Polymorphic Loci in *Arabidopsis lyrata* subsp. *kamchatica* in Alaska

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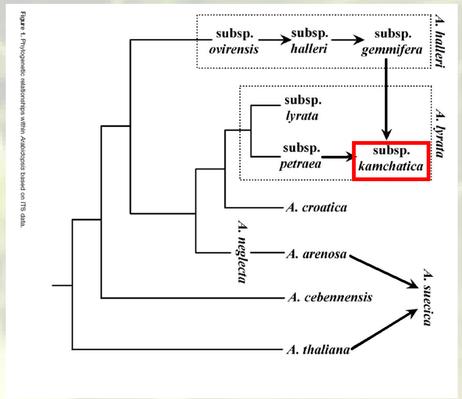
Abstract

Genetic markers are useful because one is able to use them to study population genetics, such as the rate of self-fertilization and out-crossing. Different primer pairs were tested to find polymorphic loci to be used as genetic markers. With self-fertilization, it is predicted that there would not be much diversity in the eight DNA samples tested. I used PCR to amplify microsatellites. Gel electrophoresis showed the success of the PCR, while capillary electrophoresis provided a more detailed fragment analysis. One polymorphic locus, location was discovered. Since *Arabidopsis lyrata* subsp. *kamchatica* is self compatible, the polymorphism was an exciting find. In the future, we will use these polymorphic markers to collect population genetic information such as the rate of self-fertilization and possibly paternity.

Introduction

Arabidopsis lyrata subspecies *kamchatica*:

- Found in the Asia and North America, usually not above the Arctic Circle.
- Is a tetraploid, meaning it has four of each chromosome, unlike humans, who regularly have two of each chromosome.
- The parent plants are *Arabidopsis halleri* and *Arabidopsis petraea*.



This study is important for understanding the evolution of reproductive strategy. It will help determine the rate of out-crossing and self- fertilization, or selfing. This data will help understanding of patterns of population genetics.

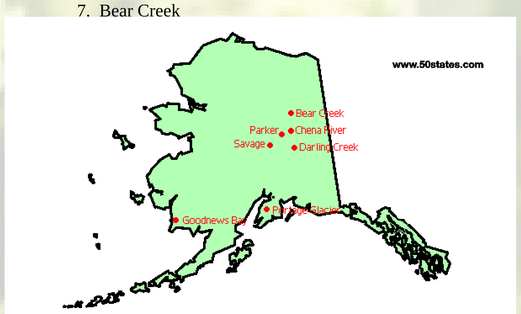
Microsatellites, irregular repetitious genetic sequences, serve as genetic markers. I attempted to find the polymorphic loci which define these microsatellites.

I used microsatellites to find DNA polymorphisms, which are changes in the DNA sequence. Capillary electrophoresis was my main tool for analyzing fragment size.

Methods

I received eight samples of extracted DNA from the plants' normal habitat in Alaska:

1. Goodnews Bay
2. Mount Parker
3. Portage Glacier – 2 samples
4. Chena River
5. Darling
6. Savage
7. Bear Creek



I tested sixteen different sets of primer pairs which were described by Clauss et al., 2002. These primers were tested using PCR to see which of them successfully amplified microsatellites in *A. lyrata* subsp. *kamchatica*. PCR takes a section of DNA which the primers bind to and multiplies it many, many times.

1. AthPYC
2. Att50392
3. ICE 12
4. ICE 15
5. nga151
6. nga 158
7. AthATPASE
8. ICE 13
9. MDC16
10. ICE 1
11. SLL2
12. F19K23
13. ca72
14. ADH1
15. F21J9
16. ICE 4

After the PCRs were finished, I ran a gel electrophoresis to test their success. Gel electrophoresis, "running a gel", is a tool used to observe the fragment size amplified by the PCR reaction.

When running a gel electrophoresis:

- DNA is separated by size as it slowly migrates through an agarose gel. The longer DNA strands encounter more resistance based on their size, and thus migrate a shorter distance.
- A ladder is also loaded. The known size DNA ladder bands are compared with the sample bands to determine the size of the amplified fragment.
- The DNA migrates toward a positively charged side of the gel. When the DNA is initially loaded, it is loaded at the negatively charged side. The buffer, liquid in which this electrophoresis takes place, provides free ions for the electrical current to flow

Once the electrophoresis is accomplished, it must be stained in Ethidium Bromide for approximately fifteen minutes. If the PCR was successful, then we would be able to see bands corresponding to the appropriate ladder bands.

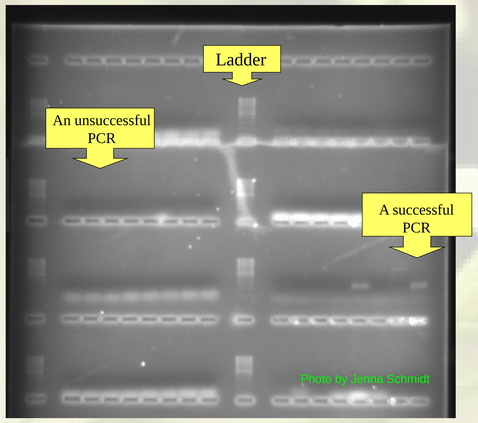


Figure 1. A image of gel electrophoresis

I was able to successfully amplify eleven out of sixteen primer pairs.

1. ICE 16
2. ICE 12
3. ICE 4
4. SLL2
5. ca72
6. F19K23
7. ICE 1
8. ADH1
9. MDC16
10. ICE 13
11. AthATPASE

Once the gel results were acquired, my next step was to put them though fragment analysis.

Fragment analysis is basically a highly accurate gel run on capillary electrophoresis machines. The results are peaks in base pairs, where the exact size can be assessed.

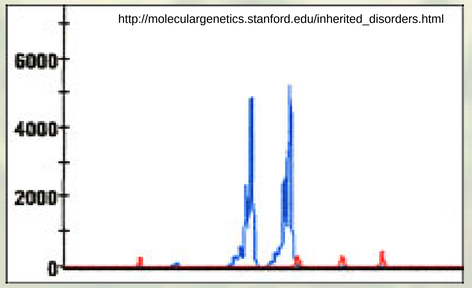


Figure 1. A fragment analysis result.

With this fragment analysis, I was able to detect DNA polymorphisms. Polymorphisms are seen as different sized fragments.

The locations of the peaks indicates the fragment size. If the peaks for the same primer pair are different sizes, then the samples are heterozygous, different.

Nine different PCRs were successful in the fragment analysis.

Results

Locus	*A. petraea* ✦	*A. halleri* ✦	*A. thaliana* ▲	*A. lyrata (k)* ▲
F19K23	0.26	0.75	0.00	0.218*
ICE 12	0.52	0.43	0.00	0.00
ca72	N/A	0.43	0.00	0.00
ADH1	0.56	0.24	0.00	0.00
ICE 15	0.41	N/A	0.00	0.00
ICE 1	0.06	0.00	0.00	0.00
ICE 4	0.00	0.00	0.12	0.00
MDC16	0.09	0.50	0.00	0.00
SLL2	0.34	0.00	0.00	0.00

▲ Self-compatible
✦ Self-incompatible
* $p<0.05$

Table 1. Expected heterozygosity for microsatellite loci within populations for *A. petraea*, *halleri*, and *thaliana*

(*A. petraea*, *halleri and thaliana* data provided by Clauss et al., 2002)

Discussion

With the discovery of this polymorphism in the locus F19K23, I now know that other populations can be investigated using the same successful primers. In the future, these markers could be used for many applications, including determination of self-fertilization rate in different populations.

There appears to be low diversity through the display of few polymorphisms in the eight populations from Alaska. This was expected, but the statistically significant primer pair raises questions as to why it is different. These questions ca be investigated further.

Research will be continued on this with DNA collected from this species in smaller areas. These primers may be useful in Asian populations to track the migration of *Arabidopsis* across the Bering Land Bridge.

Literature Cited

M. .J. Clauss, H. Cobban, T. Mitchell-Olds, 2002. Cross-species microsatellites marker for elucidating population genetic structure in *Arabidopsis* and *Arabis* (Brassicaeae). *Molecular Ecology* 11, 591-601.

Ilsan A. Al-Shehbaz, Steve L. O'Kane, Jr., 2002. Taxonomy and Phylogeny of *Arabidopsis* (Brassicaeae). *The Arabidopsis Book* 1-22.

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Photo by Jessica Beecher