Optimizing Microsatellite Markers in Four Species of *Oenothera*: *O. brachycarpa*, *O. serrulatus*, *O. lavandulafolia*, and *O. hartwegii*

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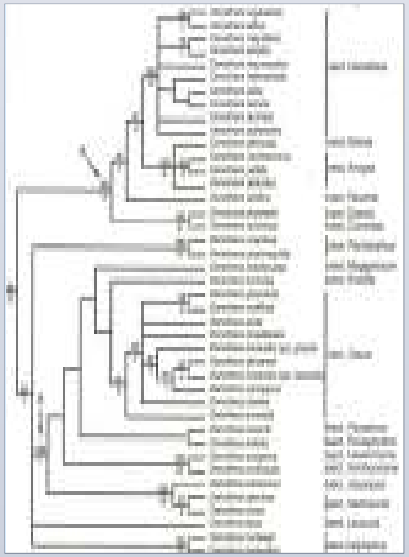
Introduction

Neutral genetic diversity within plants is often used to track important evolutionary processes including gene flow, species and population divergence, and evolutionary ancestry. By comparing the genetic diversity within and among five species of evening primrose (*Oenothera*), *O. serrulatus , O. lavindulafolius*, *O. hartwegii*, *O. brachycarpa* and *O. harringtonii*, we can track the influence of major factors such as gene flow and diversification, and shifts associated with adaptive traits such as flower morphology, floral scent, flower color, pollinator community, and geographic distribution. *Oenothera harringtonii* has a strong floral scent, white flowers, and is hawkmoth pollinated. In contrast, the remaining four species have yellow flowers, are bee pollinated and have milder scents.

In order to measure genetic diversity among these species, we need to first find marker regions that amplify consistently for each species. It is common for primers that target conserved regions to work across closely-related species (Barbara et al. 2007). The more closely related two species are the more likely they are to share these same microsatellite regions. (Barbara et al. 2007) All five species tested here belong to the evening primrose family, Onagraceae (tribe Onagreace). *Oenothera biennis* is in the section Oenothera, which is distantly related to the sections Pachylophus (containing *O. harringtonii*), Megapterium (containing *O. brachycarpa*), and Calylophus (containing *O. serrulatus, O. lavandulafolia*, and *O. hartwegii*).(Levin et al. 2004).Due to the position of each species in the most recent phylogeny (Levin et al. 2004) we expect primers developed for *O. harringtonii* to amplify in *O. brachycarpa*, *O. hartwegii*, *O. serrulatus*, and *O. lavandulafolia* more often than primers developed for *O. biennis*.



Methods

DNA from four (possibly five) species of *Oenothera – O. brachycarpa (OEBR)*, *O. serrulatus (CASER)*, *O. lavandulafolia (CALA)*, *O. hartwegii (CAHA)*, and a species suspected to be *O. serrulatus (OESER)* was extracted from silica-preserved leaf tissue using a modified CTAB method (Khasa et al. 2000). Twelve chloroplast microsatellite primers developed for *O. harringtonii (OEHA)* (Skogen et al. 2012) and 55 chloroplast microsatellite primers developed for *O. biennis* (Larson et al. 2007) were tested on all species listed. Polymerase chain reactions (PCRs) were conducted in 10-µL reactions and run for 2 min at 95° C; then 30 cycles of 95° C for 50 sec, 56° C for 1 min, and 72° C for 1 min; and a final extension of 72° C for 10 min. PCR product was mixed with a SYBR-green dye (Molecular Probes, Inc.) and loading buffer (1uL SYBR-green per 500-µL loading buffer), then loaded into 1.5% agarose gels. The gels were run for 15 min to visualize if the primers successfully amplified DNA.

Results

Seven of the 12 primers developed for *O. harringtonii* successfully amplified DNA in all species except for *O. serrulatus*, which was not tested for these primers. The other 5 primers amplified only for *O. harringtonii*. Of the 7 that did amplify, two amplified cleanly across all species, and 4 that amplified *O. brachycarpa*, 3 that amplified *O. hartwegii*, 4 that amplified *O. lavandulafolia*, and 4 that amplified *O. serrulatus* (suspected) created products that were blurry when visualized on a gel, indicating that there were sufficient differences in priming region that these primers were no longer suitable for amplifying the target regions.

Fourteen of the 55 primers developed for *O. biennis* successfully amplified DNA in all species. Other primers worked in only some of the species, 34 amplified in *O. harringtonii*, 22 amplified in *O. brachycarpa*, 29 amplified in *O. hartwegii*, 28 amplified in *O. lavandulafolia*, 27 amplified in *O. serrulates* (suspected) and 23 amplified in *O. serrulatus*. Of these, 7 that amplified in *O. harringtonii* and 3 that amplified in *O. brachycarpa*, *O. hartwegii*, 3 *O. lavandulafolia O. serrulatus* (suspected), and *O. serrulatus* were blurry.

Key	
	Clean Product
	Blurry Product
	No Product
	No Data
*	Product found in all species

	Primers Developed for *O. biennis*					
	Species					
	Primer	OEHA	OEBR	CAHA	CALA	CASER	OESER
	101						
	102						
	103						
	104						
	105						
	106						
	107						
*	108						
	109						
*	110						
*	111						
	112						
	113						
	114						
	115						
	116						
*	117						
	118						
	119						
	120						
	121						
	122						
	123						
	124						
	125						
	126						
*	127						
*	128						
*	129						
	130						
*	131						
	132						
	133						
	134						
*	135						
	20						
*	21						
*	22						
	23						
*	24						
	1						
	2d10						
	10						
	11						
	13						
	15						
	16						
	18						
	19						
	25						
	26						
	27						
*	39fn10						
	39F3						
*	3904						

	Primer	Primers Developed for *O. harringtonii*					
	JQ266367*						
	JQ266368*						
*	JQ266359*						
*	EF988083*						
	JQ266366*						
*	JQ266363*						
	JQ266361*						
	JQ266362*						
*	OB4						
*	EF988088*						
*	EF988090*						
	JQ266360*						

Conclusions

Of the primers developed for *O. harringtonii,* 58% successfully amplified in all species. Of the primers developed for *O. biennis*, 25% successfully amplified in all species. This supports our hypothesis based on the position of each in the most recent phylogeny (Levin et al. 2004); primers developed for *O. harringtonii* were more effective at amplifying DNA from *O. brachycarpa*, *O. hartwegii*, *O. lavandulafolia*, *O. serrulates* than the primers developed for *O. biennis* were. However, the utility of the primers developed for *O. harringtonii* may be limited compared to the utility of the primers developed for *O. biennis* because only 28% of the *O. harringtonii* primers that amplified did so cleanly, while 92% of the *O. biennis* primers that amplified did so cleanly.

These primers will be useful for future studies investigating gene flow within and among populations of these species, studies of their genetic structure, parentage studies, and investigating the influence of various factors on genetic diversity.

References

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