

*Draft template for Applications in Plant Sciences - Primer Note*

*Short Title for Running Head: Surname of the first author, followed, as appropriate, with the surname of a sole co-author, or et al. (if there are three or more authors) - 2 or 3 Descriptive Words [e.g., Wright and Stebbins - Antennaria microsatellites]*

## **Microsatellite primers in the native perennial herb, *Antennaria plantaginifolia* (Asteraceae)<sup>1</sup>**

*[no more than 125 characters; after a species name, include family name in parentheses]*

Sewall G. Wright<sup>2</sup> and G. Ledyard Stebbins<sup>3,4</sup>

<sup>2</sup> Department of Zoology, University of Chicago, 5812 S. Ellis Avenue, Chicago, Illinois 60637  
USA

<sup>3</sup> Department of Genetics, University of California, Davis, 310 Life Sciences, One Shields Avenue,  
Davis, California 95616-5294 USA

Email addresses: SGW: wrightsg@test.edu

GLS: stebbinslg@test.edu

Number of words: YYYY *[1200 or fewer, consisting of Introduction, Methods and Results, and Conclusions sections].*

<sup>1</sup> Manuscript received \_\_\_\_\_; revision accepted \_\_\_\_\_.

<sup>4</sup> Author for correspondence: stebbinslg@test.edu

Acknowledgments should be limited to no more than 75 words.

## ABSTRACT

The abstract should capture the interest of the general botanical community as well as specialists. The abstract is 150 words or less, written in a structured format:

- Premise of the study (why the work was done)
- Methods and Results
- Conclusions (If applicable, briefly mention other related species in which the markers also amplify.)

Avoid references; if essential, cite parenthetically with journal name, volume number, pages, and year.

Here is a sample abstract:

- *Premise of the study:* Microsatellite primers were developed in a native perennial herb, *Antennaria plantaginifolia*, to investigate potential hybridization events with related taxa promoting the evolution of polyploidy within the genus.
- *Methods and Results:* Using a non-radioactive protocol, 16 primer sets were identified in North American populations of *A. plantaginifolia*. The primers amplified di-, tri-, and pentanucleotide repeats with 1-11 alleles per locus. Most primers also amplified in *A. neglecta*, *A. solitaria*, *A. virginica*, *A. parlinii*, and *A. neodioica*.
- *Conclusions:* These results indicate the utility of primers in *A. plantaginifolia* for future studies of polyploidy and hybridization as well as their applicability across the genus.

**Key words:** *Antennaria plantaginifolia*; hybridization; perennial herb; polyploidy.

*[List 3 to 6 key words here in alphabetical order, separated by semicolons.]*

## INTRODUCTION

This section should consist of no more than two paragraphs outlining the reasons behind the study, a brief explanation of its importance, and any information regarding the species that would be of interest to the plant sciences community. Authors must also justify the need for markers; if genetic markers have already been developed in the same taxon, authors must provide appropriate citation(s) and explain why additional markers are necessary. Of particular appeal is the potential for widespread applicability of the markers or techniques to other species or systems, so authors are encouraged to test developed markers in a number of populations and/or closely related taxa.

## METHODS AND RESULTS

The combined **Methods and Results** section will consist of no more than five paragraphs. In the first one to three paragraphs of this section, the methods used to develop the genetic markers should be described. The description of methods must contain enough detail that other botanists can replicate the results; this includes the DNA extraction method, specific quantities of chemicals used for amplification, temperature conditions for amplification, and source information for chemicals and supplies (including the location [city, state/province, country] of the supplier). The number and geographic origin of samples analyzed (using GPS decimal degrees or to the nearest second) must also be included, either here within the text, as an appendix (in the case of multiple samples or populations), or as a footnote on Table 2 (see below). Voucher specimens are also required, unless their absence can be suitably justified (as in the case of an extremely rare species); this explanation should be provided in the authors' cover letter and in the manuscript text.

In the final one to two paragraphs of this section, the authors must demonstrate the usefulness of their primers, by testing all primers on a reasonable number of individuals, e.g., more than five individuals per population and two or more populations, unless the species being studied is extremely rare. Data on the number and frequency of alleles detected for at least 10 primer pairs (including both monomorphic and polymorphic primers) should be reported, in the case of microsatellite and SNP markers.

## CONCLUSIONS

In this section, the author(s) should clearly state in one paragraph the main conclusions that have been reached, focusing on the effectiveness and applicability of the markers being described.

## LITERATURE CITED

*[no more than 15; authors must cite papers reporting markers in the same species]*

GOUDET, J. 1995. FSTAT: A computer program to calculate  $F$  statistics, version 1.2. *Journal of Heredity* 86: 485–486.

STEBBINS, G. L. 1974. Flowering plants: Evolution above the species level. Belknap Press, Cambridge, Massachusetts, USA.

STEVENS, P. F. 2001 onward. Angiosperm phylogeny website, version 8, June 2007 [more or less continuously updated]. Website <http://www.mobot.org/MOBOT/research/APweb/> [accessed 00 Month Year].

TURNER, B. L., AND R. M. KING. 1977. Chromosome numbers in the Compositae. VIII. Mexican and

Central American species. *Southwestern Naturalist* 9: 27–39.

WHITE, T. J., T. D. BRUNS, S. B. LEE, AND J. W. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White [eds.], *PCR protocols: A guide to methods and applications*, 315–322. Academic Press, San Diego, California, USA.

## Tables

For marker development papers, two tables should be presented. One should contain the names of the forward and reverse primers, their DNA sequences, fragment size range, annealing temperatures, and GenBank ID. Other information should also be included as necessary for specific marker types (e.g. repeat motifs for microsatellite markers and polymorphism information [A/T] for SNPs). If primers have been developed using EST sequence data, the putative function of each locus must be given. The second table should report the number of alleles and observed heterozygosity in each population surveyed. In species expected to be primarily outcrossing, results of tests for departures from single-locus Hardy-Weinberg expectations and for departures from gametic equilibrium may be included. See example below.

Table 1. Characteristics of 16 microsatellite loci developed in *Antennaria plantaginifolia*.<sup>a</sup>

Locus	Primer sequences (5'–3')	Repeat motif	Size (bp)	$T_a$ (°C)	GenBank accession no.
Primer A1	F: CATGGGACACCATTTTAAGTG R: TCCATGTTCATCCACAATACCA	(GT) <sub>16</sub>	164	53	AJ842064
Primer B5	F: GCTGGGTAGATTGAGCTGCTT R: TCAACGATGCAATAGTGGGTA	(TC) <sub>8</sub>	219	57	AJ842074
Primer X8	F: CATCCACCAACCCACACATA R: CTAGCAACACACAGGGCATC	(GAA) <sub>5</sub>	169	55	AJ842065

Note:  $T_a$  = annealing temperature.

<sup>a</sup> All values are based on 50 samples representing North American populations located in Florida, Tennessee, and Michigan ( $N = 14\text{--}20$  for each).

Table 2. Genetic properties of the newly developed eight microsatellites of *Ranunculus bulbosus*.<sup>a</sup>

Locus	L'Etivaz ( $n = 14$ )			Erschmatt ( $n = 7$ )			Burglauenen ( $n = 245$ )		
	$A$	$H_o$	$H_e$	$A$	$H_o$	$H_e$	$A$	$H_o$	$H_e$
Rb204	4	0.077	0.589	4	0.400	0.640	14	0.163	0.742
Rb206	7	0.143	0.809	5	0.400	0.680	13	0.192	0.556
Rb302	2	0.000	0.142	2	0.000	0.278	7	0.26	0.527
Rb306	4	0.500	0.556	4	0.429	0.531	9	0.7	0.719
B127	7	0.571	0.773	6	0.857	0.755	13	0.818	0.817
B129	11	0.286	0.870	6	0.333	0.806	37	0.657	0.953
B134	16	0.615	0.920	5	0.400	0.760	63	0.588	0.974
B145	13	0.714	0.908	9	0.571	0.857	44	0.714	0.953

*Note:*  $A$  = number of alleles sampled;  $H_e$  = expected heterozygosity;  $H_o$  = observed heterozygosity;  $n$  = number of individuals sampled.

<sup>a</sup> Geographic coordinates for the populations are: L'Etivaz = 46.42964°N, 7.17680°E; Erschmatt = 46.31989°N, 7.68558°E; Burglauenen = 46.64726°N, 7.97194°E. All three populations are located in Switzerland.

## Figure and Legend

*[limited to one optional figure, which should be uploaded as a separate file; the legend should be included in the text file]*

## Appendix

[Shown below are examples of how multiple voucher specimens could be cited within an appendix.]

Appendix 2. Species, population voucher, municipality/state, country, and GPS coordinates of all samples used in this study. Abbreviations: AM = Amazonas; BA = Bahia; MG = Minas Gerais; MS = Mato Grosso do Sul; MT = Mato Grosso; PR = Paraná; SC = Santa Catarina; SP = São Paulo.

*Utricularia gibba*—UG3: Mogi das Cruzes/SP, Brazil (−23.532917, −46.143972); UG4: Mogi das Cruzes/SP, Brazil (−23.557844, −46.137386); UG5: Itararé/SP, Brazil (−24.084417, −49.201639); UG6: Guaratuba/PR, Brazil (−26.023625, −48.770411); UG7: São Bento do Sul/SC, Brazil (−26.361697, −49.388964); UG8: Corumbá/MS, Brazil (−19.008889, −57.652778); UG9: Presidente Figueiredo/AM, Brazil (−60.020556, −2.052489).

*Utricularia neottioides*—UN1: Chapada dos Guimarães/MT, Brazil (15.383333, −55.833333); UN2: Piatã/BA, Brazil (−13.151356, −41.758842); UN3: Santa Bárbara/MG, Brazil (−19.958889, −43.415000); UN4: Raudal Caldero/Amazonas, Venezuela (4.766667, −66.683333).

*Utricularia reniformis*—UR1: Salesópolis/SP, Brazil, (−23.649222, −45.677833); UR2: Biritiba-Mirim/SP, Brazil (−23.658306, −46.034556); UR3: Bananal/SP, Brazil (−22.798722, −44.377917); UR5: Itararé/SP, Brazil (−24.115472, −49.363611); UR6: Mogi das Cruzes/SP, Brazil (−23.751353, −46.126506); UR7: Campina Grande do Sul/PR, Brazil (−25.245278, −48.834167); UR8: Corupá/SC, Brazil (−26.393211, −49.354878); UR9: Morretes/PR, Brazil (−25.127778, −48.820278).

*Utricularia subulata*—US1: Salesópolis/SP, Brazil (−23.556856, −46.137842); US2: Mogi das Cruzes/SP, Brazil (−23.534294, −46.144850); US3: Jaguariaíva/PR, Brazil (−24.250833, −49.705833).

### Appendix 2. Voucher information for *Armeria* species used in this study.

Species	Voucher specimen accession no. <sup>a</sup>	Collection locality <sup>b</sup>	Geographic coordinates	No. of individuals
<i>A. caespitosa</i>	Acp-003-AG	Cabeza de Hierro, Madrid	40°47'57.14"N, 3°57'3.21"W	20
<i>A. caespitosa</i>	Acp-015-AG	Pico del Lobo, Guadalajara	41°11'0.23"N, 3°27'58.91"W	20
<i>A. bigerrensis</i>	Abg-002-AG	Morezón, Ávila	40°14'56.13"N, 5°16'11.33"W	5
<i>A. cantabrica</i>	Act-001-AG	Torrecedredo, Asturias	43°12'3.26"N, 4°50'53.19"W	5
<i>A. maritima</i>	Amt-001-AG	Cabo Mayor, Santander	43°29'26.94"N, 3°47'26.27"W	5

Note: AG = Alfredo García, collector.

<sup>a</sup> Vouchers deposited at Universidad Rey Juan Carlos, Departamento de Biología y Geología, Germplasm bank.

<sup>b</sup> Locality and Spanish province.