Fungal Genetics Reports

Volume 43 Article 16

PCR amplification of ITS rDNA from rust teliospores collected on southwestern rangeland from 1907 to 1995

Craig Liddell
New Mexico State University

Kathy Onsurez Waugh
New Mexico State University

Follow this and additional works at: https://newprairiepress.org/fgr

This work is licensed under a Creative Commons Attribution-Share Alike 4.0 License.

Recommended Citation

Liddell, C., and K.O. Waugh (1996) "PCR amplification of ITS rDNA from rust teliospores collected on southwestern rangeland from 1907 to 1995," *Fungal Genetics Reports*: Vol. 43, Article 16. https://doi.org/10.4148/1941-4765.1312

This Regular Paper is brought to you for free and open access by New Prairie Press. It has been accepted for inclusion in Fungal Genetics Reports by an authorized administrator of New Prairie Press. For more information, please contact cads@k-state.edu.

PCR amplification of ITS rDNA from rust teliospores collected on southwestern rangeland from 1907 to 1995

Abstract

Evidence of evolutionary change in the rust, *Puccinia grindeliae*, populations in New Mexico and Arizona, is revealed by use of PCR amplified ITS regions of rDNA. Polymorphic ITS5 - ITS2 fragments were found both within a single community and among geographically and temporally separated collections. These results provide evidence for genetic heterogeneity, possibly resulting from recent evolutionary change in reproductive strategy from clonal to sexual.

This regular paper is available in Fungal Genetics Reports: https://newprairiepress.org/fgr/vol43/iss1/16

PCR amplification of ITS rDNA from rust teliospores collected on southwestern rangeland from 1907 to 1995

Craig Liddell and Kathy Onsurez Waugh- Department of Entomology, Plant Pathology and Weed Science, New Mexico State University, Las Cruces, NM 88003

Evidence of evolutionary change in the rust, *Puccinia grindeliae*, populations in New Mexico and Arizona, is revealed by use of PCR amplified ITS regions of rDNA. Polymorphic ITS5 - ITS2 fragments were found both within a single community and among geographically and temporally separated collections. These results provide evidence for genetic heterogeneity, possibly resulting from recent evolutionary change in reproductive strategy from clonal to sexual.

We are using PCR amplification of DNA from herbarium specimens of fungi to study gene flow over time in biotrophic plant pathogens from natural ecosystems. In this paper we describe PCR amplification of DNA extracted from rust teliospores collected from dried herbarium material. Amplification of DNA from dried herbarium basidiomata has been achieved before but this is the first report of attempted amplification of DNA from herbarium specimens of microfungi. We are particularly interested in *Puccinia grindeliae*, a common pathogen of *Gutierrezia sarothrae* (Broom snakeweed), which is being evaluated as a potential biological control agent of this troublesome rangeland weed in New Mexico. The rust fungus *P. grindeliae* is known from only 14 citations since 1918 due to the low economic significance accorded its hosts, which are weedy shrubs in the Asteraceae.(Cummins 1978 Rust Fungi on Legumes and Composites in North America. Univ. of Ariz. Press). Our research has recently focused on better understanding the population genetics of *P. grindeliae* to help clarify the role of the rust in natural population dynamics of *G. sarothrae*.

We recently completed a study on the occurrence of *P. grindeliae* in the southwestern U.S. since 1891 using herbarium specimens to track the rust. We found 3.7% of 1,048 herbarium specimens of *G. sarothrae* were rusted and the oldest rusted collection was from 1906 (Liddell *et al.* 1995. Correlation of Climatic Factors and Occurrence of *P. grindeliae* on Herbarium Specimens of *Gutierrezia* spp. collected in southwestern states since 1891. NM Ag. Exp. Stat. Bulletin 773). We identified 25 of the 39 rusted specimens where collection sites could be accurately re-located from annotations on the herbarium sheets and attempted to visit these sites in 1993-1995 to collect fresh specimens of the rust for comparative analysis. Our objective is to compare the genetic structure of the communities at each site as far as possible to determine the type and direction of gene flow in this organism over the past 89 years. We plan to correlate these data with existing data on the genetic structure of host populations and changes in host distribution which has been well documented over the past 100 years.

We collected teliospores from diseased plant tissue by carefully excising individual telia with fine pointed forceps under a dissecting microscope. The telia were stored at room temperature in small vials until needed. We used a modified C-TAB extraction procedure to extract DNA (Ausubel *et al.* 1994 Current Protocols in Molecular Biology **1**:2.3.3). DNA was extracted by

first grinding each telium in a small etched glass tissue grinder with 5ul of CTAB extraction buffer containing 2% (w/v) CTAB; 100mM Tris-Cl, pH 8.0; 20mM EDTA, pH 8.0; 1.4M NaCl. After grinding, the extract was incubated at 65 C with occasional agitation, in a solution containing 100 ul sterile distilled water, 100 ul CTAB extraction buffer, 20 ul 10% polyvinylpyrrolidone (mol. wt. 40,000), and 2.2ul of -mercaptoethanol. After incubation, the homogenate was extracted with an equal volume of 24:1 (v/v) chloroform/isoamyl alcohol, and centrifuged at approximately 12,000 x g for 4 m. To the recovered aqueous solution, 1/10 vol 65 C CTAB/NaCl solution, containing 10% CTAB in 0.7M NaCl, was added, and mixed as before. This solution was then re-extracted with 24:1 (v/v) chloroform/isoamyl alcohol, mixed and centrifuged as before. The DNA was precipitated from the recovered aqueous extract by the addition of 3M NaOAc and ice cold 100% EtOH, and incubated at -80 C for 20 m. DNA was pelleted, washed in 70% EtOH, dried and taken up in 50-100 ul of TE. Initially, the DNA was RNase A treated for 30 m at 37 C for 30 min., followed by extraction with Tris-HCl (pH 8.0) saturated Phenol, and precipitated, washed and pelleted, dried and taken up in TE as before. However, we found that no Ribonuclease A treatment was necessary for consistent amplification. PCR parameters for both the ITS 5 -ITS 4 and ITS 5 - ITS 2 regions were as follows: Initial denaturation 94 C - 3 m, 25 cycles of 94 C - 30 s, 50 C - 30 s, 72 C - 1 m, 20 additional cycles of 94 C - 30 s, 50 C - 30 s, 72 C - 1 m 30 s, final extension 72 C - 10 m. The amplification conditions were in either a 35 ul or 50 ul reaction volume as follows: 1X Stoffel fragment buffer, 0.25mM each dNTP, 28 pmoles of each primer; 5.6mM MgCl, 0.4 units AmpliTaqreg. DNA Polymerase (Perkin Elmer N808-0038). Characterization of PCR products was done via agarose gel electrophoresis on a TBE 2% agarose gel containing 1ul/100ml of 10 mg/ml EtBr.

Puccinia grindeliae was present at only 13 of the 25 sites from which we attempted to revisit and collect fresh isolates during 1993-1995. Of these sites only one herbarium specimen collected in 1952 yielded usable rDNA and amplified satisfactorily using ITS5-ITS2 primers. We have so far been unable to amplify the remaining herbarium specimens that are from 3 to 89 years old. At least one ITS fragment has been amplified from all 13 recent (1993-1995) collections. The ITS 5 - ITS 4 region yielded a 650 bp fragment and the fragments from the ITS 5 - ITS 2 ranged from 250 to 300 bp. PCR fragments from the ITS 5 - ITS 4 region were not polymorphic on 2% agarose gels but polymorphisms were present in the ITS 5 - ITS 2 region between the small subunit and the 5.8s subunit in collections from different sites. Hence, preliminary analysis of the genetic structure of *P. grindeliae* could only be conducted using the 6 specimens that amplified using the ITS5 - ITS2 primers.

Based on preliminary analysis of the ITS5 - ITS2 fragment sizes and collection locations of amplified specimens, no spatial or temporal correlations have yet been observed. Specimen 1100 collected in 1994 from Endee, Quay County, NM had a 250 bp fragment. Specimen 969 collected in 1995 from Cornville, Yavapai County, AZ south of Flagstaff on I-17 and specimen 1106 collected in 1995 from Oracle, Pinal County, AZ north of Tucson both had 280 bp fragments. Specimen 689 collected in 1952 from Mescalero, Otero County, NM, specimen 1097 collected in 1994 from La Lande, De Baca County, NM and specimen 971 collected in 1995 from Oracle, Pinal County, AZ all yielded 300 bp fragments. It is noteworthy that both specimens 1106 and 971 were collected from the same site, 5.5 miles north of Oracle, AZ on the Mt. Lemon Rd. outside of Tucson, yet had different sized ITS5- ITS2 fragments of 280 bp and 300 bp, respectively. These results support the field observation that uredospores and other

asexual spores are very rarely produced by *P. grindeliae* and that the fungus is reproducing entirely or largely in a sexual fashion. The lack of clonal reproduction in this species could explain our observation that communities of *P. grindeliae* may be genetically quite heterogeneous.

We are currently sequencing the ITS5 - ITS2 region to determine if the 300 bp fragment from New Mexico isolates 689 and 1097 collected in 1952 and 1994 respectively, are identical. Should these fragments prove identical, this would constitute evidence that this species once reproduced clonally, perhaps by uredospores, and that the extant population has only recently begun to diverge by sexual recombination. Sequencing both the larger and smaller ITS fragments will also help to design longer sequence characterized primers that may consistently amplify ITS regions in older *P. grindeliae* telia. Complete sequences of these regions could also be used as a restriction endonuclease map, to permit rapid comparisons of isolates without the need of further sequencing. In conclusion, we have preliminary evidence that genetically heterogeneous populations of *P. grindeliae* exist in New Mexico and Arizona and we plan to use this system as a model for studying gene flow in a natural, biotrophic, host-pathogen system.

Acknowledgments: This work is being funded by the New Mexico Agricultural Experiment Station and our thanks to Dr Jennifer Riggs and Mark Waugh for providing guidance and help along the way and Dante Adorada for technical help.

Last modified 7/25/96 KMC