

Supplementary Information

ORGANIZATION OF GENETIC VARIATION WITHIN INDIVIDUALS OF ARBUSCULAR MYCORRHIZAL FUNGI

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Fungal material. *Glomus etunicatum* Becker & Gerdemann isolates were derived from individual corn (*Zea mays* L.) root systems sampled at random from an experimental maize field at Oxford Tract in Berkeley, CA, in November 1998. Individual maize root balls (soil-encased root systems) were air-dried, stratified for 30 days at 4°C to promote AM fungal spore germination, homogenized, combined with coarse sand (1:2), placed in pots (10.4 cm x 9.7 cm), and baited with two maize seedlings per pot. A total of 100 bait cultures representing 100 sampling points were established. Additionally, 10 control pots were prepared where the fungal inoculum was killed by 60 min of autoclaving at 121°C. Soil surface in all pots was covered with tin foil to prevent cross-contamination. The cultures were maintained in the greenhouse, and drip-irrigated as needed until maize plants senesced. The bait plants supported sporulation of AM fungi present in soil samples. After stratification, multiple spores of *G. etunicatum* were extracted from soil by wet sieving and sucrose centrifugation, surface-decontaminated, and used to inoculate two-week-old excised Ri T-DNA transformed carrot roots grown on the M medium modified with 10 mM MES (pH 6.0)¹. The successive generation of monoxenically raised spores served to establish single-spore cultures (isolates), each culture corresponding to a specific field sampling point.

Clonally propagated *Glomus intraradices* Schenck & Smith (DAOM 181602)² was maintained on Ri T-DNA transformed carrot roots grown in a modified minimal White's medium³.

Single spore preparation for PCR. Monoxenic spores were individually extracted from the culturing medium, crushed in 3 µl of proteinase K digestion buffer containing 1x One-Phor-All-Buffer-Plus (Amersham, Piscataway, NJ), 0.67% Tween 20, 0.67% Igepal CA-630 (Sigma St. Louis, MO), 0.7 mg ml⁻¹ proteinase K (PCR grade, Roche, Indianapolis, IN), and incubated for 10 hrs at 42°C followed by 20 min at 80°C⁹. The entire preparations were used in 20 µl PCR reactions.

Development of the PLS1 marker. To search for genetic markers variable at intrasporal level, arbitrary primers fwd AAGACCATCGTCCCAGACTC and rev CCTTTCTCAAATCGCCCC were used to PCR-amplify fragments from monoxenically raised *G. etunicatum* spores (AmpliTaq[®] DNA polymerase, Roche), (45 cycles: 94°C for 40s, 55°C for 40 sec, 72°C for 60 sec). A 327 bp fragment with sequence similarity to *Saccharomyces cerevisiae* *POL1* was extended to a 611 bp fragment using primers fwd pol4 GAATCCTTCCCAAATTGATCAGAATACTTGTT and rev pol6 AATTACAGTNARNGCNGCRTAYTTYTTYTT (AmpliTaq Gold[™] DNA polymerase; 94°C for 10 min, 15 cycles touchdown: 94°C for 40 sec, 67°C (-0.5°C per step) for 40 sec, 72°C for 2 min, 30 cycles: 94°C for 40 sec, 60°C for 40 sec, 72°C for 2 min).

Characterization of the PLS1 marker. Presence of two putative introns within the *G. etunicatum* PLSs supports their fungal origin rather than from the endosymbiotic bacteria that inhabit the spores (Figure S1).

To test the hypothesis of neutral evolution of PLS1 variants, we applied Fisher's exact test⁴ as implemented in MEGA⁵ for examining the differences between numbers of synonymous (*s*) and nonsynonymous (*n*) nucleotide substitutions observed in putative amino acid sequences of PLS1 variants and numbers of synonymous (*S*) and nonsynonymous (*N*) sites that remained unchanged in these sequences in pairwise variant comparisons (Table 1). Under the null hypothesis of neutral evolution the ratio of *n/s* was expected to equal *N/S*. The numbers of synonymous and nonsynonymous substitutions and synonymous and nonsynonymous sites were estimated using Nei and Gojobori method⁶.

Using a method by Hudson and Kaplan⁷ as implemented in DnaSP⁸, we performed a 'four-gamete' test, which is a way of inferring that at least one recombination event took place between two sites in the history of the sequences. Four 'gametic' types are represented by 16 pairs of nucleotide sites in PLS1 variants: (31, 484), (31, 485), (31, 508), (41, 92), (41, 368), (41, 484), (41, 485), (41, 508), (92, 368), (92, 483), (92, 484), (368, 484), (368, 485), (368, 508), (483, 508), (484, 508) (Figure S1). These 'gametic' types can be explained by a minimum of 4 recombination events among the PLS1 variants between the sites: (41, 92), (92, 368), (368, 484) and (484, 508). Although compelling, this evidence of recombination should not be considered as a proof of genetic exchanges in the population because it may only represent mitotic recombination.

Examination of two *G. etunicatum* individuals, PE-14-8-557 (49 clones analysed) and PE-14-8-558 (43 clones analysed), from a Minnesota population¹ revealed a pattern of PLS1 variation similar to the one observed in the California population analysed in this study (Figure S2). While three new PLS1 variants were detected in the Minnesota population, the analysed individuals harboured 13 PLS1 variants each (GenBank accession numbers AY330581, AY394011 – AY394024), which corresponded to the number of variants found in the individuals from the California population.

Microdissection of nuclei. Spores were fixed in ethanol – acetic acid (3:1) for 15 min, rinsed 3 x 15 min in water, crushed either individually between two cover slips (*G. etunicatum*) or in groups of 20 using a glass rod (*G. intraradices*), and stained on a cover slip with 2.5 μ M SYTO[®] 11 (Molecular Probes, Eugene, OR). Nuclei were observed with a Nikon Diaphot microscope (Nikon, Japan) equipped with 40x objective, 440-495 nm excitation filter, 500 nm dichroic mirror and 530 nm long-pass emission filter. Pipettes were pulled in two stages from KIMAX[®] 51 glass (No. 34500, Kimble, Vineland, NJ) to taper with a 1 μ m opening, and filled with 1 μ l of water. After positioning in a field of view with a 3-axis micromanipulator (Narishige, Japan), the pipette orifice was enlarged to about 3 μ m by breaking off the tip to accommodate the diameter of an individual nucleus. One nucleus was aspirated into the pipette tip and deposited into 3 μ l of proteinase K digestion buffer by crushing the tip. Nuclei were prepared for PCR as described earlier for spores. To control for DNA molecules freed incidentally into the nuclear suspension, samples visually devoid of nuclei were collected, and processed identically to those containing single nuclei.

PCR-amplification from individual spores and nuclei, cloning and sequencing. PLS1 528-529 bp fragments were PCR-amplified from single spore preparations with primers fwd pol4 and rev pol7 TAATAATAAAAGCCTTTCAAAAAATCCATCAATA and *PfuTurbo*[®] DNA

polymerase (Stratagene, Cedar Creek, TX) (touchdown 15 cycles: 94°C for 15 sec, 67°C (-0.5°C per cycle) for 15 sec, 75°C for 2 min, 10 cycles: 94°C for 15 sec, 60°C for 15 sec, 75°C for 2 min). The rDNA ITS1-5.8S-ITS2 fragments were PCR-amplified from single spore preparations with ITS1 and ITS4 primers¹⁰. For *G. etunicatum* we used Advantage[®]-HF 2 PCR Kit (Clontech, Palo Alto, CA), (touchdown 15 cycles: 94°C for 15 sec, 60°C (-0.5°C per cycle) for 15 sec, 68°C for 1 min, 10 cycles: 94°C for 15 sec, 53°C for 15 sec, 68°C for 1 min). For *G. intraradices* (3 randomly selected spores from a clonal culture to assess the intrasporal variation) we used *PfuTurbo*[®] DNA polymerase (touchdown 15 cycles: 94°C for 15 sec, 60°C (-0.5°C per cycle) for 15 sec, 75°C for 1 min, 20 cycles: 94°C for 15 sec, 53°C for 15 sec, 75°C for 1 min).

To recover the ITS1 variation detected earlier in individual spores, the ITS1 fragments were amplified from nuclear preparations using *PfuTurbo*[®] DNA polymerase and primers ITS1Get CGTAGGTGAACCTGCGGAAGGATCATTATAAAAT and ITS2Get CTGCGTTCTTCATCGATGCGAGAGCC in *G. etunicatum* (touchdown 15 cycles: 94°C for 15 sec, 60°C (-0.5°C per cycle) for 15 sec, 75°C for 1 min, 30 cycles: 94°C for 15 sec, 53°C for 15 sec, 75°C for 1 min), or ITS1 and ITS2Gint GCTACGTTCTTCATCGATGC in *G. intraradices* (touchdown 15 cycles: 94°C for 15 sec, 60°C (-0.5°C per cycle) for 15 sec, 75°C for 1 min, 40 cycles: 94°C for 15 sec, 53°C for 15 sec, 75°C for 1 min).

PCR amplicons were cloned with TOPO TA Cloning[®] Kit for Sequencing (Invitrogen, Carlsbad, CA) after QIAquick[®] PCR purification (Qiagen, Valencia, CA) for amplifications from individual nuclei, and addition of 3' A-overhangs in all reactions. Recombinant plasmid DNA was isolated with QIAprep[®] 96 Turbo Miniprep Kit (Qiagen). Clones were analysed by sequencing using Big Dye[™] Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and automated sequencers ABI377 and ABI3100. The number of analysed clones was

44 to 178 per spore for PLS1, 10 to 33 per spore for ITS1-5.8S-ITS2 rDNA in *G. etunicatum*, 20 to 26 per spore for ITS1-5.8S-ITS2 rDNA in *G. intraradices*, 8 to 56 per nucleus for ITS1 rDNA in *G. etunicatum* and 20 to 37 per nucleus for ITS1 rDNA in *G. intraradices*. Details on the number of PLS1 and ITS1-5.8S-ITS2 clones analysed from *G. etunicatum* spores, ITS1-5.8S-ITS2 clones analysed from *G. intraradices* spores and ITS1 clones from individual nuclei of *G. etunicatum* and *G. intraradices* are presented in Tables 2, 3, 4, 5 and 6 respectively.

Error rate estimates in PCR amplifications from individual spores. We used a fragment of beta-tubulin (GenBank accession numbers AY394025 – AY394029), which was monomorphic in our *G. etunicatum* field isolates, as an internal control for PCR-related nucleotide misincorporations in amplifications from individual spore preparations with different DNA polymerases. With primers fwd CAGAGTGGTGCTGGTAACAACCTGGGCCAAA and rev GATCAGAGTTCAGTTGACCAGGGAATCTTAAACAT, we amplified a 525 bp fragment from 6 individual spore preparations (13-4-2-K, 13-4-2-L, 21-1-4-K, 21-1-4-L, 73-3-4-I, 73-3-4-J) using *PfuTurbo*[®] DNA polymerase (touchdown 15 cycles: 94°C for 15 sec, 67°C (-0.5°C per cycle) for 15 sec, 75°C for 2 min, 10 cycles: 94°C for 15 sec, 60°C for 15 sec, 75°C for 2 min) and from 6 individual spore preparations (3-5-4-K, 3-5-4-L, 3-5-4-M, 13-4-2-M, 13-4-2-N, 13-4-2-O) using the Advantage[®]-HF 2 PCR Kit (touchdown 15 cycles: 94°C for 15 sec, 60°C (-0.5°C per cycle) for 15 sec, 68°C for 1 min, 10 cycles: 94°C for 15 sec, 53°C for 15 sec, 68°C for 1 min). We cloned and sequenced amplicons (47 sequences for *PfuTurbo*[®] and 62 sequences for the Advantage[®]-HF 2). We did not detect any nucleotide misincorporations by the *PfuTurbo*[®] DNA polymerase (error rate within the manufacturer-specified range of $<0.16 \times 10^{-5}$ errors per bp per cycle). On the other hand, the Advantage[®]-HF 2 PCR Kit yielded 5.0×10^{-5}

misincorporations per bp per cycle. To eliminate the possibility of PCR errors confounding our analysis, we only used variants that were recovered independently at least twice. PLS1 and ITS1-5.8S-ITS2 clones with unique single nucleotide polymorphisms that were recovered from individual spores only once were excluded from the analysis because the frequency of these clones did not exceed the error rates incurred in beta-tubulin PCR-amplifications. Based on the rates of PCR-induced recombination published for *Pfu* and *Taq* DNA polymerases¹¹, we also excluded from our analyses unique clones with chimeric sequences.

Counting nuclei in *G. etunicatum* spores. The nuclei were counted in 7 monoxenically raised *G. etunicatum* spores after they were fixed in buffer containing 4% formaldehyde¹², gently broken with a cover slip to release nuclei, and stained with DAPI. Three-dimensional stacks of images were collected, with optical sections 200 nm apart, on a DeltaVision imaging station (Applied Precision, Seattle, WA) using an Olympus IX70 inverted microscope with a 20x lens and a Photometrics cooled CCD (Roper Scientific, San Diego, CA). For counting, the nuclei were located automatically in the data sets by identifying local peak intensities in three dimensions using PRIISM software package¹³. All counts were verified manually.

Computer simulations and probability estimates. Estimates of probability of detecting spores genetically differentiated due to loss of PLS1 or ITS1-5.8S-ITS2 variants based on simulations of the heterokaryotic model are presented in Tables 7 and 8.

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Table 1 Summary of the numbers of putative synonymous and nonsynonymous nucleotide substitutions, synonymous and nonsynonymous positions and Fisher's exact test *P* values in pairwise PLS1 variant comparisons

Sequence 1	Sequence 2	Number of synonymous differences	Number of synonymous positions	Number of nonsynonymous differences	Number of nonsynonymous positions	Fisher's exact test <i>P</i> value
Variant 1	Variant 2	1.0	79.9	4.0	274.1	0.4257
Variant 1	Variant 3	1.5	79.6	5.5	276.4	>0.9999
Variant 1	Variant 4	1.5	79.9	4.5	274.1	>0.9999
Variant 1	Variant 5	1.0	80.7	5.0	273.3	0.5828
Variant 1	Variant 6	1.0	80.7	5.0	273.3	0.4965
Variant 1	Variant 7	1.5	79.7	5.5	274.2	0.4965
Variant 1	Variant 8	1.5	79.9	5.5	274.1	0.2970
Variant 1	Variant 9	2.0	80.8	6.0	273.2	0.6823
Variant 1	Variant 10	1.0	79.7	9.0	274.2	0.4965
Variant 1	Variant 11	1.0	79.9	7.0	274.1	0.5828
Variant 1	Variant 12	1.0	80.2	8.0	273.7	0.3492
Variant 1	Variant 13	1.0	80.5	5.0	273.5	>0.9999
Variant 1	Variant 14	2.0	80.2	6.0	273.8	0.5828
Variant 2	Variant 2	0.5	79.5	2.5	274.5	0.5828
Variant 2	Variant 4	0.5	79.8	2.5	274.2	>0.9999
Variant 2	Variant 5	0.0	80.6	5.0	273.4	0.3507
Variant 2	Variant 6	0.0	80.6	5.0	273.4	0.6641
Variant 2	Variant 7	0.5	79.7	3.5	274.3	0.6823
Variant 2	Variant 8	0.5	79.8	3.5	274.2	0.5976
Variant 2	Variant 9	1.0	80.7	6.0	273.2	0.4614
Variant 2	Variant 10	0.0	79.7	5.0	274.3	0.5386
Variant 2	Variant 11	0.0	79.8	3.0	274.2	0.3507
Variant 2	Variant 12	0.0	80.2	6.0	273.8	0.4563
Variant 2	Variant 13	0.0	80.4	5.0	273.6	0.6441
Variant 2	Variant 14	1.0	80.1	6.0	273.9	0.3507
Variant 3	Variant 4	0.0	79.5	1.0	274.5	>0.9999
Variant 3	Variant 5	0.5	80.2	4.5	273.7	>0.9999
Variant 3	Variant 6	0.5	80.2	4.5	273.7	0.4965
Variant 3	Variant 7	0.0	79.3	2.0	274.7	0.5040
Variant 3	Variant 8	0.0	79.5	2.0	274.5	0.4257
Variant 3	Variant 9	1.5	80.4	5.5	273.6	0.4965
Variant 3	Variant 10	0.5	79.3	5.5	274.7	0.4965
Variant 3	Variant 11	0.5	79.5	3.5	274.5	>0.9999
Variant 3	Variant 12	0.5	79.8	5.5	274.2	0.4960
Variant 3	Variant 13	0.5	80.1	4.5	273.9	>0.9999
Variant 3	Variant 14	1.5	79.7	5.5	274.2	>0.9999
Variant 4	Variant 5	0.5	80.6	3.5	273.4	>0.9999
Variant 4	Variant 6	0.5	80.6	3.5	273.4	0.5040
Variant 4	Variant 7	0.0	79.7	1.0	274.3	0.5040
Variant 4	Variant 8	0.0	79.8	1.0	274.2	0.5828
Variant 4	Variant 9	1.5	80.7	4.5	273.2	0.4960
Variant 4	Variant 10	0.5	79.7	5.5	274.3	>0.9999
Variant 4	Variant 11	0.5	79.8	3.5	274.2	>0.9999
Variant 4	Variant 12	0.5	80.2	4.5	273.8	0.6703
Variant 4	Variant 13	0.5	80.4	3.5	273.6	>0.9999
Variant 4	Variant 14	1.5	80.1	4.5	273.9	>0.9999
Variant 5	Variant 6	0.0	81.3	2.0	272.7	0.6763
Variant 5	Variant 7	0.5	80.4	4.5	273.6	0.6763
Variant 5	Variant 8	0.5	80.6	2.5	273.4	0.2066
Variant 5	Variant 9	1.0	81.0	3.0	272.5	0.2693
Variant 5	Variant 10	0.0	80.4	6.0	273.6	0.6763
Variant 5	Variant 11	0.0	80.6	4.0	273.4	0.5889
Variant 5	Variant 12	0.0	80.9	5.0	273.1	0.2693
Variant 5	Variant 13	0.0	81.2	2.0	272.8	0.6493
Variant 5	Variant 14	1.0	80.8	3.0	273.2	0.5889
Variant 6	Variant 7	0.5	80.4	4.5	273.6	0.5976
Variant 6	Variant 8	0.5	80.6	4.5	273.4	0.5897
Variant 6	Variant 9	1.0	81.5	3.0	272.5	0.5386
Variant 6	Variant 10	0.0	80.4	6.0	273.6	0.5976
Variant 6	Variant 11	0.0	80.6	4.0	273.4	0.6763

Table 1 continued						
Sequence 1	Sequence 2	Number of synonymous differences	Number of synonymous positions	Number of nonsynonymous differences	Number of nonsynonymous positions	Fisher's exact test <i>P</i> value
Variant 6	Variant 12	0.0	80.9	5.0	273.1	0.5897
Variant 6	Variant 13	0.0	81.2	2.0	272.8	0.7734
Variant 6	Variant 14	1.0	80.8	3.0	273.2	0.6763
Variant 7	Variant 8	0.0	79.7	2.0	274.3	0.5035
Variant 7	Variant 9	1.5	80.6	3.5	273.4	0.6441
Variant 7	Variant 10	0.5	79.5	4.5	274.5	0.5976
Variant 7	Variant 11	0.5	79.7	2.5	274.3	0.6763
Variant 7	Variant 12	0.5	80.0	3.5	274.0	0.5828
Variant 7	Variant 13	0.5	80.2	4.5	273.7	0.7734
Variant 7	Variant 14	1.5	79.9	5.5	274.1	0.5437
Variant 8	Variant 9	1.5	80.7	5.5	273.2	0.2744
Variant 8	Variant 10	0.5	79.7	6.5	274.3	0.6823
Variant 8	Variant 11	0.5	79.8	4.5	274.2	0.2066
Variant 8	Variant 12	0.5	80.2	5.5	273.8	0.2743
Variant 8	Variant 13	0.5	80.4	4.5	273.6	0.5897
Variant 8	Variant 14	1.5	80.1	5.5	273.9	0.2066
Variant 9	Variant 10	1.0	80.6	5.0	273.4	0.6441
Variant 9	Variant 11	1.0	81.7	3.0	273.2	0.2693
Variant 9	Variant 12	1.0	81.1	4.0	272.9	0.2066
Variant 9	Variant 13	1.0	81.3	3.0	272.7	0.5386
Variant 9	Variant 14	2.0	81.0	4.0	273.0	0.2693
Variant 10	Variant 11	0.0	79.7	2.0	274.3	0.6763
Variant 10	Variant 12	0.0	80.0	5.0	274.0	0.6441
Variant 10	Variant 13	0.0	80.2	6.0	273.7	0.7734
Variant 10	Variant 14	1.0	79.9	7.0	274.1	0.6763
Variant 11	Variant 12	0.0	80.2	3.0	273.8	0.2693
Variant 11	Variant 13	0.0	80.4	4.0	273.6	0.6493
Variant 11	Variant 14	1.0	80.1	5.0	273.9	0.5889
Variant 12	Variant 13	0.0	80.7	5.0	273.2	0.6763
Variant 12	Variant 14	1.0	80.4	6.0	273.6	0.2693
Variant 13	Variant 14	1.0	80.7	1.0	273.3	0.6493

Table 2 Number of clones analyzed to assess the pattern of PLS1 variant transmission in single-spore cultures of *G. etunicatum*

Culture	Spore	# of clones analysed	Observed variants													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
3-5-4	A	60	+	+	+	+	+	+	+	+	+	+	+	+	+	-
	B	47	+	+	+	+	+	+	+	+	+	+	+	+	+	-
	C	58	+	+	+	+	+	+	+	+	+	+	+	+	+	-
	D	44	+	+	+	+	+	+	+	+	+	+	+	+	+	-
	E	60	+	+	+	+	+	+	+	+	+	+	+	+	+	-
13-4-2	A	46	+	+	+	+	+	+	+	+	+	+	+	+	-	+
	B	178	+	+	+	+	+	+	+	+	+	+	+	+	-	+
	C	45	+	+	+	+	+	+	+	+	+	+	+	+	-	+
	D	47	+	+	+	+	+	+	+	+	+	+	+	+	-	+
	E	61	+	+	+	+	+	+	+	+	+	+	+	+	-	+
21-1-4	A	81	+	+	+	+	+	+	+	+	+	+	+	+	-	+
	B	85	+	+	+	+	+	+	+	+	+	+	+	+	-	+
	C	45	+	+	+	+	+	+	+	+	+	+	+	+	-	+
	D	47	+	+	+	+	+	+	+	+	+	+	+	+	-	+
	E	72	+	+	+	+	+	+	+	+	+	+	+	+	-	+
73-3-4	A	76	+	+	+	+	+	+	+	+	+	+	+	+	-	+
	B	46	+	+	+	+	+	+	+	+	+	+	+	+	-	+
	C	69	+	+	+	+	+	+	+	+	+	+	+	+	-	+
	D	98	+	+	+	+	+	+	+	+	+	+	+	+	-	+
	E	142	+	+	+	+	+	+	+	+	+	+	+	+	-	+
Total		1407*														

* 15 of these clones contained putative PCR errors, 13 were putative PCR-generated chimeras and 38 represented PLS2 variants.

Table 3 Number of clones analyzed to assess the pattern of rDNA ITS1-5.8S-ITS2 variant transmission in single-spore cultures of *G. etunicatum*

Culture	Spore	# of clones analysed	Observed variants			
			1	2	3	4
3-5-4	F	27	+	+	+	+
	G	33	+	+	+	+
	H	32	+	+	+	+
	I	27	+	+	+	+
	J	27	+	+	+	+
5-5-4	A	12	+	+	+	-
	B	14	+	+	+	-
	C	11	+	+	+	-
	D	14	+	+	+	-
	E	15	+	+	+	-
13-4-2	F	14	+	+	+	-
	G	14	+	+	+	-
	H	14	+	+	+	-
	I	15	+	+	+	-
	J	11	+	+	+	-
21-1-4	F	17	+	+	+	-
	G	10	+	+	+	-
	H	28	+	+	+	-
	I	16	+	+	+	-
	J	16	+	+	+	-
73-3-4	F	12	+	+	+	-
	G	12	+	+	+	-
	H	15	+	+	+	-
Total		406*				

* 70 of these clones contained putative PCR errors, 2 were putative PCR-generated chimeras.

Table 4 Number of clones analyzed to assess rDNA ITS1 variation in *G. intraradices* spores

Spore	# of clones analysed	Observed variants			
		1	2	3	4
A	26	+	+	+	+
B	20	+	+	+	+
C	21	+	+	+	+
Total	67*				

* 2 of these clones were putative PCR-generated chimeras.

Table 5 Number of clones analyzed to detect rDNA ITS1 variants 1, 2 and 3 in *G. etunicatum* individual nuclei

Culture	Spore	Nucleus	# of clones analysed	Variant			
				1	2	3	
21-25	A	α	56	+	+	+	
		β	33	+	+	+	
	B	α	15	+	+	+	
		β	13	+	+	+	
		γ	24	+	+	+	
	C	α	8	+	+	+	
	Total			149			

**Table 6 Number of clones analyzed to detect rDNA ITS1 variants
1, 2, 3 and 4 in *G. intraradices* individual nuclei**

Nucleus	# of clones analysed	Variant			
		1	2	3	4
α	37	+	+	+	+
β	20	+	+	+	+
γ	21	+	+	+	+
Total	78				

Table 7 Probability estimates of spore differentiation events due to PLS1 variant loss under the heterokaryosis model

Bottleneck size	Expected frequency of genetically differentiated spores arising within one generation due to the loss of one of the 13 PLS1 variants (Figure 2c) $d (\pm SE)$	Expected frequency of genetically undifferentiated spores (no loss of PLS1 variation within one generation) $u = 1 - d$	Probability that none of the 20 spores representing four single-spore cultures undergoes genetic differentiation u^{20}	Probability that none of the three field isolates undergoes genetic differentiation during four generations of independent propagation* $(u^3)^4$	Probability that none of the three field isolates undergoes genetic differentiation during 80 generations since maize field establishment* $(u^3)^{80}$
10%	0.5643 (± 0.00744)	0.4357	6.1×10^{-8}	4.7×10^{-5}	2.6×10^{-87}
20%	0.3280 (± 0.00845)	0.6720	0.0004	0.0085	3.7×10^{-42}
30%	0.2557 (± 0.00874)	0.7443	0.0027	0.0289	1.6×10^{-31}
40%	0.1934 (± 0.00785)	0.8066	0.0136	0.0759	4.0×10^{-23}

* Probability that all lineages undergo genetic differentiation in an identical manner (by losing the same variant) is negligible.

Table 8 Probability estimates of spore differentiation events due to ITS1-5.8S-ITS2 variant loss under the heterokaryosis model

Bottleneck size	Frequency of genetically differentiated spores arising within one generation due to the loss of one of the three ITS1-5.8S-ITS2 variants d (\pm SE)	Frequency of genetically undifferentiated spores (no loss of rDNA variation within one generation) $u = 1 - d$	Probability that none of the four field isolates undergoes genetic differentiation during 80 generations since maize field establishment* $(u^4)^{80}$
10%	0.0348 (\pm 0.00304)	0.9652	1.2×10^{-5}
20%	0.0335 (\pm 0.00323)	0.9665	1.9×10^{-5}
30%	0.0165 (\pm 0.00212)	0.9835	0.0048
40%	0.0130 (\pm 0.00200)	0.9870	0.0151

* Probability that all lineages undergo genetic differentiation in an identical manner (by losing the same variant) is negligible.

	Nucleotide position																																	
	0	0	0	0	0	0	0	1	1	1	2	2	2	3	3	3	3	3	4	4	4	4	4	4	4	5	5	5						
	0	2	3	3	3	4	6	8	9	9	1	3	6	5	8	9	4	6	7	9	9	2	5	7	8	8	9	0	1	1				
	3	4	1	2	3	1	2	5	2	4	3	5	0	4	7	8	4	8	5	2	3	2	8	5	3	4	5	2	8	5	7			
1	C	G	G	C	A	G	A	C	G	C	T	T	T	C	A	G	T	C	C	A	T	A	G	T	A	T	T	G	G	A	C			
2	.	.	.	T	G	.	G	.	T	.	A	-	.	A	
3	.	.	.	A	G	.	G	A	C	.	.	T	.	A	.	A		
4	.	.	.	G	.	G	A	.	T	-	.	.	.	T	.	A	.	A	
5	.	.	A	.	G	.	G	A	.	T	
6	.	T	.	G	.	G	G	A	-	T	
7	.	.	.	G	A	G	A	.	T	
8	.	.	A	.	G	.	G	A	.	T	
9	.	.	.	G	A	G	G	A	.	T	
10	.	.	T	G	A	G	.	T	T	A	.	T	
11	.	.	T	G	A	G	A	.	T	
12	A	.	.	G	A	G	A	.	T	G	.	.	.	T	.	T	C	.	A	
13	.	.	.	G	.	G	.	A	A	.	T
14	.	.	.	G	.	G	.	A	A	.	A	.	T	.	.	G	.	A	T	C	

Figure S1. Alignment of variable nucleotide positions in PLS1 variants. Dots represent the same nucleotide as in the variant 1. Dashes indicate insertions/deletions. Shaded areas correspond to putative introns.

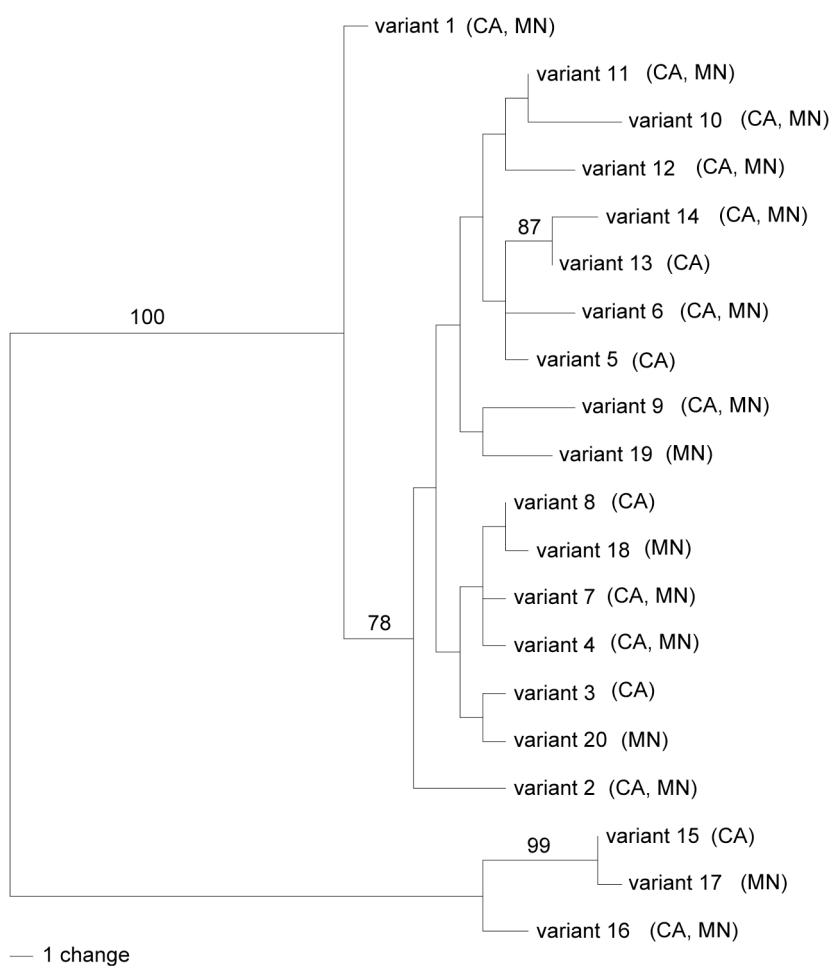


Figure S2. Single most parsimonious phylogram of the PLS variants detected in California (CA) and Minnesota (MN) populations. Numbers above branches represent bootstrap support.