Draft template for Applications in Plant Sciences - Application Article
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.

Use of single pollen grain PCR with herbarium specimens to quantify temporal and geographical genetic changes in populations[1]

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

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Number of words: YYYY *[up to 5000, consisting of Introduction, Methods, Results, and Discussion sections].*

ABSTRACT

The abstract should capture the interest of the general botanical community as well as specialists. The abstract is 200 words or less, written in a structured format:

- Premise of the study (why the work was done)
- Methods (what was done)
- Results
- Discussion

Avoid references; if essential, cite parenthetically with journal name, volume number, pages, and year. Here is a sample abstract:

- *Premise of the study:* Herbarium specimens represent an invaluable depository of historical information collected throughout time from different geographical locations, but their use in genetic studies has been limited by degradation of DNA in leaf tissue. To overcome this deficiency, DNA amplification through PCR was optimized for individual pollen grains and tested in 10 plant species within the Fabaceae and Rosaceae.

- *Methods:* Individual pollen grains for each species were obtained directly from herbarium specimens collected at least six decades apart and from three distinct locations. Pollen grains were treated and added directly to the PCR cocktail for previously developed microsatellite or SNP markers. Estimates of genetic diversity were then compared within species for each sampling period and location.

- *Results:* Using this new method, DNA could be directly amplified with microsatellite and SNP primers with high repeatability in both plant families. Genetic diversity declined over time in seven of the 10 species, but generally fluctuated across locations for any given sampling period.

- *Discussion:* PCR of pollen grains can be an advantageous tool for genetic analysis of

historical herbarium specimens with potential application to the study of global climate change.

INTRODUCTION

This section sets the stage for the study, which should illustrate the application of a new protocol, method, or software application in any area of the plant sciences within the context of a large investigation. Authors must clearly outline the reasons behind the study, an explanation of the importance of the application itself, and any other information regarding the application that would be of interest to the plant sciences community. Of particular appeal is the potential for widespread applicability of protocols, methods, or software programs to other species or systems, so authors are encouraged to include a variety of taxa in their applications when at all possible. These articles differ from Protocol Notes and Software Notes in that the emphasis is on the use of the protocol or software program, and not on the comprehensive description of a new protocol or program.

METHODS

Enough detail must be provided in this section so readers can accurately replicate the application. For protocol and methods applications, this includes specific quantities of chemicals used as well as source information for chemicals and supplies (including the location [city, state/province, country] of the supplier). For software applications, authors must include the name and versions of all software programs, including source and access information.

For any samples analyzed in the study, their number and geographic origin (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; see example below), or as a footnote on a table (see below). Voucher specimens are also required, unless their absence can be suitably justified (as in the case of an extremely rare species) in the authors' cover letter.

RESULTS

In this section, the authors must demonstrate the useful application of a protocol, method, or software program, using a reasonable number of individuals or populations. Authors may use tables and figures to support the findings. Appropriate statistical analyses must be presented to support any conclusions to be discussed below.

DISCUSSION

In this section, the author(s) should clearly state the main conclusions that have been reached, focusing on the effectiveness and utility of the application being described for the plant sciences. Suggestions for future applications in other species or systems are especially appreciated.

LITERATURE CITED *[no more than 60]*

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

Tables should be presented, when appropriate, to support the results of the study. See the example below.

Table 1. Sequencing capacity of selected next-generation platforms. The last three columns provide estimates of multiplex levels and numbers of PCR reactions for a hypothetical example assuming a 150-kbp target, amplified in thirty 5-kbp fragments at the desired coverage depth.

Platform, read length	Instrument capacity (Gbp)	Minimum samples /run[a]	Minimum/sample capacity (Gbp)	Desired coverage depth	Maximum multiplex	Amplicons (5 kb each) required to match capacity	Per-sample sequencing cost, US\$[e] (kb/\$)
Illumina GAIIx, 150 bp single-end SE	45[b]	7	4.1	50	546	16 400	2200 (1864)
Illumina HiSeq2000, 100bp SE	285[b]	7	20.4	50	2730	81 600	3000 (6800)
Roche/454 GS FLX Titanium XL+, avg. 700 bp SE	0.7[c]	16	0.04	5	58	1750	2100 (19)
ABI SOLiD 5500xl, 75 bp	56[d]	6	16.7	50	2227	66 800	2000 (8350)

SE							

[a] "Run" signifies the maximum available physical division of sequencing surface currently available. For Illumina platforms, one lane per flow cell is used for calibration/quality control purposes.

[b] http://www.illumina.com/systems/sequencing.ilmn (accessed 15 July 2011)

[c] http://454.com/products/gs-flx-system/index.asp (accessed 15 July 2011)

[d] http://www.appliedbiosystems.com/absite/us/en/home/applications-technologies/solid-next-generation-sequencing/next-generation-systems.html (accessed 15 July 2011)

[e] prices estimated for the GAIIx (http://htseq.uoregon.edu/), Roche/454 (http://www.genome.duke.edu/cores/sequencing/), and all other platforms (http://www.molecularecologist.com/next-gen-table-2b/) (all sites accessed 15 July 2011)

Figures and Legend

[Each figure should be uploaded as a separate file; the legend should be included in the text file before the tables.]

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.[a]	Collection locality[b]	Geographic coordinates	No. of individuals
A. caespitosa	Acp-003-AG	Cabeza de Hierro, Madrid	40°47′57.14″N, 3°57′3.21″W	20
A. caespitosa	Acp-015-AG	Pico del Lobo, Guadalajara	41°11′0.23″N, 3°27′58.91″W	20
A. bigerrensis	Abg-002-AG	Morezón, Ávila	40°14′56.13″N, 5°16′11.33″W	5
A. cantabrica	Act-001-AG	Torrecerredo, Asturias	43°12′3.26″N, 4°50′53.19″W	5
A. maritima	Amt-001-AG	Cabo Mayor, Santander	43°29′26.94″N, 3°47′26.27″W	5

Note: AG = Alfredo García, collector.

[a] Vouchers deposited at Universidad Rey Juan Carlos, Departamento de Biología y Geología, Germplasm bank.

[b] Locality and Spanish province.