Lobelioid Primer Testing

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Introduction

Hawaiian Lobelioids are a group six genera of rare plants; that includes Brighamia, Lobelia, Cyanea, Delissea, Clermontia, and Trematolobelia. These plants are endemic to the islands of Hawaii and many species within each genera are considered highly endangered. A number of factors such as hurricanes, invasive plant species, loss of pollinators, and human disturbances, have lead to an increase in lowering numbers of individuals in wild populations. Populations that reach low numbers face genetic issues including loss of genetic diversity, and increases in inbreeding, damaging the long-term health of these plant populations. One way to go about looking at and assessing the issues these plants face is using microsatellite primers. Microsatellites are short tandem repeats of 1-6 nucleotides. The advantages to using microsatellites as genetic marks are that microsatellites have high mutation rates (*slippage),* allowing for more information on the phylogeny of studied alleles by looking at the size of the alleles. Microsatellite primers not only provide scientist with a decent amount of background of individuals and or populations, they are easy to prepare and more cost effective compared to other forms of genetic testing.

Objective

Identify genetic markers that amplify consistently for multiple species in the Hawaiian Lobelioids.

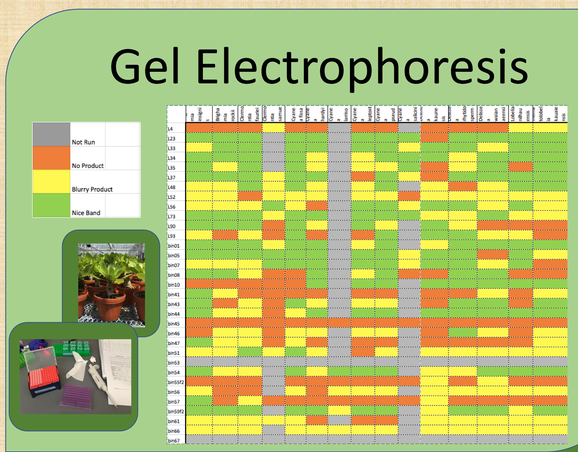
Methods

For this project, I tested over 33 primers on 26 samples of Lobelioid DNA. DNA samples of 2 species of Brighamia, 2 species of Clermontia, 6 species Cyanea, 3 species of Delissea, and 1 species for both Lobelia and Trematolobelia. The PCR products were run on 1.5% Agarose gel and visualized using SYBR green labels which fluoresces under UV lights. The gels were scored for presence and absence of a band, as well as the clarity of the band.

Discussion

The project that I had the privilege of working on this summer at the Chicago Botanic Garden, is only a small part of a larger project being conducted in the same lab. Along with my mentors, Jeremie Fant & Jordan Wood, and two other interns., we are trying to see how we can use the genetic background of the Lobelioid family to restore populations of these plants on their native islands. My project, testing the primers, can be seen as ”step 1” of the overall bigger project. Once the information of which primers work and which ones don’t , we can then run gels on the *Beckman* to look at plant paternity and genetic & allelic diversity within individuals and or populations of interest.

Gel Electrophoresis



Beckman

Genus	Species	Lob4	Lob23	Lob33	Lob34	Lob35	Lob37	Lob48	Lob52	Lob56	Lob73	Lob90	Lob93	Bin05	Bin07	Bin08
Brighamia	insignis	--	--	375	163	236-255	--	--	--	186-221	--	--	--	201-220	152	152-166
Brighamia	rockii	--	--	368	140-164	234-255	--	--	202	--	--	--	--	187-214	145-214	166-173
Clermontia	faurlei	--	--	354	160	248-258	--	--	--	220	--	--	--	187-223	127-131	127-158
Clermontia	fissa	--	--	--	--	--	--	--	210	220	--	--	--	182-222	122	165
Clermontia	samuelii spp. Hanaensis	--	--	350	154-160	270	--	--	170	220	--	--	--	181-219	120-122	156-198
Cyanea	fissa	--	--	348-358	156	166- 264	--	--	--	--	--	--	--	185-211	153-154	152-189
Cyanea	handyi	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Cyanea	lamsoonii	--	--	296-368	155	184-254	--	--	208	246-250	--	--	--	167-223	159-161	--
Cyanea	leptotegia	--	--	358	141-259	253-264	--	--	166-208	166-208	--	--	--	185-211	154-163	155
Cyanea	pesudofaurlei	--	--	349-353	170-172	261	--	--	--	132	--	--	--	--	--	162-177
Delissea	kauainsis	--	--	368	161-173	232-255	--	--	156-201	170-201	--	--	--	181-197	145-150	166
Delissea	rhytidosperma	--	--	368-383	165-175	234-255	--	--	156-201	201	--	--	--	181-225	141-225	141-167
Delissea	waianaeensis	--	--	361	--	231	--	--	--	251	--	--	--	181-224	--	122-147
Lobelia	nilhauensis	--	--	367-395	168	243-267	--	--	147-205	147-171	--	--	--	179-215	133-215	172
Trematolobelia	kauaiensis	--	--	345	160-164	250-258	--	--	171-215	240	--	--	--	--	--	152

Genus	Species	Bin10	Bin41	Bin43	Bin48	Bin45	Bin46	Bin47	Bin51	Bin53	Bin54	Bin55SP2	Bin57	Bin55SP2	Bin61	Bin66	Bin67
Brighamia	insignis	--	125	175-185	--	--	232	185	--	--	--	168-177	187-206	188	--	--	--
Brighamia	rockii	--	115	204-229	--	--	250-251	153-163	--	--	201	168	--	188	--	--	--
Clermontia	faurlei	--	97-101	198-201	--	--	244-248	181-184	--	--	--	168	--	178-184	--	--	--
Clermontia	fissa	--	--	198-225	--	--	--	182	--	--	--	--	--	--	--	--	--
Clermontia	samuelii spp. Hanaensis	--	100	201-208	--	--	230-298	177-192	--	--	138-143	168	--	168-192	--	--	--
Cyanea	fissa	--	104	191-210	--	--	210-218	--	--	--	--	163-168	--	192	--	--	--
Cyanea	handyi	--	--	--	--	--	--	--	--	--	136	168	--	--	--	--	--
Cyanea	lamsoonii	--	--	183-210	--	--	240	185	--	--	--	168	--	182-194	--	--	--
Cyanea	leptotegia	--	--	187-216	--	--	210-240	184	--	--	155-160	162-168	--	180-192	--	--	--
Cyanea	pesudofaurlei	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Delissea	kauainsis	--	134-161	196	--	--	--	178-180	--	--	--	168	--	182-191	--	--	--
Delissea	rhytidosperma	--	--	197-221	--	--	273	181-184	--	--	--	166-168	--	184-191	--	--	--
Delissea	waianaeensis	--	123	210-219	--	--	269	179	--	--	149	168	--	182	--	--	--
Lobelia	nilhauensis	--	--	183	--	--	--	206	--	--	124-189	157-168	--	171	--	--	--
Trematolobelia	kauaiensis	--	100	187-192	--	--	228-230	169-170	--	--	--	--	--	171-185	--	--	--

Results & Conclusion

Of the 33+ tested Lobelioid primers, a total of 10 seemed to work consistenly across species within the Lobelioid family. Upon first glance when looking at the gel data, you will see a vast amount of yellow and orange, indicating that the clarity of most of the band were either blurry or not present. This means that, so far, there are at least 10 primers we can use with confidence to further study this family of rare plant life.

References

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- Selkoe, Kimberly A., and Robert J. Toonen. “Microsatellites for Ecologists: a Practical Guide to Using and Evaluating Microsatellite Markers.” *Freshwater Biology*, Wiley/Blackwell (10.1111), 3 Mar. 2006, onlinelibrary.wiley.com/doi/abs/10.1111/j.1461-0248.2006.00889.x.

Acknowledgments

I’d first and foremost like to thank NSF-REU grant DBI-1757800 for support and for providing students like me with such an opportunity. Another big thanks goes to Andrea Kramer, Hilary Nobel, and Jeremie Fant for being wonderful coordinators and mentors, I have learned a great deal from you all. I’d also like to thank my mentor Jordan Wood for always being there and stepping in to help when I needed. Lastly, I want to thank all of my fellow interns for being great people to be around as well as the Chicago Botanic Garden’s educational programs.