Gene Flow in an Invasive Species of Grass, Brachypodium Sylvaticum

Trieste Dobberstein
Portland State University

Follow this and additional works at: https://pdxscholar.library.pdx.edu/mcnair

Let us know how access to this document benefits you.

Recommended Citation
https://doi.org/10.15760/mcnair.2005.95

This open access Article is distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (CC BY-NC-SA 4.0). All documents in PDXScholar should meet accessibility standards. If we can make this document more accessible to you, contact our team.
Gene Flow in an Invasive Species of Grass, Brachypodium Sylvaticum
by
Trieste Dobberstein

Faculty Mentor:
Mitchell Cruzan

Gene Flow in an Invasive Species of Grass, Brachypodium Sylvaticum

Trieste Dobberstein
Faculty Mentors: Mitchell Cruzan

Abstract

Invasive species of plants are responsible for a staggering amount of damage to the landscape and floral and faunal communities. Invasions remain largely ignored until invasive populations have reached critical levels, at which point costly and time-consuming efforts are required for containment or control. Studying the early stages of an invasion can provide insight into the mechanics behind the establishment and spread of invaders, as well as shed more light on the processes of microevolution. *Brachypodium sylvaticum* is a grass recently invasive to the Willamette Valley, and possesses many traits valuable to a migration and evolutionary study. This study has focused on the aspects of the migration patterns of B.*sylvaticum* using microsatellite DNA. Microsatellite analysis of two loci determined that the populations studied were experiencing long-distance dispersal, in which large, well-established populations provide seeds to far-away sites; migration patterns of simple diffusion were also seen. In-field observations of this invasive grass suggest that this highly damaging invader enjoys the greatest success in disturbed areas such as logging tracts, riparian areas, and alongside roads. The long-distance migration of this genetic material appears to be propelled, at least in part, by logging trucks and tourists.

Introduction

Invasive Species and Noxious Weeds: Properties of Invasives and Consequences of Invasion

The official U.S. definition of an invasive species is “an alien species whose introduction does or is likely to cause economic or environmental harm or harm to human health.” (Executive Order 13112) While only a small fraction of the total number of alien organisms introduced to the United States each year becomes invasive, the damage they cause is staggering. Estimates of the cost of controlling, preventing, and repairing the damage caused by invasives run from 100 to 300 billion dollars annually. Around one-third of the economic damage caused by invasives is the result of invading plants (Pimental et. al, 1999). Aside from monetary losses, invading organisms in the U.S. represent threats to many endangered species of flora and fauna.
By encroaching on habitat, utilizing available resources, and altering fire and hydrology regimes, invasive plants prove to be tough competition for the indigenous natives (Sakai, Allendorf et al. 2001; Rodosevich, Stubbs et al. 2003). Alien species may attain invasive status by possessing any one of a list of properties: a profound ability to colonize disturbed areas, a shorter germination time, earlier flower and seed production, faster modes of seed dispersal, or lack of predators in their new habitat (Rejmánek 1996; Goodwin 1999). Many of these characteristics enable an invasive plant to take advantage of high rates of colonization in disturbed areas, a trait that increasingly gives them an edge as humans manage land for agricultural and developmental resources (Harrod 2001; With 2002; Chornesky and Randall 2003). This tendency of many invasive plants to take advantage of an already distressed area, inhibiting natural or mechanical restoration, is one of the characteristics that make them so detrimental. In highly disturbed areas, such as in and around logging sites, along roads and in newly developed areas, it is often easy to spot invasive plants; even an inexperienced observer can easily point to the floral invaders of a system, as they will usually be the ones forming large monocultures, allowing little or no establishment of competitive natives.

The lack of biodiversity seen after an invasive takes hold affects ecosystems at many levels. Many types of animals and insects are specialists, unable to utilize a variety of plants for energy or habitation. In Hawaii, for example, many endemic species of birds have been threatened or exterminated due to the decrease in numbers of native tree populations; the trees themselves have been decimated by the invasive feral European boar, *Sus scrofa*, population (Stone 1989). Invasive plants may edge out the natives by using too much of one or a few resources, such as available water or soil nutrients. One invasive, saltcedar, actually lowers the water table in the Great Basin region, causing drought conditions that make it difficult or impossible for native willows to compete; this ability has consequences that extend beyond the boundaries of the saltcedar populations, affecting plants, aquatic and terrestrial animals outside of the saltcedar habitat as well as its immediate neighbors (D'Antonio 1992; Sala 1996). An area’s fire regimes can be strongly impacted by changes in the floral community structure: Invading *Bromus tectorum*, or cheatgrass populations in the Great Basin have been determined to be the cause of an increase in regional wildfire frequencies from one every 50 to 100 years to one every 1 to 2 years. As the area’s native shrubs are not fire tolerant, their populations are devastated with every fire, providing ample disturbed space for the cheatgrass to continue invading (Stewart and Hull 1949; Young and Allen 1997).

Much of the Pacific Northwest, with wet, mild seasons, provides an easy, comfortable environment for both flora and fauna. The geographic isolation supplied by the surrounding
mountain ranges may have granted the native species of the region protection from colonizing aliens, lowering the competitive abilities of natives and making them extra vulnerable to invasion. The European explorers began introducing many invasive species well before they began altering landscapes for agricultural uses (Harrod 2001). Early exploration expeditions proved that it takes only fleeting contact to launch a devastating invasion. Before Europeans landed, North America was uninhabited by, among other things, the common house mouse, black and brown rats, starlings, pigeons, some earthworms, and smallpox virus. All of these organisms have drastically altered northern landscapes and natural populations, from the native human populations, (in the well-known cases of smallpox pandemics), to the extreme change caused in the carbon cycle by the introduction and subsequent invasion of earthworms (Bohlen 1997).

Examining an Early Invasion: Management Regimes and Evolution

The destructiveness of invasive plants and animals, as well as the rapidity with which aliens may become invasive, makes prevention and/or early detection necessary for effective management. However, relatively little is known about the mechanisms leading up to invasion; usually, an invasive species goes ignored or undetected until it poses a serious problem—there has been as yet little opportunity to observe an invasion at its commencement. Information about the early stages of an invasion could prove invaluable to the development of management regimes. The rather rare occurrence of an alien species becoming invasive points to the existence of some event, or events, that makes invasion possible for these organisms. Describing these events can tell managers a great deal about regimes needed to control invasions, as well as which regions need to be more or less strictly policed and protected from aliens (Dietz, Fischer et al. 1999; Rejmánek 1999; Eckert, Massonnet et al. 2000; Mack, Simberloff et al. 2000; Rejmánek 2000; Sakai, Allendorf et al. 2001).

The study of early invasions can tell us more than just how to control invasions; the events leading to the invasiveness of a species must have some genetic and evolutionary components, and the study of these rapid evolution events can lead to a more complete understanding of evolution in general. Some invasions may, in fact, be made possible by the development of a new species or Evolutionarily Significant Unit, an important event of which the study can provide many insights (Blossey and Notzold 1995; Lee 2002; Levin 2003).

There are a few possibilities for the events that make an invasion possible. Alien species may go through random mutation events that lead to some phenotype that allows them to spread rapidly through their environs (Maron, Vila et al. 2004). Invasive genotypes may evolve through
hybridization or long out-crossing with natives or other invasives (Dachler and Strong 1997; Ellstrand and Schierenbeck 2000; Bonnin, Ronfort et al. 2001; Pooler, Dix et al. 2002; Collin and Shykoff 2003; Petit, Bodenes et al. 2004). The arrival of relatively few alien immigrants may, through random chance, cause a founder event or events that confers on a native environment a high frequency of individuals that are already highly pre-adapted to that particular environment (Tsutsui, Suarez et al. 2000; Mack 2003; Parker, Rodriguez et al. 2003). Finally, the release from predators that an alien species can enjoy in their new environment may allow them to allocate available energy to reproduction and growth rather than defense mechanisms, giving the invaders an edge over their native counterparts that might not have existed before the opportunity for change presented itself (Herlihy and Eckert 2002; Wolfe 2002; Lee, Remfert et al. 2003; Mitchell and Power 2003; Maron, Vila et al. 2004).

**After Establishment of an Invasive Species: Genetic Dispersal and Gene Flow**

Once a plant has become established in an alien environment and develops or begins to exhibit the trait or traits necessary to spread and compete with natives, its mode of dispersal can provide information as to how to manage and contain the spread (Mack, Simberloff et al. 2000; Matlack 2002; Clark, Lewis et al. 2003; Filipe and Maule 2004). Simple diffusion, in which new generations progress outward from their source in a gradual expansion pattern, would require a mode of biological control different from “guerilla” dispersal, in which small populations far from the source are founded with genetic information from one or a few main source populations. The mode of dispersal can, to a large degree, be determined by the genetic diversity seen within populations and the differentiation between populations: If gene flow can be observed between two relatively isolated populations, conclusions can be drawn that long-distance seed (or pollen) dispersal is taking place, or that the populations share a common source (Nichols and Hewitt 1994; Cain, Milligan et al. 2000; Muller-Landau, Levin et al. 2003; Walker, Hulme et al. 2003; Paetkau, Slade et al. 2004). Genetic diversity can also provide clues as to how long populations have been established (the more diverse a population is, the older it is likely to be), as well as the relative ages of populations (more genetically diverse populations may be older than the less diverse ones) (Gautschi, Jacob et al. 2003; Telfer, Piertney et al. 2003).
Examining Genetic Diversity Using Microsatellites

Microsatellite analyses look at the actual lengths of specific fragments of DNA in order to determine genetic diversity. Microsatellites are short tandem repeating sequences of DNA. Microsatellites can be present in alternate forms, or alleles, distinguished from one another by variations in the length of repeating segments. Different individuals may possess different or identical alleles of microsatellites, just as individuals may possess different or identical alleles of other genes, such as those coding for hair or eye color. The microsatellites of *Brachypodium sylvaticum* were identified by Alisa Ramakrishnan (unpublished).

Because microsatellites do not code for any proteins, most are under no selective pressure and enjoy relatively high rates of mutation (around one mutation per fifty generations). This lack of selective pressure means that microsatellite alleles are passed to offspring without any limits or barriers; the movement and presence of microsatellite alleles can describe the gene flow among populations (Ouborg, Piquot et al. 1999; de Jong, Guthridge et al. 2003). Gene flow refers to the movement of genes, or alleles, from one population to another population; alleles are able to move with the migration of individuals or through the transport of gametes via wind, water, pollination, or some other means.

An Invasive Grass in Oregon: *Brachypodium sylvaticum*

This project is part of a larger study, spearheaded by Alisa Ramakrishnan of Portland State University, examining the invasion of *Brachypodium sylvaticum* in Oregon. Native to North Africa and Eurasia, (Hitchcock 1969) *B. sylvaticum* is a newly invasive species of grass that has become noxious in the Willamette Valley region of Oregon, displacing typical understory (e.g. ferns, flowers, and native grasses,) in many areas of the invasion (Kaye 2001). My research is focusing on the gene flow of *B. sylvaticum* from the central, founding populations, to the populations located on the periphery of the region experiencing the invasion. From our results we can determine both how *B. sylvaticum* is dispersing, whether it is spreading through simple diffusion and/or “jumping” from one location to another via some vector; we may even be able to get an idea of which populations from which the genetic material is coming, depending on the microsatellite alleles found in populations. We can also get a general idea of how old, relative to the central populations, the peripheral populations are—based on the amount of genetic diversity found.
Materials and Methods

Experimental Design

For this study, 18 central populations will eventually be sampled. We hope to collect from five to ten peripheral sites; a few of the reported peripheral populations visited so far have yielded no specimens. From each site, 25 individuals are sampled, with two leaves taken from each individual. Because *B. sylvaticum* is a bunch grass, a minimum of five feet was maintained between sampled individuals to avoid resampling of the same or closely related plants. As yet, four central populations have been analyzed: two outside of Corvallis, and two outside of Eugene. The two analyzed populations outside of Eugene will be called populations A and B; those outside of Corvallis, C and D. The geographic locations of the four sampled populations are shown in Figure 1. The four populations were analyzed using two loci of microsatellites. (A locus is the position on a chromosome of a gene or other chromosome marker.)

Figure 1

*Locations of Analyzed Brachypodium sylvaticum Populations*
Molecular Analysis

Microsatellites, once identified, require the polymerase chain reaction, or PCR, to amplify enough fragments of the specific loci so that they can be analyzed and sorted by size. There are three main steps to PCR: First, the genomic DNA must be denatured, or broken apart into two single strands; second, primers anneal to the areas of specific genomic DNA to be amplified. (Primers are short sequences of DNA that give the DNA polymerase the starting point needed to initiate synthesis of DNA.) After the primers anneal to the loci being amplified, repeated cycles of hot and cold temperatures of the PCR enable the DNA polymerase to synthesize the microsatellite loci for which the primers are specific.

One of the microsatellite loci, 2-3A1, consisted of a trinucleotide repeating sequence; the other, 3-4E8, contained a tetranucleotide repeat. For the results discussed here, we will only use those obtained from locus 2-3A1, as the data obtained from 3-4E8 were incomplete. (We were not able to generate enough working fragment analyses to render our results statistically viable.) Mutations and variations at these loci result in varying lengths of the repeating sequences from one generation to the next. The primers used in amplification of the fragments were designed by Alisa Ramakrishnan. I used the primers to amplify only those areas of the extracted DNA that represent the microsatellite loci.

After the polymerase chain reaction was used to amplify the microsatellite loci, the fragments were analyzed for length, (amount of base-pairs found within the microsatellite fragments,) on an ABI 310 Genetic Analyzer. The alleles found in each population were compared in an analysis evaluating the similarity between populations; genetic distance between populations was calculated using the equation $d_{ij} = \sum (x_i - x_j)^2 / x_i + x_j$, where $x$ is the allele frequency in population $i$ or $j$.

Results

Field Observations

Due to time constraints limiting the amount of genetic data available, I would like to discuss a bit of the field observations that were made: First, Brachypodium sylvaticum appears able to out-compete nearly all competitors. The invasive Himalayan blackberry (Rubus discolor) seemed in most cases to inhibit growth of B. sylvaticum, except in places where the B. sylvaticum was extremely well-established: in forests with the largest B. sylvaticum populations, nothing was seen growing within the dense swaths of grass except trees.
Large numbers of *B. sylvaticum* populations were found in recently logged areas (within the last two decades); the largest populations were found near the site of original introduction, in MacDonald Research Forest, (located near population A as shown in Figure 1.) The immediate area is the site of current logging tracts, as well as many past logging ventures. I would hypothesize that further genetic analysis will reveal that even geographically isolated populations will be close genetically, in instances where both populations are located at or near recent logging sites. Populations of *B. sylvaticum* that exist near recent logging sites are shown in Figure 2. *Brachypodium sylvaticum* populations were also found in many areas frequented by tourists: Along the Rogue River Wilderness Trail in southern Oregon, at the summit of Cape Perpetua on the Oregon coast, at rest areas and campgrounds on Highways 58 and 126 east of Eugene, and in a state park near Redmond, Oregon. Populations of *B. sylvaticum* at sites frequented by tourists are shown in Figure 3.

Figure 2

*Populations Near or At Logging Sites*
Molecular Results

Though the study so far includes only four populations and one microsatellite locus, the results were intriguing: Genetic distances between the four populations, calculated using the aforementioned equation, between the four populations are represented in Table 1.

Table 1
Genetic Distances Between Populations for Microsatellite Locus 2-3A1

<table>
<thead>
<tr>
<th>Populations</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>1.1388710</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>1.0128235</td>
<td>8.6764512</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>7.4321583</td>
<td>9.0525293</td>
<td>7.8121575</td>
</tr>
</tbody>
</table>

A value of zero indicates identical allele frequencies; higher values show the amount of relative genetic distance, or dissimilarity, between the populations. As is shown by the data, population D is very genetically different from the other three populations; A is similar to both populations B and C, but B and C are very different from each other: Population A contained alleles found in both B and C; however, population B had very few alleles also found in population C; population D possessed
alleles not found often in any of the other three populations. The geographic relationship of the four analyzed populations is shown in Figure 1. For the locus 3-4E8, the results of which were not included due to the lack of completely satisfactory data from population A, the genetic distances between populations B, C, and D resembled those seen in Table 1.

**Discussion**

The results of the genetic analysis indicate that both guerilla dispersal and simple diffusion are taking place: The relatedness of population A to both B and C suggests that population A served as the source of genetic material (via seeds) for both of these populations. Population A is (geographically) relatively close to populations B, illustrating simple diffusion of genetic material and certain individuals, while being geographically distant from population C. The large genetic distance between populations B and C implies that a bottleneck took place during the establishment of these populations; only a small number of alleles from population A made it to the new sites of establishment for B and C, and the alleles contained in the genetic material used to establish population B were different from those contained in the genetic material that established C. Due to the presence of genetic material not seen at the other sites, population D, near Eugene, was established from some different source.

The proximity of populations A and C to logging sites hints at seed dispersal via logging equipment, and may provide an explanation for why these two populations share genetic information. Population B was near a residential area, and would have less of an opportunity to “communicate” genetically with population C.

The establishment of *B. sylvaticum* at areas frequented by tourists suggests that tourists, their vehicles, clothes, and/or their hiking and camping equipment are acting as vectors for the dispersal of this aggressive invader. A few of these tourist spots, such as Cape Perpetua and the Rogue Wilderness Trail, are highly protected and are not located near any recent logging sites; a larger genetic analysis could show whether or not the populations located near tourist sites were experiencing gene flow mostly between one another, or whether genetic material was being passed regardless of populations’ proximity to tourist sites. The same question could be asked in regard to gene flow among populations located near logging sites: If logging equipment is acting as a main vector, more gene flow should be seen between populations near logging tracts than between those populations and any others.
A more in-depth study of gene flow between populations is also required to illustrate with certainty the patterns of migration and dispersal of this grass. I will continue to work on the genetic aspect of this project with Alisa Ramakrishnan and Mitch Cruzan at Portland State University.
References


