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Impact of Suburban Landscape Features on Gene Flow of the Model Invasive Grass, *Brachypodium sylvaticum*

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Impact of Suburban Landscape Features on Gene Flow of the Model Invasive Grass,

Brachypodium sylvaticum

by

Tina Marie Arredondo

A thesis submitted in partial fulfillment of the
requirements for the degree of

Master of Science
in
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Thesis Committee:
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ABSTRACT

Rapid range expansion of newly invasive species provides a unique opportunity for studying patterns of dispersal and gene flow. In this thesis, I examined the effect of landscape features on gene flow in the invasive grass *Brachypodium sylvaticum* at the edge of its expanding range. I used genome-wide Single Nucleotide Polymorphism (SNP) surveys of individuals from 22 locations in the Clackamas Watershed in the Portland, Oregon metropolitan region to assess genetic diversity and structure, to identify putative source populations, and to conduct landscape genetic analyses. Resistance surfaces were created for each landscape feature, using ResistanceGA to optimize resistance parameters. My STRUCTURE analysis identified three distinct clusters, and diversity analyses support the existence of at least two local introductions. Multiple Regression on distance Matrices (MRM) showed no evidence that development, roads, canopy cover, or agriculture had a significant influence on genetic distance in *B. sylvaticum*. The effect of geographic distance was marginal and reflected geographic clustering. The model of rivers acting as a conduit explained a large portion of variation in genetic distance. Results indicate that rivers influence patterns of dispersal of *B. sylvaticum* by human recreational activity centering on use of rivers, and possibly due to movement of deer.

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CHAPTER 1: INTRODUCTION

Global exchange and establishment of invasive species is occurring at an unprecedented rate. Invasive species cause extensive economic and environmental harm worldwide (Pimentel, Zuniga, & Morrison, 2005). Despite extensive work to better understand the ecology and evolution of these species, we have a limited understanding of how invasive species are able to undergo seemingly unlimited range expansion (Hastings et al., 2005). Inbreeding depression from drift and genetic bottlenecks are expected to hamper species undergoing such rapid expansion (Excoffier, Foll, & Petit, 2009). Some recent studies suggest that gene flow due to high propagule pressure in invasive species may allow populations to evade inbreeding depression (Lockwood, Cassey, & Blackburn, 2005). One such species is *Brachypodium sylvaticum* (slender false brome), a newly invasive perennial bunchgrass currently undergoing rapid range expansion in the US and Canada.

B. sylvaticum is diploid, self-compatible, and relatively easy to control in a greenhouse setting, making it an attractive model for invasive plant evolution and genetics. *B. sylvaticum* was recognized as naturalized (reproducing outside of captivity) in Oregon in 1966. Since then, it has spread to Washington and Northern California, with separate expansion fronts in New York state and California (Daniel & Werier, 2010; Rosenthal, Ramakrishnan, & Cruzan, 2008). *B. sylvaticum* is known to prefer low-light conditions such as those found in forest understories, and is most commonly found near trails and other corridors for human movement (Holmes, Roy, Reed, & Johnson, 2010).

Previous work by Rosenthal et. al. (2008) found that the original introduction into Oregon occurred in the Eugene-Corvallis region. This region exhibited high levels of

genetic diversity, which likely resulted from the introduction of multiple individuals with a diverse genetic background (Rosenthal et al., 2008). Source regions in the native range were investigated for their similarity to the invasive genotype, and it was found that the invasion likely originated from Western Europe, though the invasive genotype is a result of admixture from multiple locations in the native range (Rosenthal et al., 2008). This study also concluded that the invasion in California is the result of a separate introduction event (Rosenthal et al., 2008); the origin of the *B. sylvaticum* invasion in New York state is currently unknown.

The spread of *B. sylvaticum* following initial invasion of central Oregon has been found to follow a non-continuous dispersal pattern (Ramakrishnan, Musial, & Cruzan, 2010). New populations become established ahead of the expansion front via long-distance dispersal, then experience a period of time before contributing to establishment of new populations in the surrounding area (Ramakrishnan et al., 2010). As populations on the range edge are known to experience genetic bottlenecks and loss of genetic diversity during dispersal, this secondary lag phase is thought to be associated with the accumulation of genetic diversity. In the study by Ramakrishnan et al. (2010), only populations which had accumulated high relative levels of genetic diversity contributed to the establishment of new populations.

An initial period of lag prior to the beginning of range expansion is common in invasive species, and is thought to be associated with demographic processes or evolution of the invasive genotype (Aikio, Duncan, & Hulme, 2010; Sakai et al., 2001). There is some evidence that continued expansion following the secondary lag phase found in *B. sylvaticum* is caused by purging of genetic load. Inbreeding followed by periodic gene

flow was found to increase rates of range expansion in a simulation of *B. sylvaticum* expansion (Marchini, Sherlock, Ramakrishnan, Rosenthal, & Cruzan, 2016). If periodic gene flow allows for range-edge populations to continue expansion, then it is imperative to understand the processes influencing gene flow between invasive populations.

B. sylvaticum is known to disperse via large ungulates in the invasive range (Heinken & Raudnitschka, 2002). However, association with trails and movement along rural roads found from previous studies demonstrates a shift in dispersal modes in the invasive range (Holmes et al., 2010; Ramakrishnan et al., 2010). The association between *B. sylvaticum* and rivers has even led some invasive species managers to believe that its seeds disperse via water (personal communication). As the range of *B. sylvaticum* now extends into heavily human-dominated areas, it is possible that the species has again shifted to a new method of dispersal. In the following chapter, I outline my work to determine the primary landscape features influencing dispersal in a set of range-edge populations of *B. sylvaticum*.

CHAPTER 2: EVIDENCE FOR HUMAN-MEDIATED RANGE EXPANSION AND GENE FLOW

Background

Rapid range expansion of invasive species provides natural experiments for studying the impact of landscape features on gene flow and dispersal. In native systems, historical range expansion, contraction, and ongoing meta-population processes can complicate interpretation of genetic relatedness among populations (Richardson, Brady, Wang, & Spear, 2016). In contrast, population differentiation in newly invasive species undergoing range expansion primarily reflects diversity of the initial colonization events (Lee, 2002). However, recurrent gene flow will generate patterns of similarity even in recently colonized regions (Dlugosch & Parker, 2008), allowing identification of landscape features influencing dispersal pathways. While all species have expanded their ranges at some point in the past, this expansion is more evident in invasive species. Species that are invading new ranges exhibit genetic signatures of range expansion and present an opportunity to evaluate colonization processes and to identify landscape features influencing range expansion and dispersal.

Invasive plants are particularly tractable for studying the impact of landscape features on gene flow, as their stationary nature simplifies population delineation and geographic sampling. Long distance dispersal is disproportionately important for expanding plant ranges (Clark et al., 1998; Hewitt, 1999; Levin, Muller-Landau, Nathan, & Chave, 2003). However, long distance dispersal often results in severe genetic bottlenecks because newly established populations are isolated and established by few propagules (Bialozyt, Ziegenhagen, & Petit, 2006). For self-compatible species just one

individual is required to establish a new population (Baker's Law; Baker & Stebbins, 1965), creating an extreme bottleneck. After establishment, subsequent gene flow among populations can boost genetic diversity, alleviating initial fitness reductions from low genetic diversity (Courchamp, Clutton-Brock, & Grenfell, 1999; Frankham, 2015; Marchini et al., 2016).

Human activities often facilitate the spread of invasive species, particularly by increasing their dispersal frequency (Mack et al., 2000). Movement of invasive propagules by humans directs dispersal towards human-disturbed areas, where heightened propagule pressure allows invasive species to become established (Colautti, Grigorovich, & MacIsaac, 2006; Lockwood et al., 2005; Simberloff, 2009; L. A. V. Taylor & Cruzan, 2015). If humans are moving plant propagules, we may also expect long distance dispersal events to be more common, while also directing dispersal towards areas dominated by human activity. Directed dispersal of plant propagules to disturbed areas should further reinforce the effects of human-mediated dispersal (Howe & Smallwood, 1982; Wenny, 2001).

A direct correlation between increasing geographic distance and increasing genetic distance, termed Isolation by distance, IBD (Wright, 1943), has been found in a wide range of plant species (Jenkins et al., 2010; Sexton, Hangartner, & Hoffmann, 2014). However, landscape features can influence the movement of dispersal vectors of pollen and seeds, resulting in a more complex relationship between genetic distance and geographic distance. Isolation by resistance (IBR) assigns landscape features relative resistances and estimates overall landscape connectivity using circuit theory (McRae & Beier, 2007). Circuitscape calculates a resistance value across a landscape for all pairs of

populations (McRae, Dickson, Keitt, & Shah, 2008). These pairwise landscape resistance values can be compared to pairwise genetic distances to identify which landscape features best predict the patterns of genetic diversity seen in the species (Jaquiéry, Broquet, Hirzel, Yearsley, & Perrin, 2011). Patterns of genetic distance can then be used to determine which dispersal agents cause a landscape feature to act as a conduit or a barrier to gene flow among populations.

We used the recently introduced invasive grass, *Brachypodium sylvaticum* (Huds.) Beauv. (Poaceae), to study potential impacts of land use and landscape features on gene flow at the edge of an expanding range. *Brachypodium sylvaticum* is a diploid, self-compatible perennial bunchgrass (Steinwand, Young, Bragg, Tobias, & Vogel, 2013). *Brachypodium sylvaticum* has been naturalized in the wild in North America for approximately eighty years and is actively expanding its range throughout the Pacific Northwest of North America (B. M. Miller, Aitken, Oldham, & Reznicek, 2012; Rosenthal et al., 2008). In addition, populations have been found in California, Virginia, and New York (Daniel & Werier, 2010). Long-distance spread of *B. sylvaticum* in its invasive range is suspected to be linked to movement of logging equipment (T. N. Kaye & Blakeley-Smith, 2006). As its range of has expanded, *B. sylvaticum* has encountered increasingly urbanized areas. While *B. sylvaticum* is dispersed primarily by large ungulates in its native range (Heinken & Raudnitschka, 2002), its occurrence along roadsides and waterways has raised the question whether of its invasive-range dispersal is linked to human use of these features (Holmes et al., 2010; T. Kaye, 2003; Ramakrishnan et al., 2010).

The relatively short history of *B. sylvaticum* in its invasive range coupled with its active expansion presents the opportunity to study interactions between newly established populations and landscape features in human-dominated areas. Here, we investigate the interaction between *B. sylvaticum* gene flow and landscape features to understand how both man-made and natural landscape features influence and potentially facilitate dispersal at the range edge. We ask (1) what is the invasion history in this watershed, (2) what landscape features have influenced gene flow among invasive populations, and (3) if there is evidence for human involvement in this movement. These analyses provide an assessment of the primary vectors responsible for range expansion and gene flow in this newly invasive grass.

Methods

(a) Sampling

A total of 22 *B. sylvaticum* locations were sampled to span the diversity of landscape features represented in the Clackamas Watershed in the Portland, Oregon metropolitan region (Fig. 1). The Clackamas Watershed encompasses a diversity of land use types, varying from heavily developed urban areas to natural secondary growth evergreen forests. At each sampling location, we collected approximately three centimeters of leaf tissue from 5-10 individuals for a total of 176 individuals. Individual plants were sampled at a minimum of 1 m apart to prevent double sampling of the same genet, as it was often difficult to delineate individuals growing in dense monocultures. This distance was considered adequate because *B. sylvaticum* individuals form dense clumps of tillers that are rarely larger than 25 cm in diameter.

Leaf tissue was stored at -4°C for up to 24 hours prior to extraction of total genomic DNA using DNeasy Plant 96 Kits (Qiagen, Leusden, The Netherlands). We used Genotyping-by-Sequencing (GBS) to concentrate sequencing around restriction enzyme cut sites, allowing genome-wide discovery of Single Nucleotide Polymorphisms (SNPs) while maintaining sufficient read depth and consistency across samples for genotyping (Elshire et al., 2011). GBS libraries were constructed using the ApeKI (GCWGC) restriction enzyme by the Cornell University Institute of Biotechnology Genomics Facility. Samples were sequenced on Illumina HiSeq 2500, yielding 100 bp single-end reads.

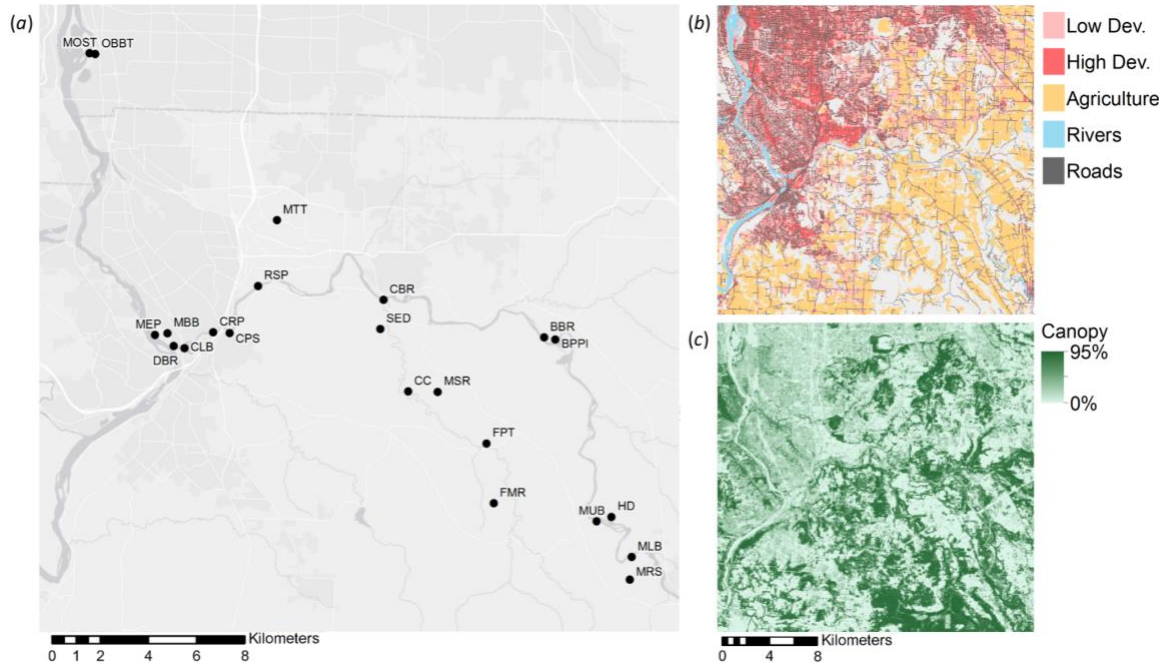


Figure 1. Geographic distribution of *B. sylvaticum* sampling locations (a), landuse (b), and canopy cover (c).

(b) Bioinformatics

Sequence data were filtered for quality and analyzed for accurate genotyping using a reduced-representation mock reference to identify reliable variants. Use of a mock reference was necessary due to a lack of a full genome reference for *B. sylvaticum*. We used the GBS-SNP-CROP pipeline for quality control, generation of the reference, and variant calling (Melo, Bartaula, & Hale, 2016). Illumina Truseq 3 SE adapters were removed using IlluminaClip in Trimmomatic 0.35 (Bolger, Lohse, & Usadel, 2014) with a clip threshold of 10 and allowing up to 2 bp mismatches between the read and the adapter. Reads were trimmed when their average quality score dropped below 30 in a 4 bp sliding window; leading and trailing bases were trimmed if their quality score fell below 30. Any reads below 32 bp in length following trimming were considered too short for analysis and were discarded. A reduced-representation mock reference consensus

sequence for regions directly surrounding GBS cut sites was generated using USEARCH with a nucleotide identity cutoff of 93% (Edgar, 2010). Trimmed reads from all samples were aligned to the mock reference using BWA (Li & Durbin, 2009). Unmapped reads and reads with poor (<30) alignment quality were removed using Samtools view (Li et al., 2009). Variant SNPs were then called using SAMtools mpileup with minimum mapping quality of 30, the coefficient for downgrading the quality score of reads with excessive mismatches set to 50, and with probabilistic realignment of base alignment quality disabled (Li et al., 2009).

Variants were filtered following recommendations for diploid data in the GBS-SNP-CROP pipeline (Melo et al., 2016). SNPs were retained if found in at least 75% of individuals across all populations with an average depth of 10-200 reads, and with a minimum of nine reads in three separate individuals supporting existence of the alternate allele. Non-biallelic variants were excluded from analysis by removing SNPs with a proportion of alternate reads to other non-primary below 0.92. An individual was called homozygous at a locus when there were at least 5 supporting reads if there were no reads showing the alternate allele; when there was one alternate read, a minimum of 20 primary-allele reads were required to call the individual homozygous. A minimum of 5 alternate reads were required to call an individual heterozygous, with a minimum ratio of primary reads to alternate reads of 0.3. SNPs with a proportion of heterozygous individuals greater than 0.5 or a minor allele frequency below 0.05 were removed from analysis, as these likely resulted from misalignment or collapse of homologous regions in the mock reference and do not represent a true variant. 2178 SNPs remained following filtering and were used in subsequent analysis.

(c) Population Structure

Population statistics (H_o , H_s , F_{is}) were calculated using Hierfstat in R (Goudet, 2005). The number of alleles exhibiting significant interallelic linkage disequilibrium (%LD) was found using Adegenet in R. We conducted a Structure analysis to characterize the number of subpopulations (K) in the watershed and to assess the amount of recent admixture among sampling locations (Pritchard, Stephens, & Donnelly, 2000). Structure version 2.3.4 was run with a burn-in of 50,000 and a run of 100,000 for values of K ranging from 1-20; five replicates were run for each value of K and the optimal K was found using the Evanno method (Evanno et al., 2005).

(d) Landscape Features

We compiled GIS layers representing landscape features likely to influence the behavior of dispersal vectors and converted them into hypothetical landscapes in which features resisted or conducted gene flow. Roads were represented by the National Transportation Dataset (NTD; Witt, 2015). Polylines in the NTD were converted to rasters using ArcMap's Polygon to Raster tool (ESRI, Redlands, CA). The National Land Cover Dataset, NCLD (Homer et al., 2015), represented land use and major water features in the study area (Fig. 1). Cells in NCLD identified as pasture, hay, and cultivated crops in NCLD were combined into one class representing agricultural activity. Developed areas were classified based on degree of impervious surface cover, with >50% impervious surfaces classified as highly developed (eg. apartment complexes, commercial/industrial areas, dense single family homes) and <50% classified as low development (golf courses, large-lot single family homes, recreational areas). Finally, we used the NCLD Tree Cover Analytical raster to represent percent canopy cover in 30 m²

areas (Fig. 1). All layers were represented at a 30 m resolution and clipped to a 2 km buffer surrounding the study locations to avoid edge effects in circuit modeling.

(e) Statistics

Data were analyzed to evaluate the relationship between our hypothetical resistance landscapes and genetic distance. We calculated Nei's genetic distance using Adegenet in R (Jombart, 2008; Nei, 1972). Optimal resistance values for landscape layers were found using ResistanceGA (Peterman, 2014). ResistanceGA iteratively varies the resistance of features and evaluates their performance using a regression against genetic distance method, choosing the resistance which maximizes the Akaike information criterion (AIC). Resistance surfaces with categorical identification of landscape features (rivers, roads, agriculture, natural areas, high and low development) were prepared for input into ResistanceGA by setting cells representing the landscape feature of interest to 10 and all other cells to 1. Canopy was quantified on a gradient and was input directly into ResistanceGA without modification of cell values. ResistanceGA version was run with 10,000 steps and a convergence p-value of 0.05 for each landscape feature.

As landscape features were optimized separately, substantial collinearity in circuit distances due to spatial autocorrelation between different landscape models was expected. To control for this collinearity, we evaluated model significance using the Multiple Regression on distance Matrices function in the Ecodist package in R (MRM; Lichstein, 2007). This allowed us to partition variation in genetic distance among the different models and to remove insignificant features from analysis using a backwards selection model until only significant landscape models remained. Variance Inflation (VIF) was calculated using fmsb in R for each MRM model to determine the degree of

collinearity between features in the model (Nakazawa, 2015). Our initial model included all landscape resistance models, and we then used backwards model selection to remove insignificant features.

Results

(a) Invasion history

Patterns of diversity in range-edge populations of *B. sylvaticum* were consistent with expectations in a recently-colonized area. Individual populations varied from very low genetic diversity (MBB, $H_s=0.068$) to very high diversity to the point of heterozygosity excess (CC, $H_s=0.297$; Table 1). A previous study on *B. sylvaticum* populations established ranges of genetic diversity associated with different stages of invasion, inferred from population genetic parameters such as genetic diversity (H_s) (Ramakrishnan et al., 2010). Overall genetic diversity in our study area ($H_s=0.175$) fell within the moderate age range described previously for *B. sylvaticum* (H_s 0.1-0.25). Four sampling locations fell within the highest age rank described by Ramakrishnan et. al (2010; $H_s >0.25$), indicating that these are the oldest sampled populations and the likely points of local introduction. Three of these high-diversity locations (CBR, CC, RSP) are clustered in the center of the study area near Clear Creek while one (MLB) is at the southeast edge in McIver State Park (Fig. 1).

Structure analysis identified $K=3$ as the optimum number of clusters in the study area (Fig. 2). A large number of individuals were assigned to one cluster by Structure, however, the majority of sampling locations were composed of individuals with mixed assignment probabilities (Fig. 3). Only three locations were assigned entirely to one cluster (CPS, MBB, MRS; Fig. 3); all three had very low genetic diversity and low interallelic genetic disequilibria ($H_s <0.1$, %LD<0.05; Table 1). Low genetic diversity and disequilibrium indicates that these locations were recently established from a single source (Ramakrishnan et al., 2010). Sampling locations at the southeast edge of the

watershed clustered together in Structure analysis as cluster 1 (Fig. 3), which is largely absent from the rest of the area, while the three central sampling locations identified as original introduction points are primarily composed of Structure clusters 2 and 3 (CBR, CC, RSP; Fig. 3). From this and our population genetic analysis, we can identify two likely areas of introduction into the Clackamas watershed, one in McIver State Park (location MLB) and a second in the region surrounding Clear Creek (sampling locations CBR, CC, RSP).

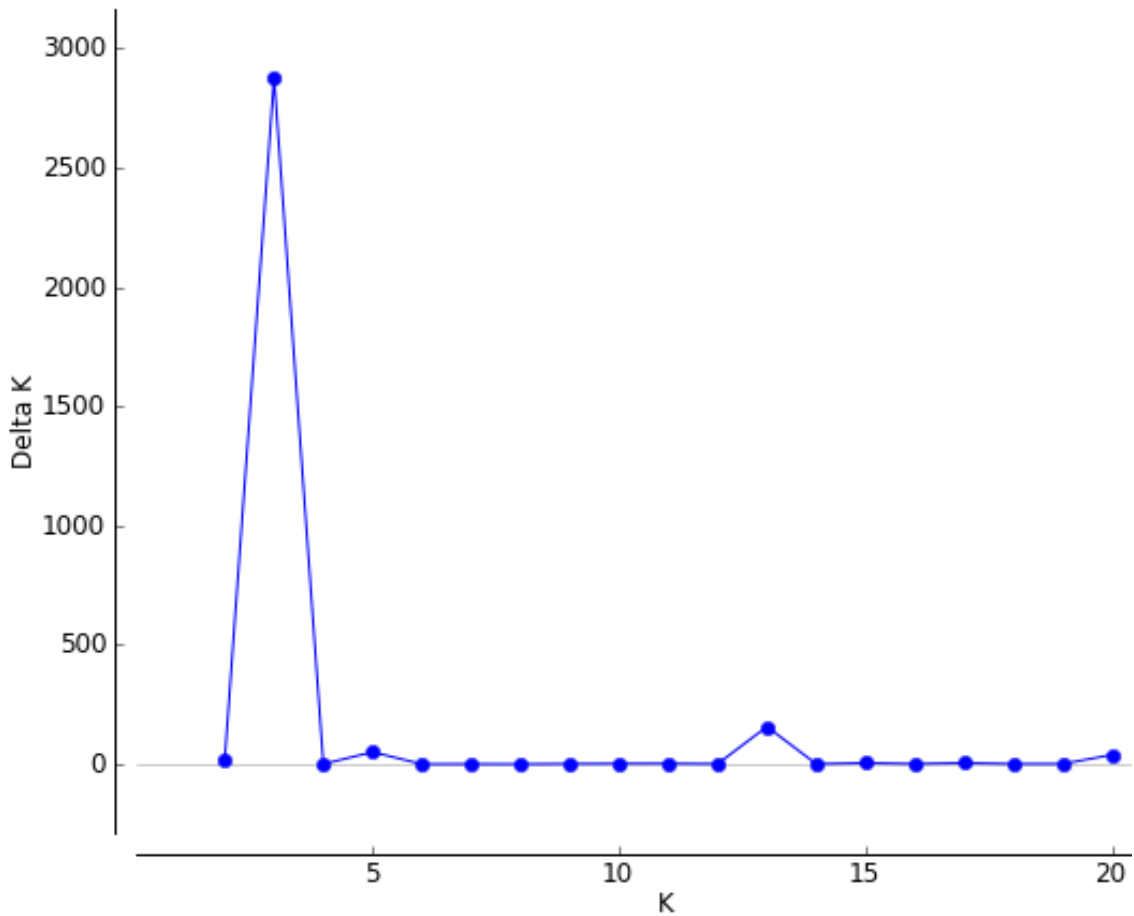


Figure 2. Delta K calculated using output from Structure following Evanno (2005) for K=1-20. Peaks represent optimum values of K.

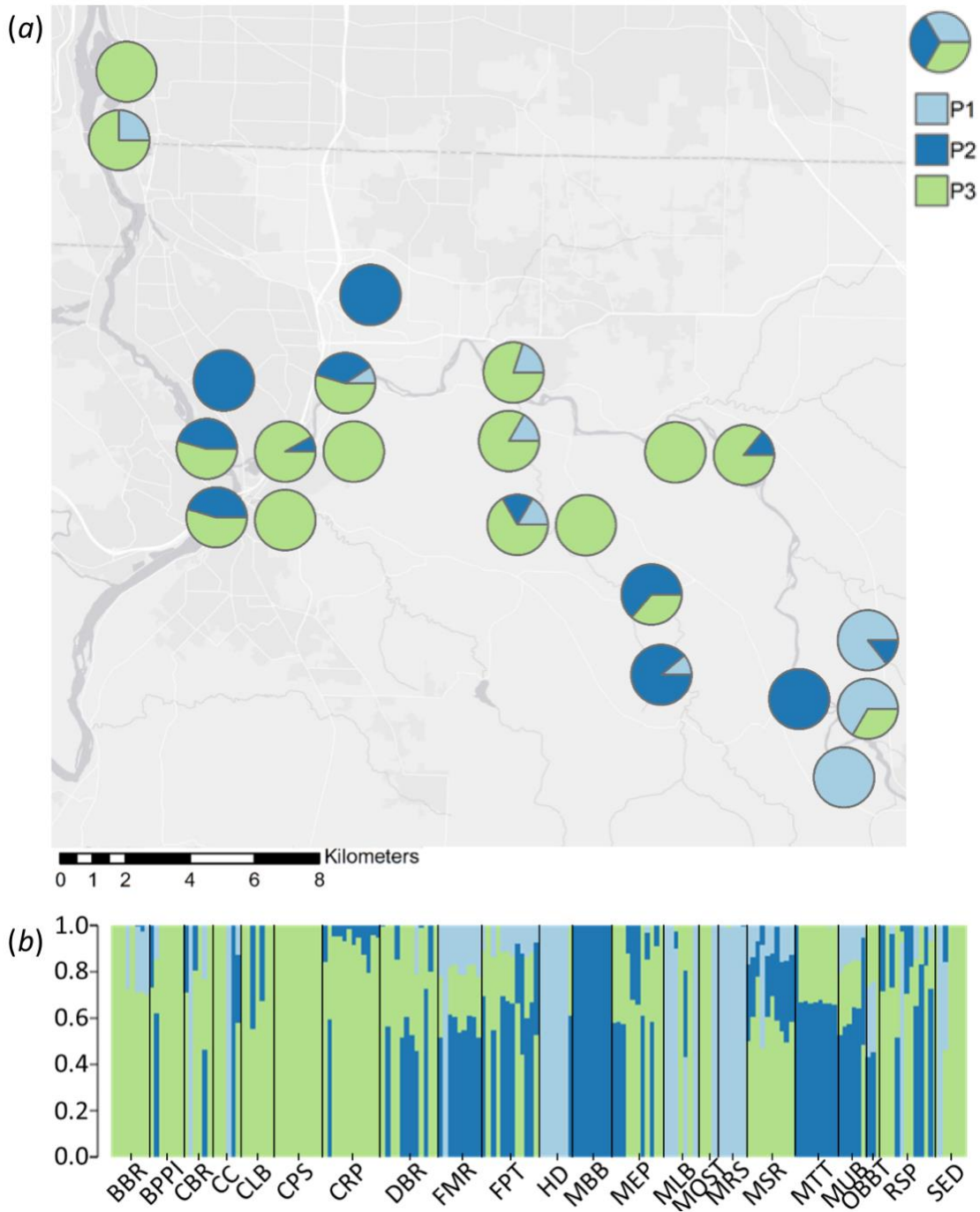


Figure 3. Sampling location cluster composition in relation to geography of the study area (a) and individual assignment probabilities to clusters P1-P3 from Structure arranged by sampling location (b).

Table 1. Average observed heterozygosity (H_o), gene diversity (H_s), inbreeding coefficient (F_{is}), and proportion of loci with significant linkage disequilibria ($p < 0.05$, %LD) by population.

| Population | Latitude | Longitude | H_o | H_s | F_{is} | %LD |
|------------|----------|------------|-------|-------|----------|-------|
| BBR | 45.37986 | -122.41015 | 0.164 | 0.161 | 0.013 | 0.046 |
| BPPI | 45.37919 | -122.40415 | 0.087 | 0.123 | 0.280 | 0.048 |
| CBR | 45.39243 | -122.49561 | 0.133 | 0.255 | 0.369 | 0.086 |
| CC | 45.35845 | -122.48145 | 0.109 | 0.297 | 0.501 | 0.102 |
| CLB | 45.37249 | -122.60039 | 0.089 | 0.126 | 0.295 | 0.031 |
| CPS | 45.37861 | -122.57660 | 0.072 | 0.044 | -0.413 | 0.004 |
| CRP | 45.37874 | -122.58533 | 0.084 | 0.097 | 0.269 | 0.033 |
| DBR | 45.37320 | -122.60605 | 0.145 | 0.231 | 0.311 | 0.119 |
| FMR | 45.31748 | -122.43463 | 0.129 | 0.188 | 0.359 | 0.101 |
| FPT | 45.33974 | -122.43922 | 0.144 | 0.196 | 0.279 | 0.098 |
| HD | 45.31340 | -122.37220 | 0.148 | 0.151 | 0.156 | 0.061 |
| MBB | 45.37794 | -122.60950 | 0.129 | 0.068 | -0.882 | 0.007 |
| MEP | 45.37716 | -122.61611 | 0.127 | 0.202 | 0.299 | 0.095 |
| MLB | 45.29860 | -122.36108 | 0.142 | 0.288 | 0.430 | 0.121 |
| MOST | 45.48172 | -122.65471 | 0.095 | 0.246 | 0.503 | 0.048 |
| MRS | 45.29014 | -122.36169 | 0.176 | 0.092 | -0.830 | 0.007 |
| MSR | 45.35858 | -122.46577 | 0.213 | 0.231 | 0.071 | 0.081 |
| MTT | 45.42118 | -122.55317 | 0.105 | 0.084 | 0.003 | 0.018 |
| MUB | 45.31162 | -122.38004 | 0.197 | 0.207 | 0.023 | 0.036 |
| OBBT | 45.48150 | -122.65171 | 0.100 | 0.192 | 0.345 | 0.001 |
| RSP | 45.39648 | -122.56226 | 0.196 | 0.270 | 0.250 | 0.148 |
| SED | 45.38149 | -122.49701 | 0.107 | 0.151 | 0.374 | 0.138 |

(b) Isolation by Resistance

Most landscape features were assigned resistance values lower than their surrounding areas by ResistanceGA, suggesting these features are potentially acting as conduits to gene flow (Table 2). Conduit features include rivers, both high and low levels of development, and roads. Agriculture was assigned a value higher than surrounding areas, indicating that it may act as a barrier to gene flow (Table 2). Original values for percent canopy cover were assigned an Inverse Ricker relationship to their final circuit resistance values by ResistanceGA (Fig. 4).

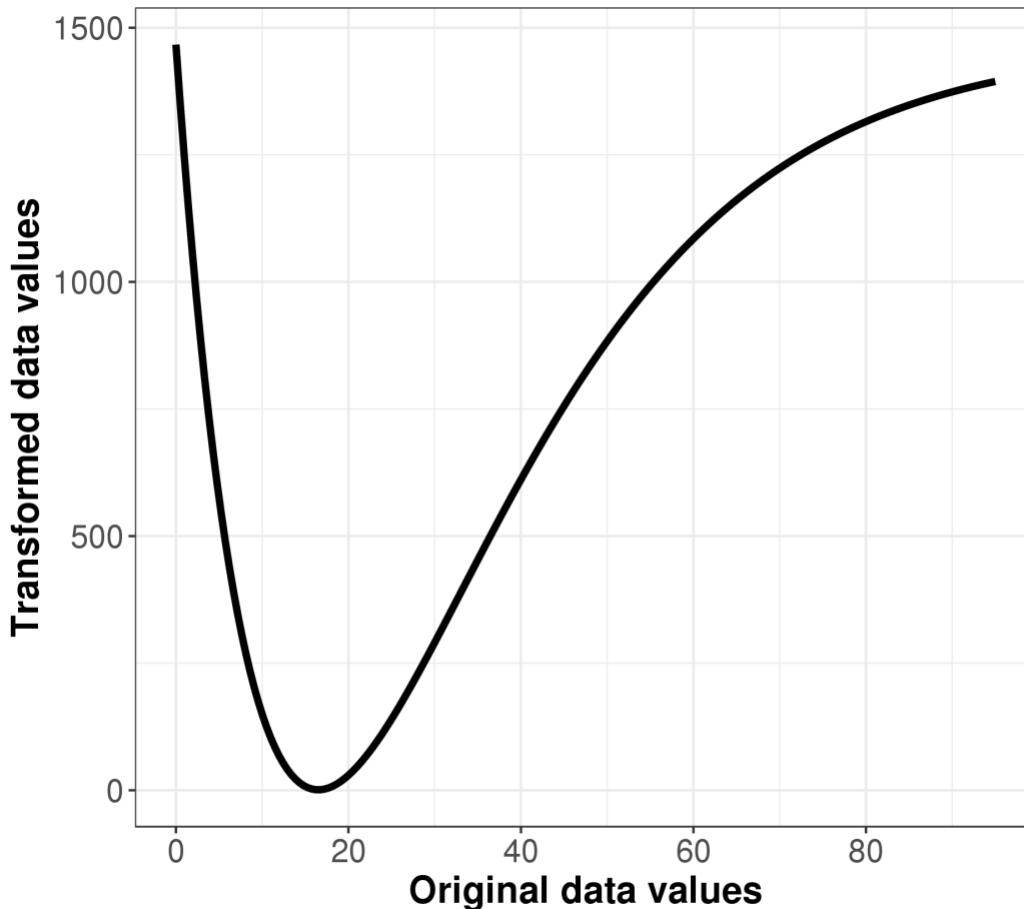


Figure 4. Original data values for canopy cover layer and their transformed values following Inverse Ricker transformation by ResistanceGA.

Optimized landscape models representing the impact of development, roads, canopy cover, and agriculture all were insignificant in our initial model (Table 2). Only geographic distance and rivers acting as a conduit were significant and carried forward into the final model. Rivers as a conduit continued to have a significant relationship with genetic distance in the final model (Coeff.=0.006, p-value<0.001,), while geographic distance dropped to marginal significance (p-value=0.063; Table 2). While it would be possible to run an additional MRM demonstrating the impact of rivers alone, keeping geographic distance in the model allows us to control for the impact of spatial autocorrelation on these results. ResistanceGA's optimization of circuit values on this

landscape assigned rivers a resistance value approximately 60 times lower than surrounding areas and the final model explained a large portion of variation in genetic distance (Table 2; $R^2=0.333$, $p\text{-value}<0.001$).

Table 2. Backwards selection results for Isolation by Resistance (IBR) models optimized using *ResistanceGA*. All feature layers were analyzed with the natural log of geographic distance as a co-variable in *MRM* to correct for Isolation by Distance (IBD). Most features acted as conduits for dispersal (Feature Resistance = 1) and had lower resistance values for the surrounding landscape (Matrix Resistance), while Agriculture had a high Feature Resistance indicating that it acted as a dispersal barrier. Significant features in bold. †Canopy cover was a continuous surface with an Inverse Ricker relationship to optimize circuit resistance (see Fig. 4).

| Landscape Feature | Feature Resist. | Matrix Resist. | Initial Model | | | Final Model | | |
|-------------------|-----------------|----------------|---------------|--------------|--------|-------------|------------------|-------|
| | | | Coeff. | Prob. | VIF | Coeff. | Prob. | VIF |
| Geo. Dist. | NA | NA | -0.056 | 0.022 | 4.636 | -0.034 | 0.063 | 2.420 |
| Rivers | 1 | 67.61 | 0.006 | 0.019 | 5.029 | 0.006 | <0.001 | 2.420 |
| Dev. (High) | 1 | 124.51 | 0.004 | 0.075 | 11.681 | --- | --- | --- |
| Roads | 1 | 69.66 | -0.003 | 0.181 | 2.166 | --- | --- | --- |
| Agriculture | 364.85 | 1 | -0.043 | 0.193 | 6.316 | --- | --- | --- |
| Dev. (Low) | 1 | 70.86 | 0.000 | 0.987 | 6.657 | --- | --- | --- |
| Canopy † | NA† | NA† | 0.000 | 0.988 | 5.506 | --- | --- | --- |

To determine if gene flow along rivers occurs primarily in the direction of water flow or bidirectionally, we regressed the genetic diversity (H_s) of sampling locations within 1 km of the Clackamas River against their distance along the river from farthest upstream location. If gene flow was moving in the direction of river flow, then we would expect to see a gradient of decreasing genetic diversity in populations close to the water moving down river. There was no significant relationship between distance along the river and genetic diversity ($R^2<0.001$, $p\text{-value}=0.993$; Fig. 5), indicating that gene flow along rivers is bidirectional. We conducted MRM with distance along the river as a predictor of genetic distance and found a significant relationship ($R^2=0.155$, $p\text{-}$

value=0.005), indicating the presence of IBD along rivers in addition to IBR with rivers acting as a conduit.

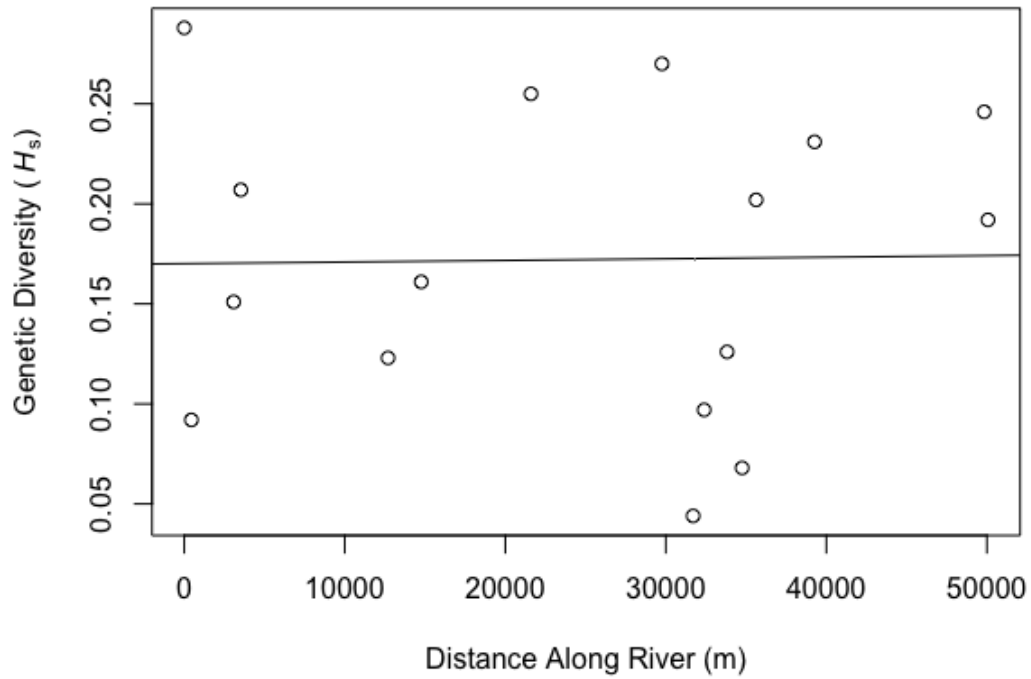


Figure 5. Nei's unbiased gene diversity (H_s) in populations within 1000m of the river versus their distance from the farthest upstream population.

Discussion

Our investigation of the history of invasion of *B. sylvaticum* in the Portland metropolitan area revealed two areas likely to be the original points of introduction into the study area. We also found evidence of extensive ongoing gene flow, represented in a large number of individuals with mixed assignment probabilities across the watershed, as well as sites that appear to have been recently colonized. While analysis of landscape features identified rivers as the single most important conduit for dispersal, it is not clear that seed movement along the rivers is due to water dispersal of seeds. Evidence for bidirectional gene flow along rivers indicates that river-mediated dispersal may be more likely due to the association between the rivers and human recreation activities, and possibly movement of other animals along riparian corridors.

Invasion History

Two areas were identified as potential local introduction points for *B. sylvaticum* in the Clackamas watershed; the area surrounding Clear Creek (CC) and Milo McIver State Park (MRS). We cannot determine precisely how many times *B. sylvaticum* was introduced into these areas. Sampling locations in the Clear Creek area contained individuals assigned to two distinct genetic clusters. This could have resulted from multiple introductions near Clear Creek. However, the original introduction into Oregon occurred in two locations and the invasive genotype is the result of admixture between multiple source locations (Rosenthal et al., 2008). It is possible that the individuals which originally colonized Clear Creek carried with them the signature of admixture between the original invasive lineages. We can confidently state that the significant genetic

structure found in the watershed is very unlikely to have arisen since local colonization, and therefore there were at least two independent colonization events.

Following introduction, additional dispersal into the watershed and subsequent gene flow generated patterns of local genetic similarity. This pattern of multiple introductions via “jump” dispersal at the edge of the range is consistent with results in other invasive species (Genton, Shykoff, & Giraud, 2005; Kirk, Paul, Straka, & Freeland, 2011; Parisod & Bonvin, 2008; Petit et al., 1997; Suarez, Holway, & Case, 2001). While founder events lower genetic diversity at the range edge, multiple introductions increase diversity and evolutionary potential on the edges of the range (Berthouly-Salazar et al., 2013; Bialozyt et al., 2006). Gene flow between the multiple invasive lineages present in the Clackamas watershed has likely alleviated loss of genetic diversity from founder events, potentially providing the diversity needed to continue expanding the range.

Landscape Influences on Gene Flow

While we have found that rivers are significant corridors for gene flow, it is not clear that seed movement along the rivers is due to water dispersal of seeds. There are multiple possible explanations for rivers acting as a conduit for gene flow in invasive *B. sylvaticum*. First, seeds could be dispersed by the flow of water. Second, it is possible that the gaps created in canopies by rivers act as conduits for pollen dispersal. Finally, recreationalists or deer could be transporting seeds among river access points.

There is no evidence of directional gene flow along the river, as would be expected if seeds were being moved by water (N. P. Miller & Matlack, 2010). There is also no evidence of decreasing genetic diversity (H_s) at sites further downriver, as would be expected when seeds are dispersed by water (Nilsson, Brown, Jansson, & Merritt, 2010).

While this does not completely rule out *B. sylvaticum* seed movement by water, we can conclude that movement of seeds by river flow is not likely to be the primary dispersal mechanism. Similarly, we see no effect of canopy cover on genetic distance independent of rivers, as would be expected if pollen dispersal by wind in the river's canopy opening over were the primary cause of rivers acting as conduits (Imbert & Lefèvre, 2003; Young, Boyle, & Brown, 1996). While pollen movement is very likely contributing to the overall high amount of gene flow in the study area, it is unlikely that pollen dispersal above rivers is the cause of rivers acting as a conduit for gene flow.

River-mediated dispersal is more likely related to movement along rivers by humans and other animals. *B. sylvaticum* seeds possess a barbed awn and are known to disperse via large animals in the native range (Heinken & Raudnitschka, 2002). White-tail deer are present in large numbers in the Pacific Northwest and are known to disperse a wide range of both native and invasive plant species (Myers, Vellend, Gardescu, & Marks, 2004; Vellend, Myers, Gardescu, & Marks, 2003). Deer have been shown to move preferentially in riparian corridors (Finder, Roseberry, & Woolf, 1999), meaning deer-mediated dispersal could underlie the pattern of genetic diversity seen in our landscape analysis. In addition to dispersal, disturbance caused by deer could facilitate the success of *B. sylvaticum* (Knight, Dunn, Smith, Davis, & Kalisz, 2009; L. A. V. Taylor, Hasenkopf, & Cruzan, 2015). However, as deer movement is known to respond to suburban land use and other habitat features (Felix, Walsh, Hughey, Campa, & Winsterstein, 2007; Kilpatrick & Spohr, 2000), we would expect to see an effect of other land use features on genetic distance if deer were the primary dispersal vectors.

The lack of a clear gradient of genetic diversity could likewise reflect the bi-directional movement of people and their dogs among river access points. The primary water feature in the study area is frequented by recreational fishermen, boaters, and rafters (Shelby, Johnson, & Brunson, 1990). *Brachypodium sylvaticum* is common at river access points throughout the area, often lining boat ramps and informal paths leading to the water. There is ample opportunity for recreationalists to come into contact with and incidentally disperse *B. sylvaticum*, and dispersal of seeds in clothing has been demonstrated in other weedy grasses (Ansong & Pickering, 2014; Vibrans, 1999). Particularly considering that dogs frequently accompany humans visiting these recreational areas, the opportunity for seed transport becomes very high. Dogs have been shown to be effective dispersers of seeds in forest habitats (Heinken, 2000), and are particularly adept at dispersing seeds with morphological features associated with epizoochory grown on tall inflorescences such as *B. sylvaticum* (Graae, 2002). The sheer prevalence of *B. sylvaticum* in recreational access points and its relatively low abundance in other areas points to movement of seeds in dog fur and recreational gear as the primary cause of gene flow along rivers.

All known populations of this invasive grass in the Portland metropolitan region are associated with human activity—they are most commonly distributed along roads in pullouts, along hiking trails, and at river access points. Given that humans are likely contributing to movement of *B. sylvaticum* propagules, we should expect to see some movement of seeds along roads in addition to along the river. Dispersal of invasive plant seeds by vehicles is known to occur (Veldman & Putz, 2010; von der Lippe & Kowarik, 2006; Zwaenepoel, Roovers, & Hermy, 2006). However, a significant effect of roads was

not seen in our analysis. An assumption of Circuitscape analysis is that genetic distance should increase proportionally with distance through the circuit, with shorter dispersal distances occurring more frequently than long distances. However, if seeds are transported on clothing and pet fur then we can expect long distance movement to be more common than short-distance dispersal. It is possible that circuit analysis is unable to detect seed movement from jump dispersal events resulting from movement of people by cars at this scale.

Newly invasive plants are excellent models for understanding processes affecting range expansion. Humans both create habitat for *B. sylvaticum* through disturbance and disperse seeds directly to these disturbed locations. Once introduced into an area, facilitation of *B. sylvaticum* dispersal by recreation creates a network of invasion foci and creates gene flow among invaded locations. Human involvement in dispersal can drastically increase and direct gene flow of invasive species to suitable habitat and may have a major influence on the abundance and distribution of alien plant species. We suggest that the dispersal of *B. sylvaticum* by humans both to new areas and between existing populations will aid its continued range expansion and success as an invasive species.

CHAPTER 3: CONCLUSIONS

The research presented here incorporates population genetic data with spatially explicit models of movement to infer patterns of dispersal at the edge of an expanding range. While this is a single-species study, spatially explicit studies of range expansion like the one presented here are necessary for understanding the process of invasion generally. *B. sylvaticum* has been found to disperse via large ungulates in its native range, along roads in rural central Oregon, and now via recreational movements in the more population dense Clackamas watershed. This shifting of dispersal mode in *B. sylvaticum* demonstrates the alarming versatility of invasive species ecology.

While the data presented here is sufficient to identify recreation as the most likely vector creating gene flow, the nature of landscape genetic analyses is correlative. Genetic sampling of populations only captures realized seed dispersal, potentially biasing my findings towards the vector most closely related to our sampling locations. Confirmation of these findings using direct observation or experimental manipulation is needed to completely rule out alternate scenarios of dispersal. While direct observation misses long distance dispersal events, I believe that it is the most practical option for confirming my findings. Direct observation could be achieved by finding relative quantities of seeds moved by potential vectors at river access points (i.e. by counting seeds found on dogs, deer, and boots). Further, seed traps experiments could be conducted to confirm my finding that hydrochory is not a significant mode of dispersal for *B. sylvaticum*.

Likewise, my identification of local source populations relies heavily on the assumption that population age corresponds to genetic diversity. While this is based on previous work in *B. sylvaticum*, there are other potential reasons for a population to have

higher genetic diversity relative to surrounding populations. High levels of directional gene flow into a population could inflate its diversity relative to populations of the same age. For instance, in my study the sampling location at Riverside County park (RSP) was identified as high-diversity and a candidate introduction point. However, this location is a popular boat launch point close to the City of Gladstone. The sheer amount of recreation occurring at this location may have increased dispersal into the site, causing higher genetic diversity than would be expected for the age of the site. In contrast, the high-diversity sampling site Clear Creek (CC) is on private property in a rural location and is unlikely to be high diversity due to asymmetrical gene flow.

Locations identified as likely introduction points into the Clackamas watershed (McIver State Park and Clear Creek) are both secondary growth forests. Logging equipment has the potential to move large numbers of seeds (Veldman & Putz, 2010), making movement of logging equipment for timber harvest a likely vector for introduction into these locations. Records of logging on private land are not well kept or readily available, making it difficult to confirm this hypothesis. A future experiment could confirm that *B. sylvaticum* was introduced to the study area by establishing that population age of the original locations coincided with the last timber harvest using stand age from dendrochronology and genetic population age estimates.

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