

**Final Report:**  
**Evolution and migration of invasive species: molecular marker development**  
(awarded 2004)

**Problem Description/Literature Review**

Invasive plants are not often a threat immediately upon arrival to a new environment. Most invasive plants undergo a lag period of several decades before becoming aggressively invasive (reviewed in Sakai *et al.* 2001). If and when an alien species exits the lag period, it starts to spread with amazing rapidity. Mathematical models (Lewis 2000) and pathogen studies (de la Cruz and Whiting 2003, Rivas *et al.* 2004) have found that long-distance dispersal events combined with simple diffusion dispersal rapidly increase rates of spread (Figure 1).

Invasive plant species are only studied when they have become a significant threat. At this point, due to high rates of migration and admixture of populations (Travis and Dytham 2002) it can be difficult to empirically trace the early evolution and migration of aggressive invasives. The presence of many large source populations can also make a comprehensive study very expensive and/or time-consuming (i.e. Mack 1981, Gaskin and Schaal 2002). While some studies in non-plant species have provided detailed genetic information specifically on recent invasion events and migration patterns (Davies *et al.* 1999a, Rivas *et al.* 2004), no such study has been conducted for a plant species.

**Original Goal:**

Sequence about 1000 clones, for a total of about 10 sequences, and screen 100 samples for each locus.

**Results Summary:**

- Successfully sequenced about 350 clones from two genomic libraries, each enriched for different microsatellite motifs (i.e., CA vs CAG)
- The first library has about 15% redundancy
- The second library has about 25% redundancy
- Overall, we get about 2-3% usable loci, twice what we expected

Of loci with sufficient quality flanking DNA to design primers, 17 amplified poorly, three were monomorphic, and 7 are confirmed as working well. Eight more loci, not listed here, are in the final stages of testing for amplification and polymorphism. We expect two to three of these loci to be adequate for population analyses, and we hope to have about 15 loci total by the end of the summer.

Our locus development was slowed by some unexpected problems in the sequencing process during the fall that took about 3 months to resolve. We will be able to make up the cost by using a new protocol that cuts our cost by \$0.50 per sequence. We also have an undergraduate assisting us now, and hope to surpass our original goal of 10 loci by the end of the summer.

Table 1, Locus Summary: This table indicates the number of alleles per locus, allele size range, number of individuals screened, the repeat motif sequence being amplified, the sequences of the primers used to amplify each locus, and the melting temperatures (T<sub>m</sub>)

Locus	Allele #	Allele Sizes	N	Motif	Primer Sequences	T <sub>m</sub>
2-3A1	29	183-357	775	(GAA)21	For: AGAGGGATTGCATTGTCATCAG Rev: TTCGGAGGATAGCTTGGTCACTC	56.4°C 59.6°C
3-4E8	16	236-316	775	(CAAA)9*	For: ACATGGTAAGAACCAGAATCGG Rev: TGAATTCGGCACGTCTGGATCC	59.1°C 59.5°C
3-4F9	6	246-285	775	(GATT)3 (GATT)3	For: GCTCAGCTTGTTCTTTTACCCATATC Rev: TTGCCACCGCCTCTTAACATAC	56.0°C 57.4°C
2-3D12	2	236-248	29	(GAGT)3 (GAAAA)2 polyA	For: TGTGACAGCCATAGATATCGGC Rev: ATCACTCGTTAATATTCCCTACTAGTG	56.6°C 53.8°C
3-2A7	3	152-179	12	(ATCT)6	For: CTTATGCCTTTCCAGGACGA Rev: CCTGCACTGCTAATCAACCA	60.2°C 59.9°C
3-2C4	2	148-152	12	(GTT)3 (TTTG)4	For: GTGTGCTAGGCAATGCATTTAT Rev: TGAAGCATAATGTATTACAGATGAGAA	59.2°C 58.4°C
2-3B5	2	256-258	12	(GT)5	For: TTCTGCTCTGTCTCACTCCCTCTAC Rev: AGAGGTAAGAGATCAGCACTGAACTAG	59.2°C 57.2°C

of each primer.

### **Preliminary population genetic analyses:**

In preparation for an international scientific meeting we attended in June (Society for the Study of Evolution, Fairbanks, AK), we conducted some preliminary analyses. Three loci, amplified on a total of about 775 individuals from both Oregon and USDA accessions, indicate that *B.sylvaticum* appears to experience moderate rates of long-distance dispersal events, and was most likely introduced during a limited space of time into the center of the current range.

Our preliminary results for some Oregon populations are summarized in Figure 1: The central populations have more diversity than the peripheral populations, as expected when migration rates are low to moderate. The peripheral populations differ remarkably from each other, with the eastern-most population being quite different than the western-most population. Such a pattern is expected with long-distance dispersal, though further analyses are needed to accurately track the dispersal processes that have led to the current distribution.

These results have been used to help identify putative source populations in the Middle East, and will be invaluable when compared with results from research on morphological variation. These phenotypic analyses are to be conducted in part this year, in addition to studies on outcrossing rates.

Figure 1: Three-locus genotype frequencies for some populations in Oregon. Each pie chart represents a different population, with the inset being central populations.

