Polymorphic microsatellite loci in Polemonium brandegei and P. viscosum (section Melliosoma, Polemoniaceae)1

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Polymorphic microsatellite loci in *Polemonium brandegei* and *P. viscosum* (section *Melliosoma*, Polemoniaceae)[1]

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- *Premise of the study*: Microsatellite markers were isolated in *Polemonium brandegei* and *P. viscosum* to be used in future studies of mating system evolution, population structure, and hybridization.
- *Methods and Results*: Six loci were used in a preliminary genetic diversity study in two populations each of the closely related *Polemonium brandegei* and *P. viscosum*. We found 39 alleles across the six loci (average 7 per locus), with overall levels of observed heterozygosities ranging from 0.067 to 0.867 in *P. brandegei* and 0.000 to 0.666 in *P. viscosum*. Additional primers are reported, but require further design and optimization.
- *Conclusions*: The reported markers will aid in further studies of mating system evolution, population structure, and hybridization in *P. brandegei* and *P. viscosum*.

Key words: genetic diversity; microsatellite; *Polemonium brandgei*; *Polemonium viscosum*.

The genus *Polemonium* L. (Polemoniaceae) includes between 19 and 42 species (Worley et al., 2009; Grant, 1989; Davidson, 1950). *Polemonium* is of particular interest to evolutionary biologists as several closely related taxa display contrasting floral morphologies and pollination strategies (Grant and Grant, 1965). *Polemonium brandegei* (A. Gray) Greene displays floral traits associated with both hawkmoth- and hummingbird-pollinated species and is frequented by both types of pollinators (Kulbaba and Worley, 2008). However, a close relative of *P. brandegei*, *P. viscousm* Nutt., is primarily pollinated by bees and large flies (Galen et al., 1991) and displays a markedly different floral morphology (Grant, 1989).

To explore how this pollinator shift has influenced the floral design of *P. brandegei*, we developed microsatellite markers to study patterns of paternity and therefore selection through male function (siring success) under hawkmoth and hummingbird pollination.

METHODS AND RESULTS

Genomic DNA of *Polemonium brandegei* was extracted with a FastPrep homogenizer and FastDNA extraction kits (QBioGene, Solon, Ohio, USA). Microsatellite loci were isolated using a biotin/streptavidin-enrichment process (Khasa et al., 2000). Genomic DNA was digested separately with restriction endonucleases Hae III, Rsa I, and Alu I (each with PshA1), and oligonucleotide adaptors M28 (5′-CTCTTGCTTGAATTCGGACTA) and M29 (5′-TAGTCCGAATTCAAGCAAGAGCACA) were added using T4 DNA ligase. Linker-adapted fragments were then enriched by two rounds of hybridization with 5′ biotin (GA)10, (TG)10, (CATA)5, and (GATA)5 followed by purification with streptavidin paramagnetic bead (M270S, Invitrogen, Carlsbad, California, USA). After amplification with linker-specific primer M28 and digestion with Eco R1, the enriched genomic DNA fragments were cloned into plasmid vectors (pGEM3Z+, Promega, Madison, Wisconsin, USA), and single colonies containing microsatellites were identified by dot blot hybridization. Inserts from positive colonies were amplified with m13 universal forward and reverse primers, treated with Exonuclease I and Antarctic alkaline phosphatase (New England Biolabs, Ipswich, Massachusetts, USA), and then sequenced from one or both orientations using ABI 3730 capillary electrophoresis (NAPS Service, University of British Columbia). Primers were then manually designed using Primer 3.0 (v. 0.4.0, Rozen and Skaletsky, 2000).

PCR amplification of microsatellite loci was performed in 25-μL volumes containing 0.2 NEB Taq polymerase (New England Biolabs), 0.25 μM of both primers, 1.5 mM $MgCl_2$, 0.2mM of each dNTP. PCR reactions began with a 3 min denaturation step at 94°C, followed by 35 cycles of 30 s at 94°C and 30 s at 55°C, followed by 1 min 30 s at 72°C. The PCR cycle completed with a final extension step of 5 min at 72°C. Amplification products were run on an ABI 3730 Genetic Analyzer and manually scored in GeneMapper 4.0 (Applied Biosystems, Foster City, California, USA).

We sampled 15 individuals from two populations from each of *Polemonium brandegei* (Taylor Canyon, CO: 39°34′33″N, 104°22′26″W; and Vedauwoo, WY: 44°46′57″N, 116°18′50″W) and *P. viscosum* (Cottonwood Pass, CO: 38°46′34″N, 106°13′3″W; and Loveland Pass, CO: 39°39′48.92″N, 105°52′44.94″W). Voucher information is in Appendix 1. Therefore, we screened a total of 60 individuals. Of the 19 identified loci (Table 1), six (Pbra6, Pbra18, Pbra21, Pbra27, Pbra43, and Pbra13t) were selected for a population study of genetic diversity, and therefore had dye-labels appended to the forward primer (6-FAM, PET, HEX, NED; Applied Biosystems). These six loci were chosen because they displayed the highest degree of polymorphism during preliminary surveys of *P. brandegei* and were most readily amplified and scored in both species. As *P. brandegei* and *P. viscosum* are both self-sterile, we tested the six loci for deviations from Hardy–Weinberg equilibrium, evidence of linkage-disequilibrium, as well as the number of observed and expected heterozygotes in GenePop v3.4 (Raymond and Rousset, 1995).

A total of 39 alleles were found across the six dye-labeled primers (see Table 1). One locus (Pbra27) in *Polemonium brandegei* and two loci in *P. viscosum* (Pbra27 and Pbra13t) showed significant departures from Hardy–Weinberg equilibrium (Table 2). This may be a result of heterozygote deficiency, potentially resulting from the presence of null alleles and/or population structure. None of the 15 pairwise comparisons among loci displayed significant linkage disequilibrium.

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TABLE 1. Primer sequence and configuration of 19 microsatellite loci developed for *Polemonium brandegei*.

Locus	Primer Sequence (5′–3′)	Repeat Motif	T_a (°C)	Microsatellite Size Range or expected size (bp)	GenBank Accession No.
Pbra6	F: GCCTGCTGCTACTATGACTGG R: GACACCATGGATGGACCTCT	$(CA)_8$	56	234–342	HQ197909
Pbra10	F: TAGGGCAGCGCGTCTCTC R: CCCAAAATCACTTAAGTGATTTTC	$(GA)_{21}$	–	95	HQ197910
Pbra18	F: CCCTAGAATTGGTCGAACTG R: GAGGCTAAGCAAATTAGGAAAACA	$(TG)_7$	56	167–175	HQ197911
Pbra21	F: GGAAAACAGAGGCAACGAGA R: CTCCGAAGCCCAGACATC	$(TC)_5$	55	151–174	HQ197912
Pbra27	F: GAATTCTATGTTTTACCTGTGTT R: TGCAATAAAACCTCTCTCTCT	$(TC)_{14}$	54	166–253	HQ197913
Pbra43	F: GAGAGACAGATCGGGCAGAG R: CCTCTCTATTTTCCCCTCGATT	$(TC)_9$	55	103–244	HQ197914
Pbra2t	F: GAATTCTGTTAGAGTTCACGTG R: GAATTCCCTTCTGTCTGTCT	$(TCTG)_5$	–	174	HQ197915
Pbra5t	F: TAGATGTTCCCAACGGCTTG R: TTTGGGCGAATCCAAATAAA	$(CATA)_6$	–	151	HQ197916
Pbra16t	F: CTTTCACGATAGTAGTAGTAG R: AATGGATCGGTCTCACTTGT	$(CTA)_{22}$	–	223	HQ197917
Pbra9	F: GGTTAAACGTTGTCAATTATGTG R: CTCGGTAGATGCTATGTTATA	$(TG7GA)_{23}$	–	279	HQ197908
Pbra11	F: CTTCTCGACAGTAAATCTCTCT R: AACTAGTAACAGTCGGTGAAC	$(GA)_{25}$	–	218	HQ197902
Pbra17	F: CGCTTGGTCTACTATGTACAA R: TTAGTATAACAGGACAATGGGAAA	$(TG)_{13}(GA)_n$	–	483	HQ197903
Pbra18.1	F: CAAAAGAGAAAAGACGGTGTCA R: GTGTACCCAAGTGGCACAAA	$(TG)_{>14}$	–	728	HQ197918
Pbra18.2	F: GGTTTGGTGTATTTGTGTTTGAA R: CTATAAGGCCATCCACTATATAA	$(CAA)_{36}$	–	308	HQ197919
Pbra22	F: ATCGAGTGCTCCGATCTTG R: GAATTCCAGGCAAGTTACAG	$(TC)_{26}$	–	152	HQ197904
Pbra33	F: CTCCACCCTAAAACCACCA R: TCTGCGGTACCCATCTCTC	$(TC)_{36}$	–	140	HQ197905
Pbra39	F: GAATTCTCCAATGCAAATTAAC R: CTGTCCCATTGAATACACCTT	$(TG)_{19}$	–	135	HQ197906
Pbra11t	F: TCGGTCGTATCGCTAGAATG R:TGACATAAATTTTACCTTCATTGTTGA	$(GTA)_{16}$	–	184	HQ197921
Pbra13t	F: CCCGTCAACCAGGGATAGTA R: TCATGTTTGGGTAAACAATCCA	$(CATA)_{18}$	54	105–240	HQ197922

T_a optimized annealing temperature

CONCLUSIONS

Of the 19 microsatellite loci reported, six were characterized in two species of *Polemonium*. The remaining loci will be further explored (e.g., primer design) for use in future population studies. These markers will be used for population levels of genetic diversity, measuring selection through male function in pollination experiments, and studies examining hybridization between *P. brandegei* and *P. viscosum*. These six primers developed for *P. brandegei* have been demonstrated to consistently cross-amplify in *P. viscosum*.

TABLE 2. Summary of allele counts, and expected and observed heterozygosity for two populations of *Polemonium brandegei* and *P. viscosum*.

	Polemonium brandegei				*Polemonium viscosum*			
	Taylor Canyon, CO		Vedauwoo, WY		Loveland Pass, CO		Cottonwood Pass, CO	
Primer	No. of Alleles	H_O/H_E	No. of Alleles	H_O/H_E	No. of Alleles	H_O/H_E	No. of Alleles	H_O/H_E
Pbra6	3	0.333/0.535†	2	0.800/0.496	3	0.466/0.604	4 (1)	0.400/0.540
Pbra18	3 (1)	0.467/0.387	3	0.733/0.545	3	0.400/0.439	3	0.267/0.239
Pbra21	5	0.533/0.664	4 (1)	0.666/0.522	4	0.666/0.655	3	0.467/0.480
Pbra27	3	0.733/0.680	3 (1)	0.400/0.615*	5 (1)	0.600/0.640	3 (1)	0.200/0.649*
Pbra43	8(2)	0.600/0.829	2	0.067/0.186	6	0.400/0.690	4	0.667/0.671
Pbra13t	9 (2)	0.867/0.885	2	0.200/0.287	7	0.600/0.834*	1	0.000/0.000n/a

Number of unique alleles are in parentheses. H_O / H_E observed and expected heterozygosity, respectively.

* Significant departure from Hardy–Weinberg equilibrium ($P < 0.05$).

† Marginally did not deviate from Hardy–Weinberg equilibrium ($P = 0.0514$).

n/a Could not test for departure of Hardy–Weinberg equilibrium, allele was fixed.

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APPENDIX 1. Voucher information for taxa used in this study. All voucher specimens are deposited in WIN.

Species – Country and Locality, Collection

***Polemonium brandegei* (A. Gray) Greene– ***USA, Taylor Canyon, Colorado, 09006. USA, Vedauwoo, Wyoming, 09008

***Polemonium viscosum* Nutt. –** USA, Cottonwood Pass, Colorado, 09012. USA, Loveland Pass, Colorado, 09015.

*Indicates locality used to develop microsatellites.