansion for long-lived perennials on temporal scales shorter than those used in phylogeography could also aid in our understanding of the immediate consequences that will shape the longer lasting patterns that we see when we look at post-pleistocene range expansion.

Literature Cited

Amsellem L., J.L. Noyer, T. Le Bourgeois and M. Hossaert-McKey. 2000. Comparison Of Genetic Diversity Of The Invasive Weed *Rubus alceifolius* Poir. (Rosaceae) In Its Native Range And In Areas Of Introduction, Using Amplified Fragment Length Polymorphism (AFLP) Markers. *Molecular Ecology*. 9: 443-455.

Archibald-Shaw, R. 2009. Shrinking the Janzen-Connell Doughnut: Consequences of an Invasive Multiplier (*Microstegium vimineum*) on the Mid-canopy in a Mixed Pine-Oak Forest. (M.S. Thesis, Virginia Commonwealth University).

Austerlitz F., B. Jung-Muller, B. Godelle and P-H. Gouyon. 1997. Evolution Of Coalescence Times, Genetic Diversity And Structure During Colonization. *Theoretical and Population Biology*. 51: 148–164.

Avise, J.C. 2004. What is the Field of Biogeography, and Where is it Going? *Taxon*. 53: 893-898.

Baker H. 1974. The Evolution of Weeds. *Annual Review of Ecology and Systematics*. 5: 1-24.

Barrett S.C., R.I. Colautti and C.G. Eckert. 2008. Plant Reproductive Systems and Evolution During Biological Invasion. *Molecular Ecology*. 17: 373-383.

Bonin A., E. Bellemain, P. Bronken Eidesen, F. Pompanon, C. Brochmann and P. Taberlet. 2004. How to Track and Assess Genotyping Errors in Population Genetics Studies. *Molecular Ecology*. 13: 3261-3273.

Besnard G., P. Henry, L. Willw, D. Cooke and E. Chapuis. 2007. On The Origin of The Invasive Olive (*Olea europaea* L., Oleaceae). *Heredity*. 99: 808-819.

Caetano, S. D. Prado, R.T. Pennington, S. Beck, A. Oliviera-Filho, R. Spichiger and Y. Naciri. 2008. The History of Seasonally Dry Tropical Forests in Eastern South America: Inferences from the Genetic Structure of the Tree *Astronium urundeuva* (Anacardiaceae). *Molecular Ecology*. 17: 3147-3159.

Cheplick G.P. 2006. A Modular Approach to Biomass Allocation in an Invasive Annual (*Microstegium vimineum*; Poaceae). *American Journal of Botany*. 93: 539-545.

Dyer, R.J. and J.D. Nason. 2004. Population Graphs: the Graph Theoretic Shape of Genetic Structure. *Molecular Ecology*. 13: 1713-1727.

Dyer, R.J., R.D. Westfall, V.L. Sork and P.E. Smouse. 2004. Two-Generation Analysis Of Pollen Flow Across A Landscape V: A Stepwise Approach For Extracting Factors Contributing To Pollen Structure. *Heredity*. 92: 204-211.

Dyer R.J., 2009. GENETICSTUDIO: A Suite Of Programs For Spatial Analysis Of Genetic-Marker Data. *Molecular Ecology Resources*. 9: 110-113.

Excoffier L., P.E. Smouse and J.M. Quattro. 1992. Analysis of Molecular Variance Inferred from Metric Distances Among DNA Haplotypes: Applications to Human Mitochondrial DNA Restriction Data. *Genetics*. 131: 479-491.

Excoffier L. and N. Ray. 2008. Surfing During Population Expansions Promotes Genetic Revolutions and Structuration. *Trends in Ecology and Evolution*. 23:347-351.

Fairbrothers D.E. and J.R. Gray. 1972. *Microstegium vimineum* (Trin.) A. Camus (Gramineae) in the United States. *Bulletin of the Torrey Botanical Club*. 99: 97-100.

Felsenstein J. 1993. PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle.

Garrick R.C., J.D. Nason, C.A. Meadows, R.J. Dyer. 2009. Not Just Vicariance: Phylogeography of a Sonoran Desert Euphorb Indicates a Major Role of Range Expansion Along the Baja Peninsula. *Molecular Ecology*. 18: 1916-1931.

Gilchrist G.W., R.B. Huey, J. Balanyà, M. Pascual and L. Serra. 2004. A Time Series Of Evolution In Action: A Latitudinal Cline In Wing Size In South American *Drosophila subobscura*. *Evolution*. 58: 768-780.

Grabherr G., M. Gottfried and H. Pauli. 1994. Climate Effects on Mountain Plants. *Nature*. 369: 448.

Hamrick, J.L., Y.B. Linhart and J.B. Mitton. 1979. Relationships Between Life History Characteristics and Electrophoretically Detectable Genetic Variation in Plants. *Annual Review of Ecology, Evolution and Systematics*. 10: 173-200.

Hamrick J.L. and M.J.W. Godt. 1996. Effects Of Life History Traits On Genetic Diversity In Plant Species. *Philosophical Transactions: Biological Sciences*. 35: 1291-1298.

Hengeveld R. 1989. Dynamics of Biological Invasions. Chapman and Hall: New York, New York.

Hewitt G. 2000. The Genetic Legacy Of The Quaternary Ice Ages. *Nature*. 405: 907–913.

Huff, D.R., R. Peakall, P.E. Smouse. 1993. RAPD Variation Within and Among Natural Populations of Outcrossing Buffalograss *Buchloe dactyloides* (Nutt) Engelm. *Theoretical and Applied Genetics*. 86: 927-934.

Ibrahim, K.M., R.A. Nichols, G.M. Hewitt. 1996. Spatial Patterns of Genetic Variation Generated by Different Forms of Dispersal During Range Expansion. *Heredity*. 77: 282-291.

van Kleunen M., M. Fischer and S.D. Johnson. 2007. Reproductive Assurance Through Self-Fertilization Does Not Vary With Population Size In The Alien Invasive Plant *Datura stramonium*. *Oikos*. 116: 1400-1412.

Klopfstein, S., M. Currat, L. Excoffier. 2006. The Fate of Mutations Surfing on the Wave of a Range Expansion. *Molecular Biology and Evolution*. 23: 482-490.

Mantel N. 1967. The Detection of Disease Clustering and a Generalized Regression Approach. *Cancer Research*. 27: 209-220.

Mayr E. 1963. Animal Species and Evolution. Cambridge, MA: Harvard Univ. Press.

McLachlan J.S., J.S. Clark and P.S. Manos. 2005. Molecular Indicators of Tree Migration Capacity Under Rapid Climate Change. *Ecology*. 86: 2088-2098.

Nason J.D., J.L. Hamrick and T.H. Fleming. 2002. Historical Vicariance and Postglacial Colonization Effects On The Evolution of Genetic Structure In *Lophocereus*, A Sonoran Desert Columnar Cactus. *Evolution*. 56: 2214-2226.

Nei, M. 1978. Estimation of Average Heterozygosity and Genetic Distance from a Small Number of Individuals. *Genetics*. 89: 583-590.

Novak S.J. and R.N. Mack. 2001. Tracing Plant Introduction and Spread: Genetic Evidence from *Bromus tectorum* (Cheatgrass). *Bioscience*. 51: 114-122.

Oswalt C.M., S.N. Oswalt and W.K. Clatterbuck. 2007. Effects of *Microstegium vimineum* (Trin.) A. Camus on Native Woody Species Density and Diversity in a Productive Mixed-Hardwood Forest in Tennessee. *Forest Ecology and Management*. 2442: 727-732.

Pappert R.A., J.L Hamrick and L.A. Donovan. 2000. Genetic Variation in *Pueraria lobata* (Fabaceae) An Introduced, Clonal, Invasive Plant Of The Southeastern United States. *American Journal of Botany*. 87: 1240-1245.

Parisod C. and G. Bonvin. 2008. Fine-Scale Genetic Structure And Marginal Processes In An Expanding Population of *Biscutella laevigata* L. (Brassicaceae). *Heredity*. 101: 536-542.

Parmesan, C. 2006. Ecologically and Evolutionary Responses to Recent Climate Change. *The Annual Review of Ecology, Evolution and Systematics*. 37: 637-669.

Peakall, R. and P.E. Smouse. 2006. GENALEX 6: Genetic Analysis in EXCEL. Population Genetic Software for Teaching and Research. *Molecular Ecology Notes*. 6: 288-295.

Pritchard, J.K., M. Stephens and P. Donnelly. 2000. Inference of Population Structure Using Multilocus Genotype Data. *Genetics*. 155: 945-959.

Shannon C.E. 1948. A Mathematical Theory of Communication. *Bell System Technical Journal*. 27: 379-423 and 623-656.

Slatkin, M. 1993. Isolation by Distance in Equilibrium and Non-equilibrium Populations. *Evolution*. 47:457-462.

Suarez A.V., D.A. Holway and T.J. Case. 2001. Patterns of Spread in Biological Invasions Dominated by Long-Distance Jump Dispersal: Insights from Argentine Ants. *Proceedings of the National Academy of Science USA*. 98: 1095-1100.

Taberlet P., L. Fumagalli, A. G. Wust-Saucy and J. F. Cosson. 1998. Comparative Phylogeography And Postglacial Colonization Routes In Europe. *Molecular Ecology*. 7: 453–464.

Ward, S. 2006. Genetic Analysis of Invasive Plant Populations at Different Spatial Scales. *Biological Invasions*: 541-552.

Williams D.A., E. Muchugu, W.A. Overholt and J.P. Cuda. 2007. Colonization Patterns of the Invasive Brazilian Peppertree, *Schinus terebinthifolius*, in Florida. *Heredity*. 98: 284-293.

Wilson, J.R.U, E.E. Dormontt, P.J. Prentis, A.J. Lowe and D.M. Richardson. Something in the Way You Move: Dispersal Pathways Affect Invasion Success. *Trends in Ecology and Evolution*. 24: 136-144.

van de Wouw M., P. van Dijk and Ad.H.L. Huiskes. 2008. Regional Genetic Diversity Patterns In Antarctic Hairgrass (*Deschampsia antarctica* Desv.). *Journal of Biogeography*. 35: 365-376.

Wright, S. 1943. Isolation By Distance. *Genetics*. 28: 114-138

Tables

Table 1: Amplified fragment length polymorphism adapters and primer sequences that were used for ligation, pre-selective amplification and screening for selective amplification of Microstegium vimineum genomic DNA. Primers with an asterisk (*) were primers used for final analysis.

Adapters	
EcoRI Forward	5’ CTC GTA GAC TGC GTA CC 3’
EcoRI Reverse	5’ AAT TGG TAC GCA GTC 3’
MseI Forward	5’ GAC GAT GAG TCC TGA G 3’
MseI Reverse	5’ TAC TCA GGA CTC AT 3’
Pre-Selective Primers	
MseI+C	5’ GAC GAT GAG TCC TGA GTA A-C 3’
EcoRI+A	5’ GAC TGC GTA CCA ATT C-A 3’
Selective Primers	
MseI+CAA*	5’ GAC GAT GAG TCC TGA GTA A-CAA 3’
MseI+CAC	5' GAC GAT GAG TCC TGA GTA A-CAC 3'
EcoRI+AGC (TET)*	5’ AC TGC GTA CCA ATT C-AGC 3’
EcoRI+AAC (TET)	5' AC TGC GTA CCA ATT C-AAC 3'
EcoRI+ACG (FAM)*	5’ AC TGC GTA CCA ATT C-ACG 3’
EcoRI+ACA (FAM)	5' AC TGC GTA CCA ATT C-ACA 3'

Table 2: Sampling locations, number of individuals collected from each population, spatial coordinates and regional membership determined by STRUCTURE for *Microstegium vimineum* populations sampled. Populations without an assigned region are out-groups.

Sampling Site	Sample Size	Latitude	Longitude	Region
Caledon Natural Area (CNA)	24	38.3534	-77.1516	-
Douthat State Park (DSP)	20	37.9358	-79.7818	Western JRB
Alleghany County (AC)	10	37.8253	-79.6633	Western JRB
City of Buena Vista (BV)	20	37.7314	-79.3118	Western JRB
Amherst County (AMC)	15	37.7361	-79.2887	Western JRB
James River State Park (JRSP)	20	37.6264	-78.802	Western JRB
James River Wildlife Management Area (JRWMA)	9	37.6688	-78.7265	Western JRB
Holiday Lake State Park (HLSP)	15	37.3955	-78.6404	Western JRB
Feather Fin Wildlife Management Area (FFWMA)	9	37.3612	-78.5797	Western JRB
Briery Creek Wildlife Management Area (BCWMA)	10	37.1772	-78.4473	Western JRB
Bear Creek Lake State Park (BCSP)	12	37.5314	-78.2648	Western JRB
Powhatan Wildlife Management Area (PWMA)	20	37.5512	-78.0148	Western JRB
Pulaski County (PUL)	19	37.0355	-80.5582	-
Mason Neck State Park (MNSP)	18	38.6404	-77.1978	-
Sailor's Creek State Park (SCSP)	11	37.3072	-78.2281	Central JRB
Twin Lake State Park (TLSP)	20	37.1761	-78.2764	Central JRB
Amelia County (AMELC)	15	37.2473	-78.0095	Eastern JRB
Chesterfield County (CC)	12	37.3376	-77.7216	Eastern JRB
Pocohontas State Park (PSP)	20	37.3664	-77.5744	Eastern JRB
VCU Rice Center (RICE)	25	37.3312	-77.208	Eastern JRB
Chickahominy Wildlife Management Area (CWMA)	10	37.3124	-76.9338	Eastern JRB
Chippokes Plantation State Park (CHP)	20	37.1456	-76.7383	Eastern JRB
York River State Park (YRSP)	20	37.4119	-76.7093	Eastern JRB
Hog Island Wildlife Management Area (HI)	8	37.1419	-76.6891	Western JRB
New Quarter Park (NQP)	20	37.2954	-76.6354	Eastern JRB
York County High School (YHS)	18	37.2024	-76.4997	Eastern JRB
Back Bay National Wildlife Refuge (BBNWR)	24	36.6719	-75.9161	Eastern JRB

Table 3: Percent polymorphic loci and Shannon's *I* derived from analysis of amplified fragment length polymorphism for all populations of *Microstegium vimineum* sampled.

Population	P	I
BCWMA	19.44%	0.089
CNA	27.78%	0.148
CWMA	27.78%	0.176
CC	30.56%	0.183
HI	30.56%	0.183
JRWMA	33.33%	0.182
AMELC	33.33%	0.193
BBNWR	36.11%	0.196
CHP	38.89%	0.205
BCSP	38.89%	0.235
YRSP	41.67%	0.23
RICE	44.44%	0.239
AC	44.44%	0.253
HLSP	44.44%	0.254
JRSP	47.22%	0.248
BV	47.22%	0.305
YHS	52.78%	0.273
FFWMA	52.78%	0.29
PUL	61.11%	0.332
NQP	63.89%	0.343
SCSP	63.89%	0.361
AMC	63.89%	0.365
PWMA	63.89%	0.379
MNSP	66.67%	0.367
DSP	69.44%	0.373
TLSP	72.22%	0.38
PSP	77.78%	0.35
Mean	47.94%	0.264

Table 4: Analysis of variance table for heteroscedasticity of all *Microstegium vimineum* populations sampled.

Source	***df***	**SS**	**MS**	**F**
Among	26	501.6744	19.2952	21.694
Within	332	295.2888	0.8894	
Total	358	796.963	20.1846	

Table 5: Pair-wise comparisons of heteroscedasticity for populations of *Microstegium vimineun.* Significant difference between populations are denoted by (X). Numbers above populations correspond to population numbers in Figure 1.

		26	1	2	3	4	5	6	7	8	9	10	12	11	13	27	14	15	16	17	19	18	20	22	21	23	24	25
		CNA	PUL	DSP	AC	BV	AMC	JRSP	JRVMA	HLSP	FFVMA	BCVMA	BCSP	TLSP	SCSP	MNSP	PVMA	AMELC	CC	PSP	CVMA	RICE	CHP	YRSP	HI	NGP	YHS	BBNWR
26	CNA	0																										
1	PUL	X	0																									
2	DSP	X	X	0																								
3	AC		X	X	0																							
4	BV		X	X		0																						
5	AMC	X	X		X	X	0																					
6	JRSP		X				X	0																				
7	JRVMA		X	X			X		0																			
8	HLSP		X	X			X			0																		
9	FFVMA		X				X				0																	
10	BCVMA	X	X	X		X	X	X			X	0																
12	BCSP		X	X			X					X	0															
11	TLSP	X	X		X	X			X	X		X	X	0														
13	SCSP	X	X		X	X				X		X			0													
27	MNSP	X	X		X	X			X	X		X				0												
14	PVMA	X	X		X	X				X		X					0											
15	AMELC	X			X	X		X	X	X	X	X	X					0										
16	CC		X	X			X					X		X	X	X		X	0									
17	PSP	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	0									
19	CVMA		X	X			X					X		X				X		X	0							
18	RICE		X									X		X				X		X		0						
20	CHP	X			X	X		X	X	X	X	X	X						X		X	X	0					
22	YRSP	X	X		X	X			X	X		X							X	X				0				
21	HI		X	X			X	X					X	X	X	X	X	X		X		X	X	X	0			
23	NGP	X			X	X		X	X	X	X	X							X		X	X			X	0		
24	YHS	X	X		X	X	X		X	X		X							X	X					X		0	
25	BBNWR		X	X			X							X	X	X	X	X		X			X	X		X	X	0

Table 6: Pair-wise Nei's genetic distance for all populations of *Microstegium vimineum* (Above diagonal). Pair-wise Φ_{ST} values for all populations of *Microstegium vimineum* (Below diagonal).Numbers above populations correspond to population identification on Figure 1.

		26	1	11	27	13	19	23	24	22	17	25	21	16	20	15	18	14	12	7	6	8	9	4	5	3	2	10
		CNA	PUL	TLSP	MNSP	SCSP	CWMA	NQP	YHS	YRSP	PSP	BBNWR	HI	CC	CHP	AMELC	RICE	PWMA	BCSP	JRWMA	JRSP	HLSP	FPWMA	BV	AMC	AC	DSP	BCWMA
26	CNA	0	0.347	0.194	0.288	0.261	0.377	0.281	0.326	0.326	0.344	0.341	0.287	0.358	0.361	0.364	0.357	0.242	0.322	0.349	0.355	0.341	0.262	0.259	0.27	0.324	0.226	0.257
1	PUL	0.706	0	0.183	0.183	0.328	0.258	0.115	0.19	0.223	0.089	0.311	0.274	0.294	0.252	0.25	0.247	0.074	0.133	0.154	0.104	0.17	0.13	0.124	0.104	0.173	0.144	0.263
11	TLSP	0.611	0.467	0	0.086	0.095	0.211	0.139	0.156	0.18	0.135	0.247	0.302	0.244	0.178	0.181	0.208	0.168	0.257	0.254	0.248	0.272	0.178	0.242	0.212	0.298	0.217	0.317
27	MNSP	0.67	0.386	0.348	0	0.154	0.276	0.186	0.249	0.261	0.132	0.323	0.315	0.332	0.263	0.288	0.295	0.137	0.16	0.155	0.15	0.18	0.138	0.206	0.208	0.244	0.225	0.286
13	SCSP	0.701	0.526	0.267	0.433	0	0.308	0.268	0.259	0.244	0.291	0.306	0.418	0.3	0.246	0.261	0.274	0.307	0.423	0.426	0.43	0.413	0.34	0.413	0.332	0.431	0.321	0.437
19	CWMA	0.827	0.6	0.602	0.62	0.663	0	0.051	0.035	0.051	0.127	0.049	0.119	0.049	0.006	0.119	0.03	0.189	0.311	0.289	0.301	0.338	0.214	0.294	0.353	0.353	0.337	0.417
23	NQP	0.688	0.394	0.487	0.496	0.545	0.299	0	0.027	0.052	0.043	0.094	0.139	0.096	0.083	0.098	0.07	0.086	0.197	0.201	0.18	0.246	0.123	0.195	0.207	0.236	0.204	0.327
24	YHS	0.768	0.566	0.565	0.6	0.617	0.344	0.29	0	0.028	0.099	0.062	0.138	0.057	0.052	0.071	0.032	0.155	0.293	0.288	0.279	0.314	0.187	0.255	0.285	0.315	0.26	0.359
22	YRSP	0.765	0.481	0.519	0.558	0.562	0.362	0.245	0.261	0	0.142	0.058	0.145	0.062	0.061	0.105	0.018	0.186	0.319	0.309	0.308	0.329	0.217	0.278	0.288	0.349	0.255	0.391
17	PSP	0.674	0.281	0.468	0.399	0.518	0.406	0.266	0.398	0.356	0	0.219	0.242	0.214	0.149	0.152	0.168	0.066	0.155	0.147	0.124	0.201	0.09	0.178	0.173	0.219	0.223	0.346
25	BBNWR	0.81	0.675	0.666	0.684	0.728	0.447	0.403	0.437	0.449	0.524	0	0.057	0.013	0.009	0.108	0.038	0.216	0.316	0.32	0.32	0.329	0.24	0.272	0.345	0.307	0.259	0.355
21	HI	0.752	0.458	0.549	0.54	0.603	0.469	0.36	0.482	0.431	0.402	0.464	0	0.076	0.183	0.217	0.118	0.153	0.194	0.2	0.205	0.202	0.155	0.146	0.273	0.18	0.213	0.189
16	CC	0.814	0.61	0.622	0.635	0.678	0.421	0.347	0.393	0.404	0.454	0.394	0.442	0	0.079	0.104	0.035	0.211	0.322	0.336	0.323	0.326	0.246	0.274	0.338	0.304	0.256	0.347
20	CHP	0.811	0.614	0.594	0.633	0.64	0.517	0.384	0.376	0.394	0.453	0.555	0.581	0.511	0	0.026	0.061	0.208	0.329	0.331	0.31	0.345	0.208	0.302	0.295	0.314	0.252	0.409
15	AMELC	0.814	0.64	0.557	0.595	0.599	0.542	0.365	0.39	0.414	0.386	0.59	0.572	0.527	0.327	0	0.101	0.223	0.362	0.356	0.329	0.372	0.212	0.298	0.306	0.325	0.259	0.405
18	RICE	0.782	0.59	0.597	0.623	0.65	0.331	0.337	0.336	0.334	0.446	0.418	0.461	0.375	0.452	0.473	0	0.201	0.316	0.306	0.315	0.311	0.229	0.277	0.309	0.342	0.264	0.376
14	PWMA	0.626	0.281	0.501	0.442	0.559	0.562	0.436	0.562	0.509	0.357	0.604	0.372	0.561	0.598	0.564	0.564	0	0.048	0.073	0.041	0.089	0.043	0.059	0.071	0.065	0.105	0.127
12	BCSP	0.761	0.389	0.591	0.506	0.663	0.74	0.563	0.69	0.669	0.447	0.744	0.538	0.72	0.739	0.719	0.697	0.254	0	0.02	0.022	0.029	0.056	0.056	0.098	0.043	0.108	0.104
7	JRWMA	0.773	0.383	0.584	0.496	0.659	0.737	0.541	0.682	0.657	0.413	0.739	0.521	0.717	0.736	0.716	0.687	0.264	0.301	0	0.029	0.046	0.052	0.071	0.119	0.085	0.156	0.133
6	JRSP	0.75	0.391	0.611	0.517	0.684	0.721	0.565	0.693	0.668	0.447	0.726	0.545	0.702	0.717	0.698	0.691	0.29	0.314	0.314	0	0.037	0.04	0.039	0.089	0.058	0.113	0.124
8	HLSP	0.786	0.47	0.618	0.549	0.695	0.761	0.609	0.714	0.705	0.519	0.758	0.577	0.74	0.756	0.745	0.717	0.301	0.343	0.335	0.372	0	0.07	0.048	0.09	0.052	0.109	0.08
9	FPWMA	0.721	0.327	0.539	0.462	0.599	0.661	0.482	0.62	0.581	0.366	0.687	0.422	0.647	0.657	0.613	0.638	0.227	0.296	0.264	0.264	0.354	0	0.057	0.107	0.076	0.14	0.119
4	BV	0.704	0.419	0.595	0.542	0.662	0.699	0.565	0.668	0.644	0.492	0.703	0.476	0.679	0.703	0.684	0.674	0.285	0.353	0.359	0.358	0.368	0.281	0	0.056	0.054	0.07	0.07
5	AMC	0.654	0.294	0.531	0.493	0.587	0.668	0.519	0.639	0.589	0.446	0.699	0.494	0.655	0.664	0.625	0.649	0.268	0.349	0.367	0.366	0.376	0.305	0.324	0	0.103	0.048	0.135
3	AC	0.755	0.433	0.598	0.552	0.65	0.733	0.569	0.687	0.663	0.5	0.733	0.491	0.707	0.722	0.701	0.696	0.286	0.372	0.404	0.39	0.392	0.302	0.31	0.369	0	0.106	0.064
2	DSP	0.624	0.301	0.498	0.464	0.547	0.621	0.495	0.602	0.543	0.441	0.654	0.437	0.601	0.614	0.57	0.61	0.281	0.34	0.367	0.377	0.365	0.303	0.317	0.215	0.326	0	0.136
10	BCWMA	0.786	0.486	0.599	0.563	0.674	0.824	0.61	0.73	0.718	0.544	0.791	0.588	0.782	0.781	0.779	0.741	0.3	0.476	0.526	0.469	0.465	0.374	0.356	0.342	0.397	0.311	0

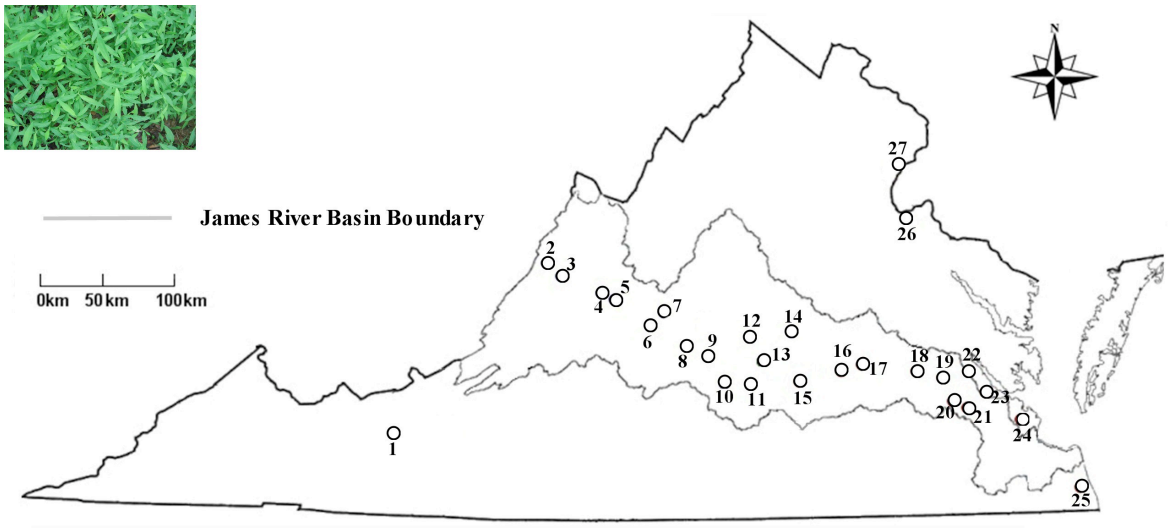
Table 7: Hierarchical analysis of molecular variance for regions inferred by Structure as well as populations of *Microstegium vimineum*.

Source	***df***	**SS**	**MS**
Among Regions	3	332.2741	110.758
Among Populations	23	141.2427	6.141
Error	332	474.5528	1.4294
Total	358	948.0696	118.3284

Table 8: Step-wise analysis of molecular variance for *Microstegium vimineum* populations and spatial coordinates.

Source	***df***	**SS**	**MS**
Latitude	1	0.0113	0.0113
Longitude	1	0.016	0.016
Among Populations	26	473.4895	18.211
Error	330	474.5528	1.438
Total	358	948.0696	2.6482

Figures



1: Pulaski Co. (PUL)
2: Douthat State Park (DSP)
3: Alleghany Co. (AC)
4: City of Buena Vista (BV)
5: Amherst Co. (AMC)
6: James River State Park (JRSP)
7: James River WMA (JRWMA)
8: Holiday Lake State Park (HLSP)
9: Featherfin WMA (FFWMA)
10: Briery Creek WMA (BCWMA)
11: Twin Lakes State Park (TLSP)
12: Bear Creek Lake State Park (BCSP)
13. Sailor's Creek State Park (SCSP)
14: Powhatan WMA (PWMA)
15: Amelia Co. (AMELC)
16: Chesterfield Co. (CC)
17: Pocohontas State Park (PSP)
18: VCU Rice Center (RICE)
19: Chickahominy WMA (CWMA)
20: Chippokes Plantation State Park (CHP)
21: Hog Island WMA (HI)
22: York River State Park (YRSP)
23: New Quarter Park (NQP)
24: York Co. High School (YHS)
25: Back Bay NWR (BBNWR)
26: Caledon Natural Area (CNA)
27. Mason Neck State Park (MNSP)

Figure 1: Geographic sampling locations of *Microstegium vimineum*. Circles with numbers correspond to population location and population identification. Size of circle does not correspond to sample size. (Inset) Picture of *M. vimineum* individuals (not to scale).

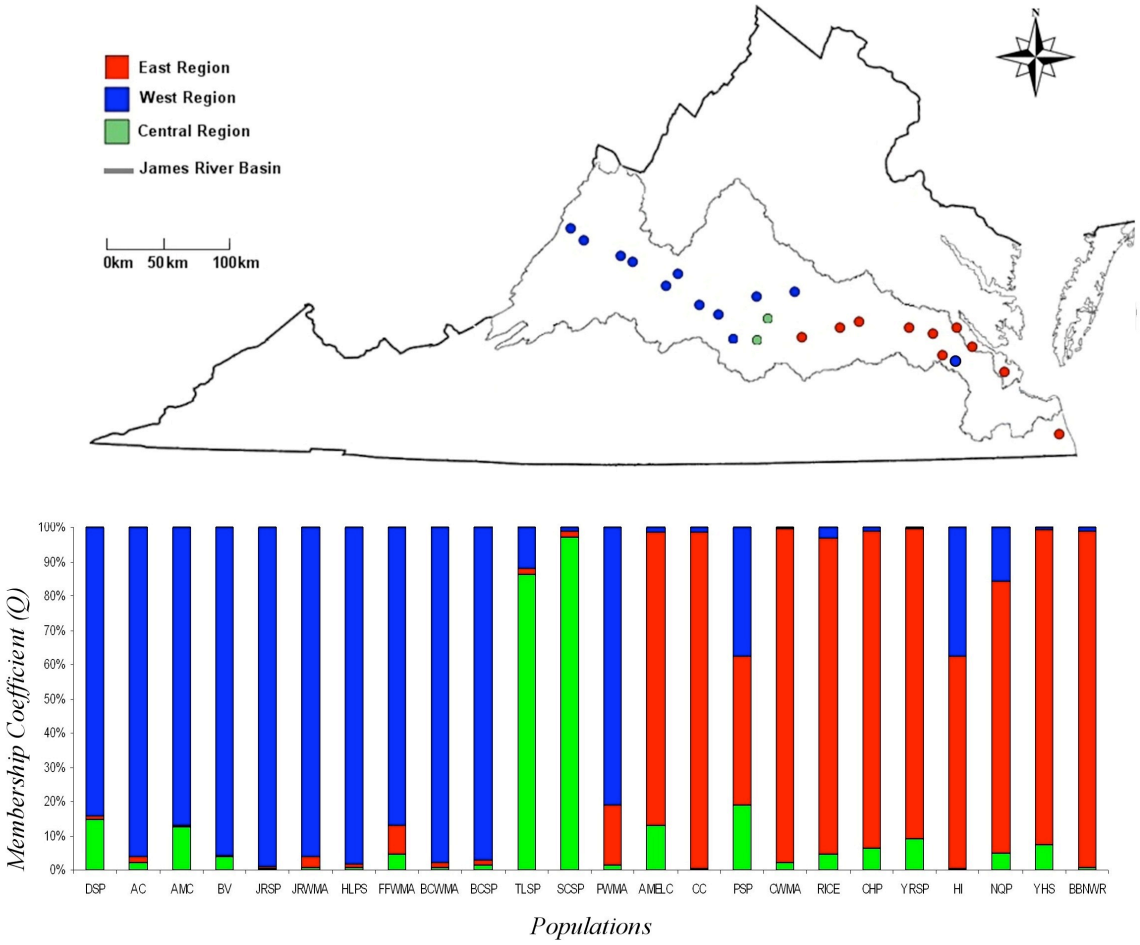


Figure 2: (Above) *Microstegium vimineum* populations within the James River Basin of Virginia where color denotes regional membership determined by STRUCTURE. (Below) Membership values (*Q*) determined by Structure for populations in the James River Basin listed in geographic order from west to east and corresponding to above map.

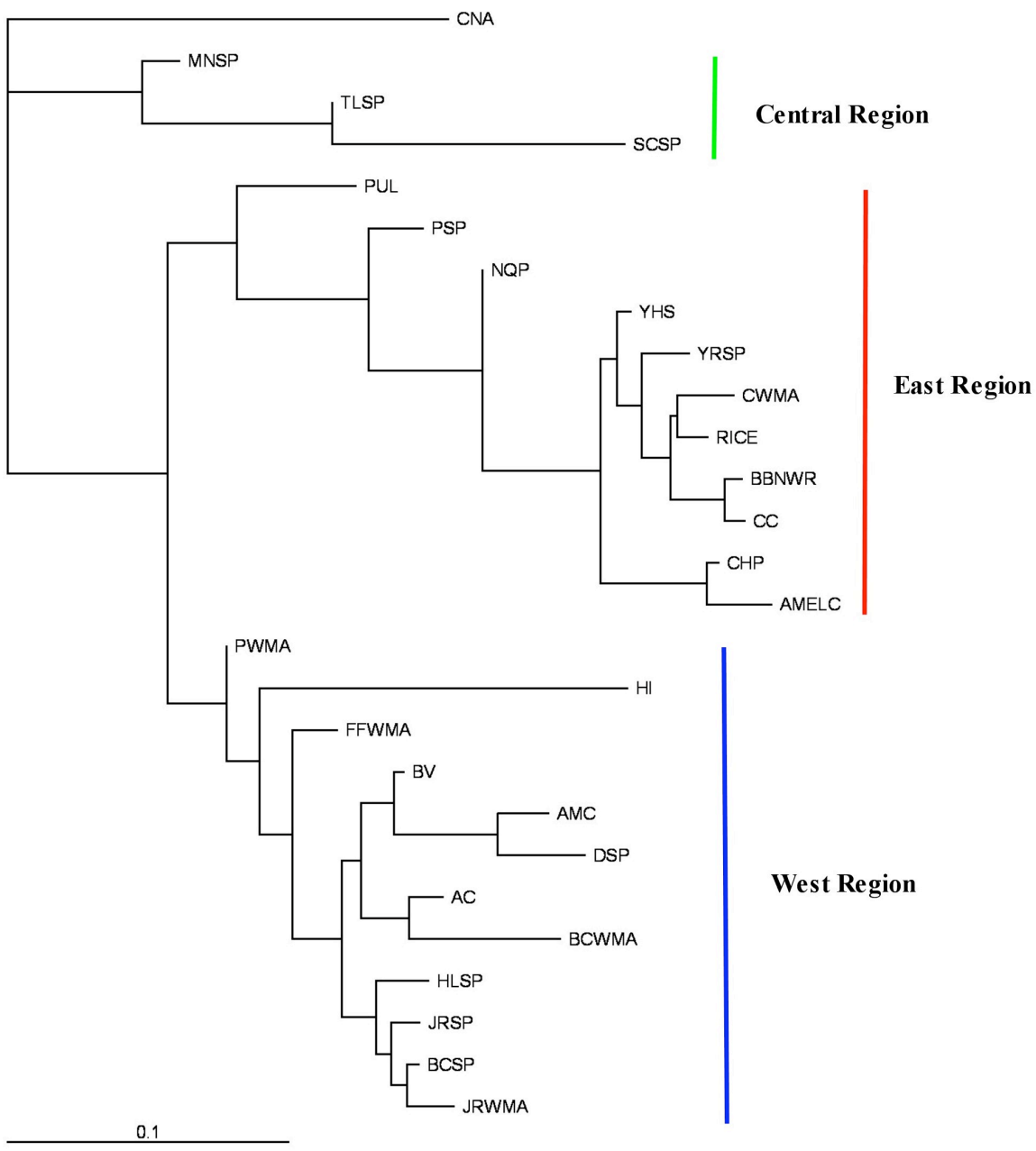


Figure 3: Neighbor-joining tree of *Microstegium vimineum* populations of the James River Basin as well as out-group populations constructed using PHYLIP from Nei's genetic distance. The east and west regions both form clades that are in agreement with regions defined by STRUCTURE.

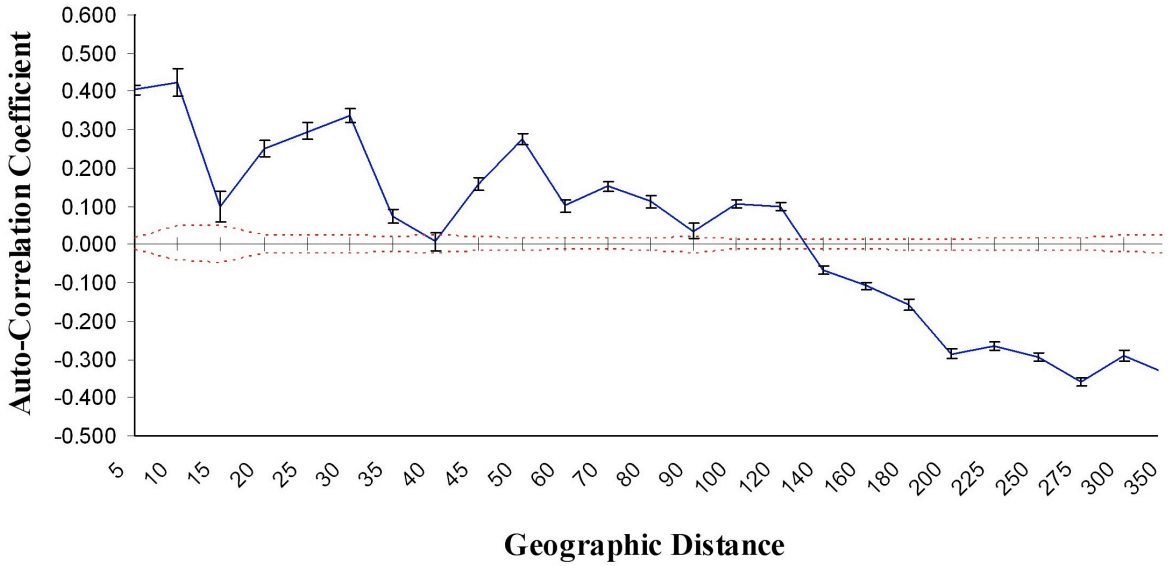


Figure 4: Spatial autocorrelation Correlogram for populations of *Microstegium vimineum* in the James River Basin indicating significant correlation of genotypes up to 40km. Dashed red lines indicate upper and lower bounds of a 95% confidence interval. Standard error bars are bootstrapped values around the observed population correlation coefficient. Correlation coefficients for distance classes are taken at the end-point to include all populations within the range of the distance class up to the start of the next distance class.

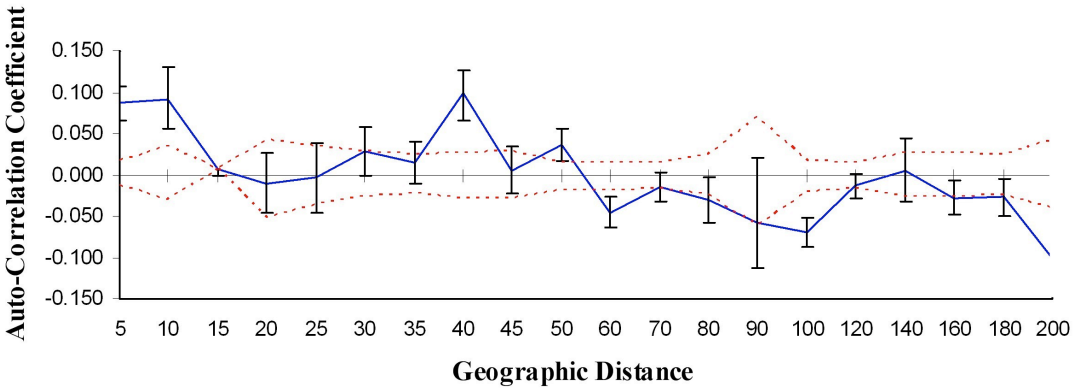


Figure 5: Spatial autocorrelation for populations of *Microstegium vimineum* in the James River Basin indicating significant correlation of genotypes up to 10km. Dashed red lines indicate upper and lower bounds of a 95% confidence interval. Standard error bars are bootstrapped values around the observed population correlation coefficient. Correlation coefficients for distance classes are taken at the end-point to include all populations within the range of the distance class up to the start of the next distance class.

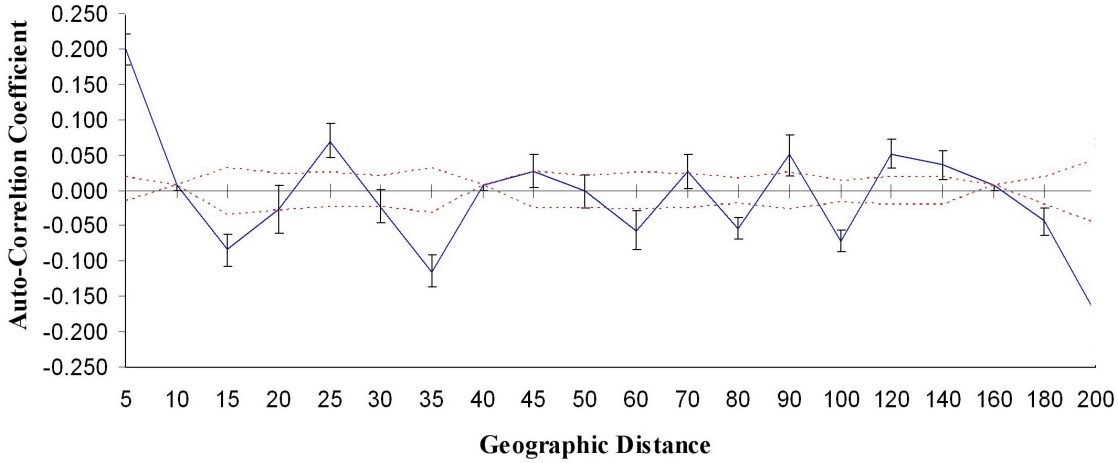


Figure 6: Spatial autocorrelation for populations of *Microstegium vimineum* in the east region of the James River Basin indicating significant correlation of genotypes up to 5km. Dashed red lines indicate upper and lower bounds of a 95% confidence interval. Standard error bars are bootstrapped values around the observed population correlation coefficient. Correlation coefficients for distance classes are taken at the end-point to include all populations within the range of the distance class up to the start of the next distance class.

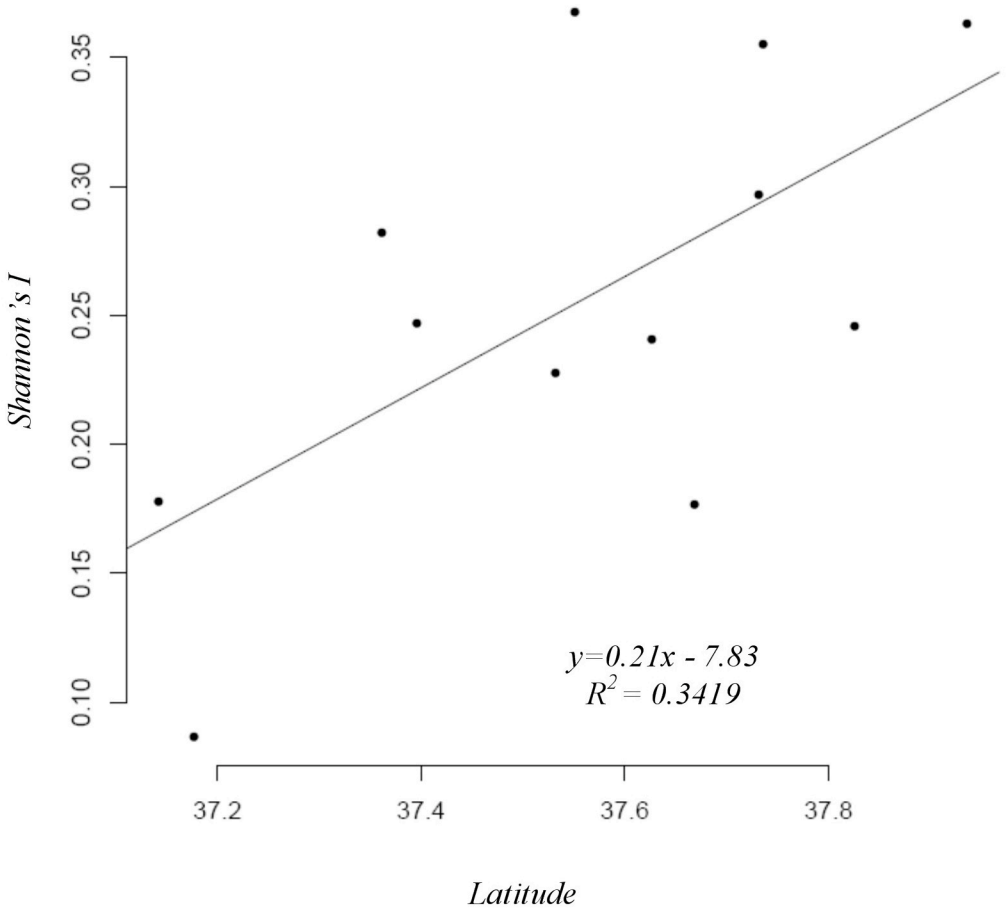


Figure 7: Regression test of Shannon's information index (*I*) for populations of *Microstegium vimineum* in the west region showing a significant positive correlation for increasing *I* with increasing latitude.

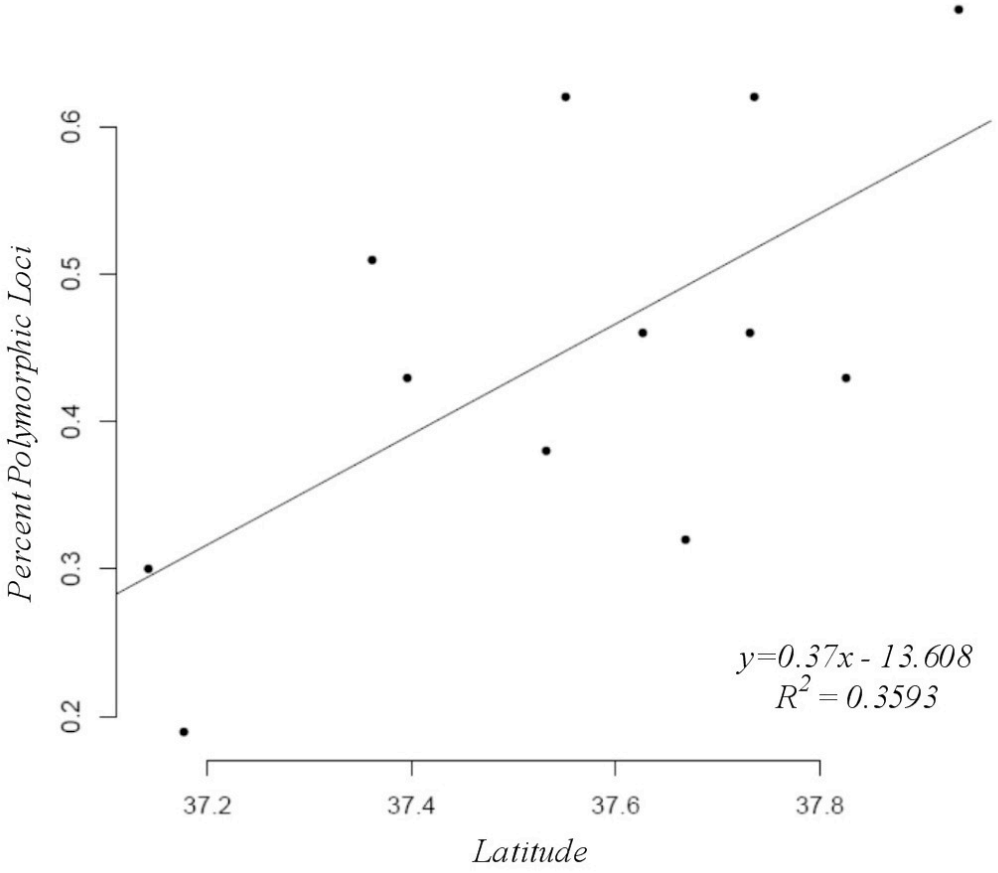


Figure 8: Regression test of percent polymorphic loci (*P*) for populations of *Microstegium vimineum* in the west region showing a significant positive correlation for increasing *P* with increasing latitude.

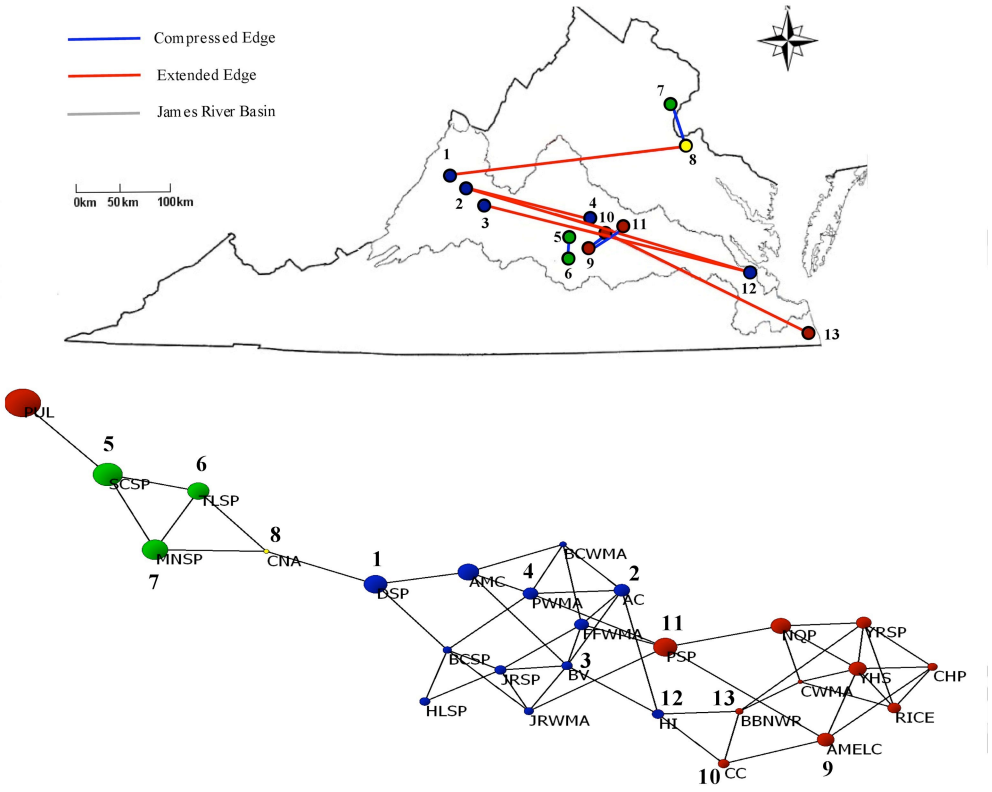


Figure 9: Population network (below) with colors corresponding to region identity inferred by STRUCTURE. Edge length corresponds to scaled genetic distance while node size is correlated to within population diversity as measured by heteroscedasticity. Nodes are numbered corresponding to the numbered populations on the population graph (above) showing extended (red lines) and compressed edges (blue lines) between populations indicating populations closer (compressed) or farther (extended) genetically than expected geographically.

VITA

Stephen Baker was born in Richmond, Virginia August 16th 1980. He graduated from J. Sargeant Reynolds Community College in 2006 with an A.S. in Science. He received his B.S. in Biology from Virginia Commonwealth University in 2006. He was the President of the Graduate Organization of Biology Students in 2008 and was also inducted into the Phi Kappa Phi Honor Society in 2008. While at VCU he taught labs for Biological Concepts, Environmental Science, Introduction to Biological Sciences II and Genetics.