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For contributions to International Oaks
contact
Béatrice Chassé
pouyouleixarboretum@gmail.com or editor@internationaloaksociety.org
Les Pouyouleix
24800 St.-Jory-de-Chalais
France
Author guidelines for submissions can be found at
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Quercus alba, The Mortom Arboretum (Charles Snyers d’Attenhoven).
Oakcoding: a Nuclear DNA Barcode for Evolutionary Studies in Oaks

E. Fitzek¹, E. Guichoux², R. Petit³, and A. Hipp¹

1. The Morton Arboretum Herbarium
   Lisle, IL 60532, USA
2. Platforme Genome Transcriptome
   INRA
   33610 Cestas, France

Oaks (*Quercus*, Fagaceae) are keystone species in forests and savannas across the Northern Hemisphere. They are a model genus for studying tempo and rate of hybridization. Single-nucleotide polymorphisms (SNP) genotyping is increasingly used to study population structure and hybridization rates within a population. The goal of OAKCODING is to develop two-multiplexes (80 SNPs) as an easy-to-use genotyping tool to distinguish a set of the most common North American White Oaks. Secondly, these SNPs will help to differentiate them from the Eurasian White Oaks with which they can hybridize in cultivation. An existing RADseq dataset for 69 samples representing the Eastern North American and Eurasian White Oaks was utilized to identify species-specific SNPs. RADami, pyRAD, STACKS were used to generate a list of potential SNPs and screen 8-plex (total of 291 SNPs) on ca. 95 DNA extractions to evaluate barcoding success rate using the SEQUENOM (INRA, Pierroton). Currently, we have 80 verified SNPs that will count towards OAKCODING to distinguish e.g., *Quercus alba* L., *Q. bicolor* Willd., *Q. macrocarpa* Michx., *Q. michauxii* Nutt., *Q. montana* Willd., and *Q. stellata* Wangenh. We then propose to utilize this toolkit to study hybridization patterns in a unique and old (> 90 years old) oak taxonomic collection at The Morton Arboretum.
Oakcoding: a nuclear DNA barcode for evolutionary studies in oaks

Elisabeth Fitzek¹, Erwan Guichoux², Remy Petit², Andrew Hipp¹
¹The Morton Arboretum, Herbarium, 4100 Illinois route 53, Lisle, IL 60532, USA
²Platforme Genome Transcriptome, 69 Route d’Arcachon INRA, Site de Pierroton/Btmt ARTIGA CESTAS, 33610 FR

Introduction
Oaks (Quercus, Fagaceae) are keystone species in forests and savannas across the northern hemisphere. Oaks are also a model genus for studying tempo and rate of hybridization (Pettit et al., 2003). Oak hybrids may lie anywhere on the morphological continuum between their parents or lack obvious morphological intermediate forms altogether (Burgarella et al., 2008). Detecting oak hybrids is thus quite challenging without large numbers of informative molecular markers. Restriction enzyme Associated DNA sequencing (RADseq) is an inexpensive and fast approach to generate a reduced-representation genome-scale dataset (Baird et al., 2008). A currently-funded NSF project has generated a RADseq dataset of more than 300 oak individuals within the Americas (Hipp et al 2014). For this project (OAKCODING), we are utilizing this existing RADseq dataset to identify species-specific single-nucleotide polymorphisms (SNPs) to develop an economical, high-throughput DNA barcode for eastern North American white oaks.

Objective
The primary focus of OAKCODING is to develop an easy-to-use genotyping tool to distinguish oak species from each other. Our current RADseq dataset (ca. 36,000 100bp loci for each of 311 oaks) represents a subsample of the oak genome sufficient for phylogenetic analysis and species identification. Our goal is to identify SNPs that are fixed or nearly fixed within species and to develop two-multiplexes (80 SNPs) as an easy-to-use genotyping tool distinguishing a set of the most common North American white oaks.

Why white oaks?
OAKCODING focuses on the white oaks because: (1) at The Morton Arboretum we have a particularly strong sample of eastern North American white oaks (17 species, represented by 52 samples) and Eurasian white oaks (15 species, represented by 17 samples); (2) white oaks are of ecological and economic importance across the northern temperate zone, in North America, Europe and Asia.

SNPs sufficient to identify at least one of the 8 white oak species screened

1. Q. alba, 2. Q. bicolor, 3. Q. macrocarpa, 4. Q. michauxii, 5. Q. montana, 6. Q. muehlenbergii/prinoides 7. Q. stellata, 8. Q. lyrata
Methods

RADseq of 69 samples (North American and Euroasian white oaks)

PyRAD, RAdami

STACKS

pSNP list

Barcode

Primer development: Assay Design Suite provided by SEQUENOM

Genotyping & Hybridization

SNP screen with SEQUENOM iPLEX technology

List of verified SNPs

Data analysis: TYPER 4.0 software

Results

PyRAD and RAdami resulted in 1698 potential SNPs of $F_{ST} = 1$ (we deliberately used a very stringent criterion for SNP selection); STACKS resulted in 1300 potential SNPs. Custom scripts were applied as pre-filter to select for SNPs that were present in at least two samples of one species to be considered for SNP screening. Altogether we considered 844 SNPs for primer development of which 344 passed and 500 failed to design suitable primers. We screened 8-multiplexes (291 SNPs) using the SEQUENOM technology at INRA (Pierroton, FR) of which more than 119 SNPs are considered for the barcode. On average, each of our 8 species is distinguished from all others by ~4 SNPs.

Barcode selection

Q. stellata

Q. montana

Q. macrocarpa

Q. bicolor

Q. lyra

Q. prinoides / muehlenbergii

Q. alba

Q. michauxii

Out of 291 screened SNPs at least 80 SNPs are considered for barcode genotyping tool kit. Maximum likelihood (Phylogeny.fr) was used to verify SNP selection.

Conclusion

The DNA barcode presented here is a potentially useful tool to identify species in hybrid hotspots, monitor hybridization progress in response to climatic change, study the fitness effects of hybridization, and help to predict which species are more likely to hybridize.

Future directions

In our next steps, we will be using the refined barcode to investigate patterns of hybridization in white oaks at The Morton Arboretum. We will be genotyping >25 acorns from each of 9 mother trees at The Arboretum from outside their range. Our expectation is that the barcode will allow us to detect $F_1$ hybrids between introduced white oaks and the surrounding native oak populations without having to undertake an exhaustive parentage study. This information will increase our understanding of how managed relocation of southern trees may impact oak population dynamics.

This project was funded by the Transnational Access to Research Infrastructures activity in the 7th Framework Programme of the EC under the Trees4Future project (no. 284181) mobility grant (LabEx COTE) to E.F.