Determination of heterozygosity among Alaskan populations of *Arabidopsis lyrata* subsp. *kamchatica* using microsatellites.

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Abstract

In this study of *Arabidopsis lyrata* subsp *kamchatica*. …

This experiment is trying to determine the polymorphism of microsatellite loci that might be used to determine the self-fertilization rate.

Microsatellite loci used- genes with high intraspecies variation.

1 sample from each of 8 separate populations were taken.

Collected from northern Interior to costal Southwest Alaska.

How the study was conducted…

Standard PCR and fragment analysis protocol were used.

What was discovered…

Only 1 microsatellite locus of 6 analyzed had heterozygosity.

There were too few samples analyzed to statistically conclude a reproductive pattern.

What it means…

Polymorphism in 1 locus might indicate ability to outcross (sexual reproduction)

The lack of diversity overall might indicate reproduction through mostly self-fertilization (selfing).

Since subsp. genetic ancestors (parents) were outcrossing (sexual reproduction), hybridization factor or mutation might have led to a reproductive preference to selfing.

Objectives…

Discover polymorphic microsatellite loci using primers designed by Clauss et. al. 2002.

Using these microsatellites, we can learn about various properties of *Arabidopsis lyrata*.

Might reveal rate of reproductive tendencies.

Arabidopsis lyrata subsp. *Kamchatica*

Photo by Jessica Beecher

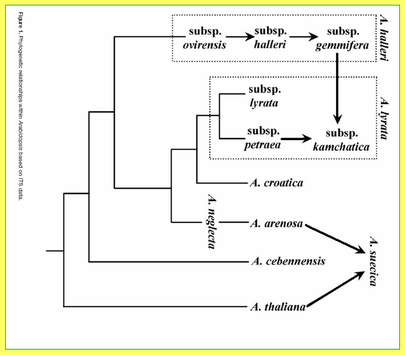

Figure 2. This phylogenetic tree portrays subsp. *kamchatica* as a descendant of *A. petraea* and *A. halleri*, both outcrossers.

Methods & Materials

The 8 samples were provided beforehand with a minimum concentration of DNA verified.

Amplified with polymerase chain reaction (PCR) using ready-made PCR beads.

Visualized using electrophoresis to confirm presence of PCR product.

Fragments analyzed with a cycle sequencing machine using the Liz500 size standard ladder. Therefore, fragments between the sizes of 50 and 500 base pairs would be seen.

Results obtained using GeneMapper™ to pinpoint microsatellite nucleotide fragment sizes to the nearest base pair.

Results

Fragment Analysis results

6 Primer Pairs Amplified

1 Pair indicates polymorphism: locus nga 112 (refer to Figure 4)

5 Pairs indicate monomorphism: no variation within loci

Within polymorphic microsatellite samples of locus **nga 112** (7 samples)

Observed Heterozygosity:

3 homozygous (43%) samples: amplified only 1 allele

4 heterozygous (57%) samples: amplified 1+ allele

Expected (calculated) frequency of genotype:

Homozygous: 28.5%...the same for each sample, no variation

Heterozygous: 71.5%...variation from other samples

Hardy-Weinberg Equilibrium (HWE): Relationship between alleles and genotypes

Assumptions include no genetic drift, no selection, random mating, and a large population size.

If samples are “out of equilibrium”, a HWE assumption is violated.

Chi Square Results

Chi Square test is a statistical test of the assumption of HWE. It is used to determine if the observed values differ from the expected values.

The results from our Chi-square did not reject the null hypothesis that these samples are in HWE. This result may be due to small sample size leading to low statistical power.

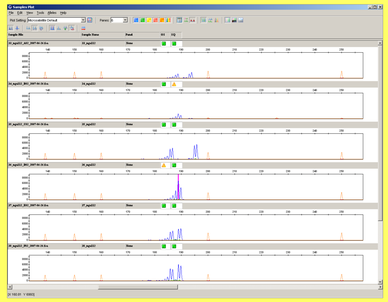

Figure 4. 6 samples of *A. lyrata* subsp. *kamchatica*, amplifying microsatellite loci nga 112. Each peak portrays 1 allele. The smaller orange peaks represent the dye ladder for base pair reference. This loci portrays heterozygosity (1+ allele) in samples 33, 35, 38, 40 (not shown). Samples 34, 36, 37 are homozygous (1 allele).

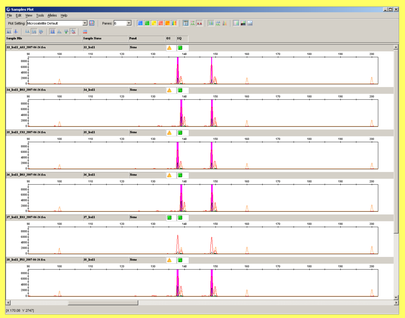

Figure 5. This fragment analysis program is showing microsatellite loci ICE 11. Though there are two peaks for each sample. This loci is monomorphic (no variation). *Arabidopsis* plants are tetraploids, and have 2 chromosomes from each parent. What is shown is the same loci on two different genomes.

Locus	*A. petraea**	*A. halleri**	*A. thaliana***	*A. lyrata* subsp. *Kamchatica***†
nga 112	no pcr	no pcr	0.00	0.714
ICE 11	0.00	0.43	0.00	0.00
Ath ACS	0.65	0.25	no pcr	0.00
F19G 10	0.32	0.00	0.00	0.00
ICE 2	0.65	0.25	no pcr	0.00
nga 249	0.00	0.50	0.12	0.00

Figure 3.

*- Self-incompatible

**- Self-compatible

†- From current study, all others from Clauss et. al. 2002. This table represents the **Expected (calculated) heterozygosity** within 4 species of the *Arabidopsis* family. The results for loci nga 112 might be flawed- the heterozygosity formula is based on the number of samples, and only 7 were amplified. Of the 15 primers studies, these were the 6 primer with results.

Conclusions

Chi Square Results

Samples may be in HWE, but cannot rejected or supported due to sample size.

Test failed to reject HWE- may still be within HWE (outbreeding)

Results based on number of samples taken

More samples amplified may have seen different results.

Of the 6 primers originally amplified, 5 primers showed no polymorphism.

Results not inconsistent similar to other ongoing *Arabidopsis* studies in the Wolf/Takebayashi lab, UAF.

Their studies conclude that most of subsp. kamchatica are self-fertilizing.

The microsatellite loci I have tested will be used in other population genetic studies in the future.

Literature Cited

Clauss, M.J., Cobban, H., and Mitchell-Olds, T., Cross-species microsatellite markers for elucidating population genetic structure in *Arabidopsis* and *Arabis* (Brassicaeae), *Molecular Biology*

Ihsan A. Al-Shehbaz and Steve L. O’Kane, Jr. “Taxonomy and Phylogeny of *Arabidopsis* (Brassicaceae).” American Society of Plant Biologists. (2002).

Naoki Takebayashi and Peter L. Morrell. “Is self-fertilization an evolutionary dead-end? Revisiting an old hypothesis with genetic theories and a macroevolutionary approach.” American Journal of Botany. 88(7) (2001): 1143-1150. July 7 2007.

Interviews with Diana Wolf, June-July, 2007.

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