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Inferred origin of several Native American potatoes from the Pacific Northwest and Southeast Alaska using SSR markers

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Abstract Certain Native Americans from the Pacific Northwest and Alaska of the USA have grown potatoes in their gardens for many generations. In this study, the origin of several potatoes collected from Native gardens was investigated. Fourteen SSR markers covering the 12 potato homologs yielding a total of 199 alleles were amplified and scored in *Solanum tuberosum* Group *Andigena* (52 accessions), *S. tuberosum* Group. *Tuberosum* (39 accessions) and wild species (6 accessions). "Ozette" from the Makah Nation on the Olympic Peninsula in Washington State was closely related to "Maria's" and "Kasaan" potatoes collected from Native Alaskan gardens in Southeast Alaska. These three potatoes were more closely related to either two Mexican and one Peruvian andigena accessions or three Chilean Group Tuberosum accessions, while being relatively less related to the old European or modern varieties and most distantly related to Group Andigenum. "To-Le-Ak" was closely related to two Chilean tuberosum accessions and one old European variety. All Native potatoes harbored T-type chloroplast genome indicating that their maternal lineage is shared with Chilean Group Tuberosum. Using genetic relationship as a guide to origin it appears plausible that the Native American/Alaskan cultivars are either directly or

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indirectly from Mexico and Chile. These Native potato cultivars present a possible second route of diffusion distinct from the South America to Europe transfer which has been assumed to the sole means by which potato was spread out of South America.

Keywords *Solanum tuberosum* · Group Andigenum · Chilean potato · Chloroplast genome · Simple sequence repeats · Phylogenetics · Makah · Quillayute · Haida · Tlingit · Ozette · Kasaan · Maria's · To-Le-Ak

Introduction

The potato was first cultivated by the natives of Peruvian and Bolivian Andes more than six thousands years ago (Hawkes 1990). The genetic patterns of potato distribution indicate that the potato probably originated in the mountainous west-central region of the continent. The latest papers by Spooner et al. (2005a, b) and Spooner and Hetterscheid (2005) conclude that there was a single origin of the cultivated potato in Northern Bolivia and Southern Peru The archaeological remains date from 4000 BC and have been found on the shores of Lake Titicaca (Hawkes 1990).

The Spanish explorers were the first Europeans to come into contact with potatoes after they arrived in Peru in 1532. They carried potatoes back to Spain around 1570. From Spain, potatoes slowly spread to Italy and other European countries. In 1845, late blight was introduced to Europe and decimated the varieties of the time. Another introduction took place, in 1861 in the form of Rough Purple Chili, a clone obtained by the reverend Chauncey Goodrich of New York State. The cultivars Russet Burbank and Early Rose were derived from this and the latter has had a pervasive ancestral contribution to potatoes bred in the late 1800s and the following century (Bryan et al. 1999). It has been assumed that "Rough Purple Chili" originated from the long-day-adapted Group *Tuberosum* of Chile. Today potatoes extant in the long day temperate or highland tropical areas of the world outside of the Andes resemble Chilean potato Group *Tuberosum*. The most important genetic marker supporting this is the chloroplast genome in which a 242 bp deletion is shared by the Chilean potato and all non-Andean potatoes (denoted T-cytoplasm). As a consequence two theories of the origin of the potato diaspora have existed for decades. One purports that central Andean potato was taken to Europe whereupon it evolved into a long day adapted form and has spread widely throughout the world. The conversion to the Chilean chloroplast genome is explained as occurring later when Chilean potato became the sole cytoplasmic donor, perhaps mainly due to the introduction of Rough Purple Chili into the breeding pool. The T-cytoplasm is often associated with male sterility, ensuring maintenance of the original maternal line. The second hypothesis simply states that Chilean potato was taken to Europe and is the sole progenitor of present day long-day-adapted varieties.

Potatoes were first introduced to North America in the 1620s when the British governor of the Bahamas sent a gift box of *Solanum tuberosum* to the governor of the colony of Virginia. While they spread throughout the northern colonies in limited quantities, potatoes did not become widely accepted. Later the potato continued its long geographical and evolutionary journey, carried by Scottish and Irish settlers to North American colonies in the 17th century. The first permanent North American potato patches were established in New England around 1719.

For thousands of years, the Makah Nation has made its home on the Northwest corner of the Olympic peninsula, in present-day Washington State bordered by the Pacific Ocean on the west, and by the Strait of Juan de Fuca on the north and northeast. Originally there were five distinct villages, but presently most Makah live in and around Neah Bay. They have grown "Ozette" potatoes in their gardens for many generations. In addition, the "To-Le-Ak" potato was grown by the Quillayute Nation of La Push, Washington on the Olympic Peninsula, "Maria's Potato" by the Tlingit Nation of Alaska, and lastly "Kasaan" by the Haida living in Kasaan, Alaska. Historical accounts indicate that the Makah/Ozette potato has been present in their gardens for over two hundred years (Swan 1868; MacDonald 1972; Wagner 1933; Suttles 1951; Gill 1983; Kirk and Alexander 1990). Determination of origin may add considerably to our knowledge of diffusion of potato from South America to the rest of the world.

Simple sequence repeats (SSRs) have been observed in a wide range of genomes, including mammals, birds, insects, fish and plants (Zane et al. 2002). SSR markers have been applied to the genetic

study of many plant species, including potato. The first generation of SSRs in potato was obtained from the identification of specific repeat motifs in gene sequences (Veilleux et al. 1995; Kawchuk et al. 1996; Provan et al. 1996; Schneider and Douches 1997). The second wave of SSRs came from screening genomic libraries enriched for repeat motifs (Milbourne et al. 1998). More recently, as reported in the present publication, the search for repeat motifs within expressed sequence tags (ESTs) from potato showed that 5% of ESTs evaluated contained SSRs. In this study, we used a highly informative and user-friendly set of SSRs.

Chloroplast DNA (cpDNA) restriction site data documented several chloroplast genotypes in *S. tuberosum*, which included Groups *Tuberosum* and *Andigena*. Group *Andigena* has all five types and native Chilean subsp. *tuberosum* has three types: A, T, and W (Hosaka and Hanneman 1988). The most frequently observed type in Chilean Group *Tuberosum* is T, which is characterized by a 241-base-pair deletion (Kawagoe and Kikuta 1991).

In this study, the origin of several potatoes, including two potatoes from Native Americans, "Ozette" (Makah Nation) and "To-Le-Ak" (Quillayute Nation), and two potatoes from Native Alaskans, Kasaan (Haida Nation), and Maria's Potato (Tlingit Nation), were fingerprinted using 14 SSR markers covering the 12 potato chromosomes.

Materials and methods

Plant material and DNA extraction

We sampled a set of 97 potato accessions representing *Solanum tuberosum* Group A*ndigena* (52 accessions), *S. tuberosum* Group *Tuberosum* (38 accessions) and several wild species ranging from South America to North America. Included in Group *Tuberosum* were old European, old American and cultivars and breeding lines bred in recent times. A number of native Mexican varieties were also included. We included 6 accessions of wild species or cultivated-wild species hybrids to serve as an outgroup for rooting phylogenies. The complete information for the plant materials, including landrace designation, germplasm bank accession numbers, and geographical information for some cultivated potatoes are listed in Table 1.

DNA was extracted from the plants, which were germinated from the seeds requested from NRSP-6 (United States Potato Genebank) at Sturgeon Bay, WI or from clonally propagated materials. DNA was extracted using a modified CTAB method (Doyle and Doyle 1987; Bonierbale et al. 1988; Gebhardt et al. 1989; Sosinski and Douches 1996). Approximately 0.2 g of young leaf tissue was harvested into 1.5-ml Eppendorf tubes held in racks suspended above liquid nitrogen. The frozen tissue was then crushed with glass rods before addition of extraction buffer. Two hundred micro liter of extraction buffer was added to the frozen tissue, and the racks containing the tubes were placed at room temperature until the extraction buffer thawed. Samples were then ground for about 5 s each with a power drill fixed with a plastic bit, rinsing the bit between samples. After grinding, an additional 550 µl of extraction buffer was added, samples were mixed, and then placed in a 65°C water bath for 20–60 min. Tubes were removed from the water bath, mixed, filled with a 24:1 mixture of chloroform and isoamyl alcohol (550–600 µl) and then placed on a shaker for 5 min. After mixing, tubes were centrifuged at 13,000 rpm for 10 m and the supernatant was removed with a pipette and placed into a new Eppendorf tube, where it was mixed with 2/3 the volume of cold isopropanol. The tubes were inverted repeatedly to precipitate the DNA, followed by another centrifugation at 13,000 rpm for 12 min to pellet the DNA. The supernatant was discarded, and the pellet was washed with 800 µl of cold 70% (v/v) ethanol, precipitated by centrifugation and dried. The pellet was re-suspended in 50 µl of TE buffer in a 65°C water bath.

SSR sequences and amplification conditions

Fourteen SSR primer pairs that covered 12 potato chromosomes and revealed high polymorphism according to Ghislain et al. (2004) were used in this study. These SSR sequences were identified through potato database searches (Provan et al. 1996), enriched genomic libraries (Milbourne et al. 1998) and expressed sequence tags developed at the Scottish Crop Research Institute, Invergowrie, UK. The degree of applicability across cultivar groups and the polymorphic index content (PIC) of SSRs were used to select a highly informative set of SSRs for cultivated potato fingerprinting and phylogenetic studies.

Table 1 DNA samples included in this study

NO	Name	Taxon (as reported in NRSP-6 database)	Origin	Notes	Chloroplast marker
1	160373	*S. andigena*	Mexico		T
2	161131	*S. andigena*	Mexico		A
3	161348	*S. andigena*	Mexico		A
4	161677	*S. andigena*	Mexico		T
5	161683	*S. andigena*	Mexico		T/A
6	161695	*S. andigena*	Mexico		T
7	161716	*S. andigena*	Mexico		A
8	161771	*S. andigena*	Mexico		A
9	186177	*S. andigena*	Peru		A
10	189473	*S. andigena*	Mexico		A
11	197757	*S. andigena*	Bolivia		A
12	197932	*S. andigena*	Colombia		A
13	205388	*S. andigena*	Argentina		T
14	214434	*S. andigena*	Peru		A
15	225635	*S. andigena*	Venezuela		A
16	230470	*S. andigena*	Ecuador		A
17	233980	*S. andigena*	Bolivia		A
18	234001	*S. andigena*	Bolivia		A
19	243361	*S. andigena*	Columbia		A
20	243400	*S. andigena*	Ecuador		A
21	243429	*S. andigena*	Colombia		A
22	243436	*S. andigena*	Colombia		A
23	255491	*S. andigena*	Bolivia		A
24	279291	*S. andigena*	Guatemala		T
25	280907	*S. andigena*	Argentina		A
26	280968	*S. andigena*	Bolivia		A
27	281032	*S. andigena*	Bolivia		A
28	281033	*S. andigena*	Mexico		A
29	281105	*S. andigena*	Peru		T
30	281119	*S. andigena*	Peru		A
31	281186	*S. andigena*	Peru		A
32	281233	*S. andigena*	Peru		A
33	281245	*S. andigena*	Peru		A
34	285019	*S. andigena*	Mexico		A
35	285023	*S. andigena*	Mexico		A
36	292073	*S. andigena*	Peru		A
37	292078	*S. andigena*	Peru		A
38	292089	*S. andigena*	Peru		A
39	292101	*S. andigena*	Peru		A
40	292128	*S. andigena*	Bolivia		T
41	306302	*S. andigena*	Guatemala		A
42	306303	*S. andigena*	Guatemala		T
43	307743	*S. andigena*	Mexico		A

Table 1 continued

NO	Name	Taxon (as reported in NRSP-6 database)	Origin	Notes	Chloroplast marker
44	324454	*S. andigena*	Mexico		T
45	324461	*S. andigena*	Mexico		A
46	365402	*S. andigena*	Mexico		A
47	473271	*S. andigena*	Argentina		A
48	473296	*S. andigena*	Argentina		A
49	473390	*S. andigena*	Bolivia		A
50	545744	*S. andigena*	Mexico		T
51	546018	*S. andigena*	Bolivia		A
52	703606	*S. andigena*			
53	595453	*S. tuberosum*	Chile		T
54	595458	*S. tuberosum*	Chile		T
55	595459	*S. tuberosum*	Chile		T
56	595460	*S. tuberosum*	Chile		T
57	700313	*S. tuberosum*	Chile		
58	A77715-5	*S. tuberosum*		USDA/ARS, Prosser, WA Breeding line	T
59	A89875.5	*S. tuberosum*		USDA/ARS, Prosser, WA Breeding line	T
60	Atlantic	*S. tuberosum*		Modern Variety	T
61	Blueberry Ripples	*S. tuberosum*		American Heirloom	T
62	Bannock	*S. tuberosum*		Modern Variety (Bannock Russet)	T
63	Chilean Aucud	*S. tuberosum*	Chile	Chilean cultivar	T
64	EDY 12-4	*S. tuberosum*		Eersteling-Duke of York (old variety-1891)	T
65	EO 34-11	*S. tuberosum*		Early Ohio (old variety-1871)	T
66	ER 34-7	*S. tuberosum*		Early Rose (old variety-1861)	T
67	GEM	*S. tuberosum*		Gem Russet (Modern Variety)	T
68	GM 34-4	*S. tuberosum*		Green Mountain (old variety-1875)	T
69	Haida	*S. tuberosum*	New Massett, Queen Charlotte Is., Canada	= Ozette	T
70	Irish Cobbler	*S. tuberosum*		Irish Cobbler (old variety bred in 1876)	T
71	Johnny Gunther	*S. tuberosum*		Oregon heirloom	T
72	PA99P20-2	*S. tuberosum*		USDA/ARS, Prosser, WA Breeding Line	T
73	PL-17 Bzura	*S. tuberosum*		Polish Variety	A
74	PL-10 Cisa	*S. tuberosum*		Polish Variety	A
75	PL11 Frezja	*S. tuberosum*		Polish Variety	T
76	R4	*S. tuberosum*		R4 gene	A
77	Ranger Russet	*S. tuberosum*		Modern Variety	T
78	RBI	*S. tuberosum*		Old variety Russet Burbank-Idaho	T
79	TRI 19-10	*S. tuberosum*		Triumph (old variety-1877)	T
80	Uma	*S. tuberosum*		Umatilla Russet (modern variety)	T
81	Mak(ID)	*Native*		Ozette in Idaho	T
82	Mak 1.2	*Native*	Neah Bay	Ozette collected in Neah Bay	T

Table 1 continued

NO	Name	Taxon (as reported in NRSP-6 database)	Origin	Notes	Chloroplast marker
83	Mak 2.2	*Native*	Neah Bay	Ozette collected in Neah Bay	T
84	OZ (Gilmore)	*Native*	Reno, Nevada	Ozette in Nevada	T
85	OZ (Kirk)	*Native*	Lacey WA	Ozette in Washington	T
86	OZ (Victoria)	*Native*	Victoria B.C. Canada	Ozette on Vancouver Island, Canada	T
87	Ozette	*Native*	Neah Bay	Ozette, Olympic Peninsula, Washington State	T
88	SB (OZ)	*Native*	NRSP-6 Sturgeon Bay, WI	Ozette in Wisconsin	T
89	Kasaan	*Native*	Kasaan, Alaska	Haida Nation, southeast Alaska	
90	Maria's Potato	*Native*	Juneau, Alaska	Tlingit Nation, southeast Alaska	T
91	To-Le-Ak	*Native*	Oil City, WA USA	Quillayute Nation, Olympic Peninsula, Washington State	T
92	SB22	*S. bulbocastanum*		2n = 24	A
93	95H3.3	*S. hjertingii* hybrid		2n = 36	A
94	95A2.8	*S. hougasii*		2n = 72, Wild species parent	A
95	96A2-1	*S. hougasii*		2n = 60, Hybrid	A
96	91E22	*S. phureja*		2n = 24	A
97	EGA9706-14	*S. phureja*		2n = 24, Polish Breeding Line, IHAR, Młochow, Poland	A

PCR reactions were performed in a 10 µl volume containing 5 µl 2 × CLP TAQ master mix a final magnesium concentration of 1.5 mM (CLP, San Diego, CA), 0.5 µM of each primer (forward and reverse), (Integrated DNA Technologies, Coralville, IA) and 10 ng of DNA templates. PCR was carried out in a PTC-200 thermocycler (MJ Research Inc., Watertown, Mass.), set to the following program: 3 min at 94°C, 2 min at annealing temperature (T a), 1 min 30 s at 72°C, 29 cycles of 1 min at 94°C, 2 min at T a, and 1 min 30 s at 72°C, with a final extension step of 5 min at 72°C. In some cases (indicated as Td.60–50 in Table 2), a modified PCR program was used: 3 min at 94°C, 16 double cycles of 1 min at 94°C, 2 min at 60°C, 1.5 min at 72°C, and 1 min at 94°C, 2 min at 50°C, 1.5 min at 72°C and one final elongation cycle of 5 min at 72°C.

The microsatellite regions were amplified by PCR with florescent-labeled primers. The PCR products labeled with 6-FAM were analyzed on an Applied Biosystems automated Genetic Analyzer (ABI 3100). PCR samples were prepared by combining 1 µl of the PCR product with 11.2 µl deionized formamide, 0.5 µl loading dye, and 0.3 µl GENESCAN 500-TAMRA size standard (Perkin Elmer/Applied Biosystems). After denaturing at 90°C for 3 min, 0.8 µl of the sample was loaded into 96 well format plates. Electrophoresis was performed with the Performance Optimized Polymer 4 (POP-4TM, PE Applied Biosystems). The auto-sampler was calibrated after setting the temperature at 60°C. Denatured working samples were transferred to sample tubes and covered with septa before placing them on the sample tray. The injection time was 5 s at 15 kV and run time was 24–36 min at 15 kV. Fragment Analysis SSR fragment sizing was performed by the "Local Southern Method" and default analysis settings of the GeneScan (Perkin Elmer/Applied Biosystems). Size standard peaks were defined by the user. Allele calling was performed with Genotyper software, version 2.5 (Perkin Elmer/Applied Biosystems). The precise size of the SSR was determined for each individual.

Chloroplast marker

The T-type chloroplast DNA was distinguished by the presence of a 241 bp deletion from the A-type chloroplast DNA found among the Andean potatoes

Table 2 The SSR primers used for this study

SSR ID	Repeat	Sequences	AT	Chrom	Copies	Type	Number of alleles	Size
STM1049	(ATA)6	CTACCAGTTTGTTGATTGTGGTG AGGGACTTTAATTTGTTGGACG	57	I	>1	3' UTR	9	184–254
STM2022	(CAA)3…(CAA)3	GCGTCAGCGATTTCAGTACTA TTCAGTCAACTCCTGTTGCG	53	II	>1	Intergenic	13	184–244
STM1053	(TA)4 (ATC)5	TCTCCCCATCTTAATGTTTC CAACACAGCATSCAGATCATC	55	III	1	3' UTR	7	168–184
STM3023	(GA)9 (GA)8 (GA)4	AAGCTGTTACTTGATTGCTGCA GTTCTGGCATTTCCATCTAGAGA	50	IV	1	Intergenic	5	169–201
STPoAc58	(TA)13	TTGATGAAAGGAATGCAGCTTGTG ACGTTAAAGAAGTGAGAGTACGAC	57	V	1	3' UTR	13	203–277
STM0019	(AT)7(GT)10(AT)4(GT)5 (GC)4 (GT)4	AATAGGTGTACTGACTCTCAATG TTGAAGTAAAAGTCCTAGTATGTG	47	VI	1	Intergenic	27	155–241
							10	83–124
STM0031	(AC)5… (AC)3(GCAC) (AC)2(GCAC)2	CATACGCACGCACGTACAC TTCAACCTATCATTTTGTGAGTCG	57	VII	1	Intergenic	11	155–205
STM1052	(AT)14 GT (AT)4(GT)6	CAATTTCGTTTTTTCATGTGACAC ATGGCGTAATTTGATTTAATACGTAA	Td.60–50	VII	1	Intron	16	212–268
STM2013	(TCTA)6	TTCGGAATTACCCTCTGCC AAAAAAAGAACGCGCACG	55	VII	2	Intergenic	20	146–172
STM1104	(TCT)5	TGATTCTCTTGCCTACTGTAATCG CAAAGTGGTGTGAAGCTGTGA	57	VIII	1	3' UTR	17	164–185
STM1106	(ATT)13	TCCAGCTGATTGGTTAGGTTG ATGCGAATCTACTCGTCATGG	55	X	1	Intron	15	131–197
STM3012	(CT)4, (CT)8	CAACTCAAACCAGAAGGCAAA GAGAAATGGGCACAAAAAACA	57	IX	1	Intergenic	8	168–213
STM0037	(TC)5 (AC)6 AA (AC)7 (AT)4	AATTTAACTTAGAAGATTAGTCTC ATTTGGTTGGGTATGATA	53	XI	1	Intergenic	13	75–125
STM0030	Compound (GT/GC)(GT)8	AGAGATCGATGTAAAACACGT GTGGCATTTTGATGGATT	53	XII	>1	Intergenic	15	122–191

(Hosaka et al. 1988; Kawagoe and Kikuta 1991). PCR amplification was performed as described previously (Hosaka 2002). PCR products from were separated by electrophoresis in 2% agarose (Fisher Scientific) gels.

Data analysis

Presence or absence of each SSR fragment was coded as "1" and "0", where "1" indicated the presence of a specific allele and "0" indicated its absence. Genetic diversity for each locus was then calculated by D_{SA} (Chakraborty and Jin 1993). We used the proportion of shared alleles distance that is free of the stepwise assumption. We used the FITCH program in the PHYLIP package with the log-transformed proportion of shared alleles distance (Felsenstein 1993). Genetic similarities between pairs of accessions were measured by the DICE similarity coefficient based on the proportion of shared alleles (Dice 1945; Nei and Li 1979). The Dice similarity coefficient = 2a/(2a + b+c), where a is the number of positive matches (presence of a band in both accessions), and b + c is the number of no matches (presence of a band either in one accession but absent in the other accession). The accessions were clustered based on a similarity matrix using an unweighted pair group method with the arithmetic average (UPGMA) algorithm. The result was used to construct a dendrogram with the TREE module. Principal components analysis for the SSR data was conducted using the NYSYSpc 2.2 and plotted using Mod3Dplot in the NTSYSpc (Rohlf 2007). The first and second principal components were plotted with identifiers relating to the major clusters seen on the UPGMA dendrograms.

Results

In this study, a total of 199 alleles were amplified and scored in a set of 97 *Solanum tuberosum* Group A*ndigena*, *S. tuberosum* Group *Tuberosum* and wild species. Amplification of the genomic DNA from these potato cultivars with fourteen SSR primer sets produced fragments ranging in size from 66 to 260 bp from 26 different loci. The number of amplified fragments was dependent on the cultivar and primer set. The total number of microsatellite alleles varied from the lowest of 28 in Irish Cobbler (Group *Tuberosum*) to the highest of 62 in PI 306303 (Group *Andigena;* from Guatemala), with the mean number of alleles per cultivar of 41. The number of amplified fragments detected by individual primer sets varied from 5 to 27. A minimum of 5 alleles were amplified with primer set STM3023, while primer set STM0019 amplified a maximum of 27 alleles from 4 loci.

The estimated genetic distance between the cultivars as calculated using Log-Shared-Allele using PHYLIP and ranged from 0.43 between EGA970614 and PI306303 to 0.02 between PI595458 and PI595459. High genetic distance values suggested a further genetic base of the cultivars tested in the present study. All 97 cultivars could be grouped into three major groups as shown in the dendrograms (Figs. 1, 2). None of the primer sets could distinguish between all 97 cultivars singly.

The phylogenetic analysis showed that Group *Andigena* was separated from Group *Tuberosum*, with some exceptions (Huamán and Spooner 2002). The wild species formed a well-defined outgroup. "Ozette" from the Makah Nation on Olympic Peninsula in Washington State was most closely related to "Maria's" and "Kasaan" potatoes collected from Native Alaskan gardens. These three potatoes, "Ozette", "Maria's" and "Kasaan", were least closely related to Central Andean cultivars, but were more closely related to either two Mexican and Peruvian *Andigena* accessions or three Chilean *Tuberosum* accessions, and less closely related to old European old American or modern varieties. They appear to be less related to most of the accessions from the Andes (i.e. Group. *Andigena*). "To-Le-Ak" was not closely related to either "Ozette" or "Maria's" or the "Kasaan" cultivars. "To-Le-Ak" was closely related to two Chilean *Tuberosum* accessions and one old European variety.

There are two types of ctDNA revealed in this study by using the PCR primer from Hosaka (1995). Among 97 accessions, 51 A-type, 44 T-types and 3 undecided were found respectively. For *Andigena* accessions, 41 A-type and 11 T-type of ctDNA were found. For Group *Tuberosum* accessions, 33 T-type and 3 A-type of ctDNA were found. In the large *Andigena* clade, all possessed A-type. All the wild species included in this study were A-type. However, in the Tuberosum clade, A-and T-types were present. Furthermore, Group *Andigena* with T-type of ctDNA were all co-related in the second clade of the Group *Tuberosum*. Mexican accessions were assigned to

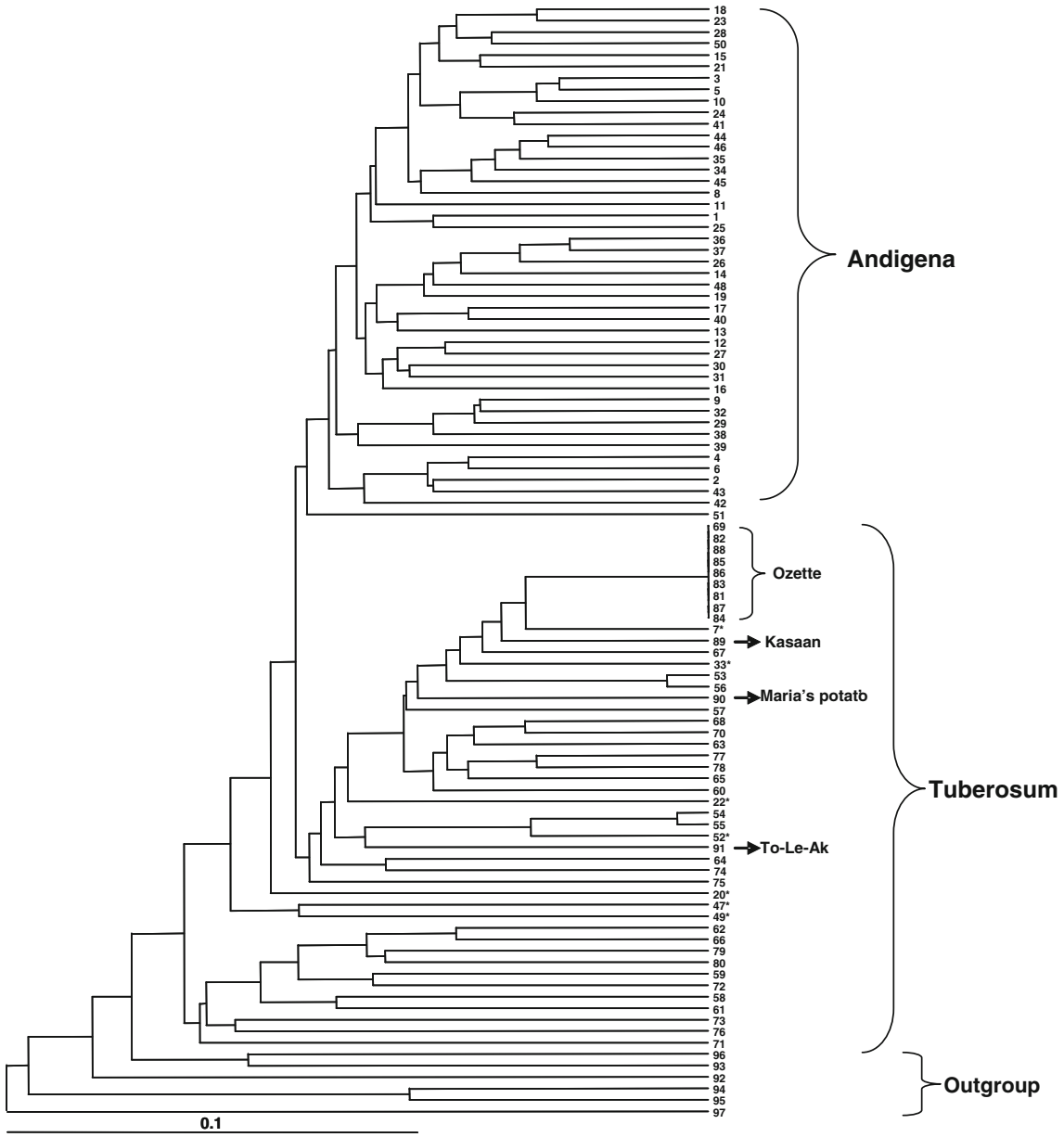

Fig. 1 The UPGMA tree resulting from phylogenetic analysis (Log-Shared-Allele in Phylip) of 97 *Solanum tuberosum* Group *Andigena*, *S. tuberosum* Group *Tuberosum* and wild species (outgroup) using 14 SSR markers

both A-type and T-types. All Native potatoes in this study all were T-type.

Discussion

Groups *Tuberosum* and *Andigena* are not strongly differentiated genetically and attribution of relationship to one or other is often difficult to support.

Recently Spooner (2005a) concluded that there was a single origin of the cultivated potato represented today by assigning certain wild species in Southern Peru and Northern Bolivia, as ancestors of monophyletic cultivated potato origin. Native cultivated potatoes or landraces are distributed widely in the Andes, although the long day adapted Chilean cultivars are supposed to be derived from secondary hybridization with most likely Bolivian and/or

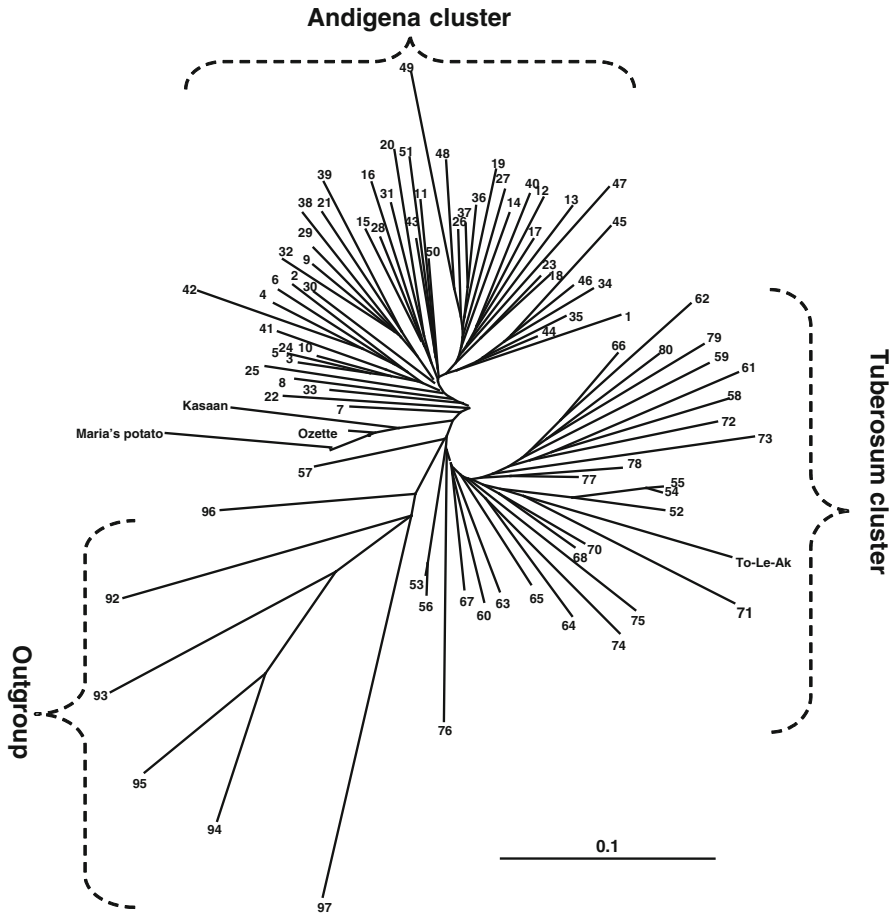

Fig. 2 The un-rooted phylogeny for 98 potato accessions using the Fitch-Margoliash method and the log-transformed proportion of shared distance (PHYLIP) of 97 *Solanum tuberosum* Group *Andigena*, *S. tuberosum* Group *Tuberosum* and wild species (outgroup) using 14 SSR primers (199 alleles)

Argentinean wild species. All previous hypotheses had proposed that the cultivated potato had developed in a number of different points from a variety of wild species. The native Chilean cultivars and the European cultivars are very similar, not only morphologically but also in their photoperiodic response. Grun and Staub (1979) originally found that the cytoplasmic constitution of Groups *Andigena* and *Tuberosum* was different, as expressed in the types of male sterility. Grun (1990) suggested that *Group Tuberosum* was distinct from Group *Andigena* based on cytoplasmic sterility factors, geographical isolation, and ecological differences. Hawkes (1990) distinguished the two subspecies by subsp. *tuberosum* having fewer stems with foliage aligned at a broad angle to the stem and having less-dissected leaves with wider leaflets and thicker pedicels. Raker and Spooner (2002) showed that Chilean potato is distinct, but still closely related to Group *Andigena* based on the SSR markers. In a separate study these researchers surveyed an assortment of heirloom potato varieties from India considered to be remnants of some of the first potatoes introduced to Europe and transferred to India during the time of the British Colonial control (Spooner et al. 2005b). They found that these descendants share specific molecular traits, including SSR's and a cytoplasmic marker that establish a closer relationship with potatoes from Chile than with Central Andean potatoes (Group *Andigena*).

In our study the phylogenetic tree divided Tuberosum and Andigena into two distinct clades. Three of the Native potatoes, "Ozette," "Maria's," and "Kasaan" fall into an intermediate position in three methods of analysis, the phylogenetic tree (Fig. 1), the unrooted phylogenetic tree (Fig. 2), and Principal Component Analysis (Fig. 3). Based on the three analyses these three clones are more closely related to several

Mexican and Chilean clones. All four clones have T cytoplasm, a fact that argues against a Central Andean origin. Their lack of similarity to the Andigena clade argues that these clones did not come from the central Andes. Thus it is likely that these three cultivars were transported on Spanish ships originating from the Port of San Blas, New Spain (e.i., modern Mexico), originating from Mexican cultivators, who had probably received them from Chile in the past. It is also possible that they came directly from Chile. However, the time that would be required to travel from Chile to the Pacific Northwest and Southeast Alaska argues against this. The Native cultivar "To-Le-Ak" falls into the Tuberosum clade and is not related to Ozette, Maria's or the Kasaan potato. The four abovementioned Native potatoes all had T-cytoplasm. It should also be noted that the Mexican Collections denoted *S. tuberosum* ssp. *andigena* in the NRSP-6 collection were a mixture of A and T type cytoplasm. This connotes that Mexico may have received cultivars from the Central Andes and Chile which existed sympatrically into the Twentieth Century, when the collections of NRSP-6 were made.

European contact with the Native Americans of the Pacific Northwest started from the beginning of the European occupation of the Western Hemisphere. Both Spanish and English mariners made landfall along the Pacific coast. The Manila route, taken by Spanish ships, consisted of voyages from the Pacific coast of Mexico to Asia, with landfall on the North American coast often occurring extremely far north of New Spain (Mexico). A southward coastal route would then be used to return to Mexico. The last half of the eighteenth century saw successful voyages up the North American coast and to Alaska in some cases. A Spanish fort was established and maintained for several months in 1792 at Neah Bay by Salvador Fidalgo (Wagner 1933; Cutter 1991). Apparently a garden had been planted the year before at the Spanish settlement of Nootka Bay, and was reported to contain potatoes among other vegetables (Wagner 1933). In the same year (1792), a Spanish (native born in the Mexico of today) naturalist, Jose Mariano Moziño accompanied the expedition of Juan Francisco de la Bodega y Quadra, and listed *Solanum tuberosum* on Vancouver Island in the report emanating from his study (Moziño 1991). James Swan, a naturalist and schoolteacher of the Makah Nation in the 1860s also mentioned the potato as a staple of their diet (Swan 1868; MacDonald 1972). Evidence also exists for the early dissemination of the potato throughout the land bordering the Strait of Juan de Fuca. A Makah word for potato, *qa·wic* (roughly pronounced "kaw-weech"), possibly referred originally to a native root, *Sagittaria*, and various forms of *qa·wic* are found in Coastal Salish languages of the region (Gill 1983).

Anthropologist Steven J. Gill reported that "Ozette" was formerly grown at the Ozette village and by almost everyone at Neah Bay and supplied to schooners by local residents. The Makah have been growing it for so long that some consider it a traditional food. Like the Makahs, the Haidas in the

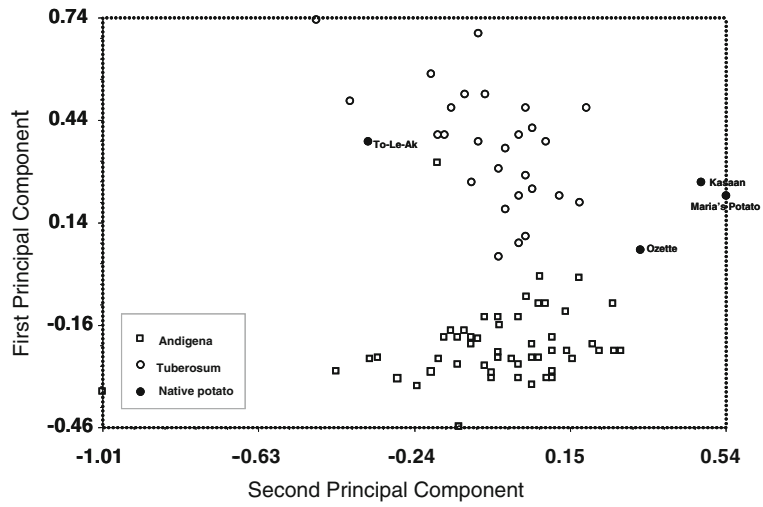

Fig. 3 The first and second principal components of the SSR fingerprint data for all of the potato clones presented based on the clustering in the dendrogram shown in Fig. 1

Queen Charlotte Islands, also grew potatoes. Dr. Nancy J. Turner, an ethnobotanist whose work deals with native peoples of British Columbia, writes that the potato was a staple crop for the Haidas by the mid-1880s (Turner 1975). Turner also reported that the potato was an early agricultural commodity, traded with vessels and others on the land (Turner 1995). Haida villagers were contracted by Russian fur seal fleets to produce potatoes for them in the early 1800s (Gibson 1999).

The Haida of Alaska and western Canada tell similar stories of pre-Columbian traffic in potatoes. These stories state that the Haida grew ancient varieties, which they have traded for centuries with northwest Pacific islanders and inhabitants on the Russian mainland. Their oral history traces the origin of one of these varieties to "Baylu" thought to be a variation of Perú (Turner 1995).

The Makah potato was collected and placed in the Potato Introduction Station Collection at Sturgeon Bay, Wisconsin in 1988. There were also several "Ozette" potatoes obtained from different sources included in this study. By using SSR marker, it was shown they are genetically identical. In this study, potato known by the name "Haida," derived from Haida gardeners on the Queen Charlotte Islands, Canada, was also identified as "Ozette."

The SSR markers and limitation of SSR markers

Microsatellites are often useful for only closely related germplasm sources, and even moderately divergent cross-species amplification can lead to false positives and provide significant distortion in genetic similarity estimates (Peakall et al. 1998; Westman and Kresovich 1998). This was demonstrated in potato when SSRs developed for modern cultivars worked very well in a cultivated species gene pool (Raker and Spooner 2002) but produced limited amplification and clearly distorted phylogenetic information in germplasm from another phylogenetic clade of tuber-bearing *Solanum* (Lara-Cabrera and Spooner 2003). However, once SSRs are identified, their high allele and genetic information content make them a highly desirable system for fingerprinting large collections of related accessions, and the system also is amenable to automation (Mitchell et al. 1997).

Although SSRs are useful for phylogenetic study, it appears there is no consensus among researchers as to which evolutionary model is most appropriate for reconstructing phylogenies based on microsatellite data (Feldman et al. 1999; Goldstein and Pollock 1997). Trees of *S. tuberosum* (Grun 1990; Miller and Spooner 1999) were constructed using both the SMM model (Goldstein et al. 1995a, b) and the IAM model of Nei (1972). Both models failed to absolutely distinguish subsp. *andigena* from subsp. *tuberosum*, or from the other cultivated species. Neither method will clearly separate subsp. *andigena* from some of its in-group relatives in the *S. brevicaule* complex and other cultivated species. Obtaining a reliable phylogeny requires a genetic distance measure that fits the pattern of mutation displayed by the microsatellites. Therefore, we used the proportion of shared alleles distance that is free of the stepwise assumption, and is widely used with multilocus microsatellite data (Matsuoka et al. 2002). We used the FITCH program in the PHYLIP package with the log-transformed proportion of shared alleles distance as implemented in the program to construct phylogenetic trees. This approach has been successfully applied in maize (Matsuoka et al. 2002). Because many microsatellites of potato and other species do not evolve in a stepwise manner, they violate the assumptions for the genetic distance measures that are based on the stepwise mutation model.

The Chloroplast marker

Two known types of ctDNA were assigned to cultivated potatoes as reported previously (Hosaka 1995). The major types were found A and T among cultivated potatoes. All the other wild species were polymorphic with W-, C-, S- or A-type ctDNA. However, only Type-A was found among the wild species included in this study. The Mexican cultivated supposedly all belong to T-type. However, in this study, the Mexican accessions were a mixture of both A- and T- types.

Origin of the Native Indian potatoes

Since the potato came to the American colonies with Scottish and Irish immigrants in the early 17th century (having made a long geographical and evolutionary journey from its Andean birthplace), it

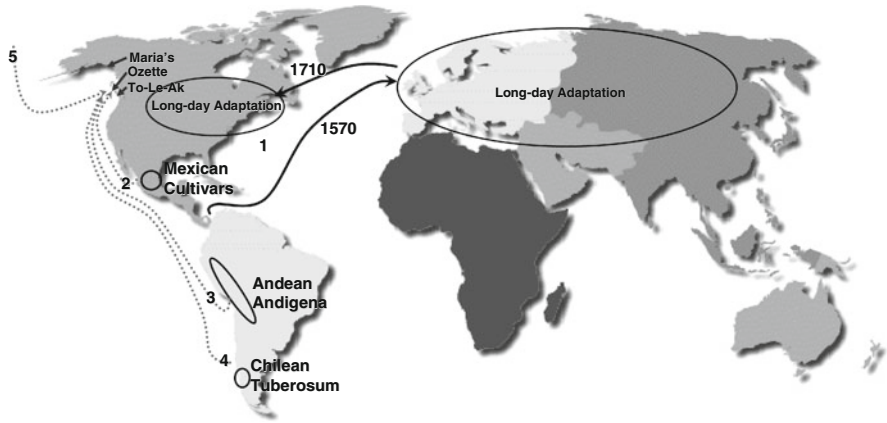

Fig. 4 The potato's round-trip journey from the New World (South America) to the Old World (Europe) and back to North America. Possible sources of the "Ozette," "Kasaan," "To-Le-Ak" and "Maria's" cultivars: (1) North American colonies and states; (2) Mexico; (3) Andes; (4) Chile; (5) Eastern Russia

is a virtual certainty that Native Indian potato comes from a different foreign donor because they are different from the old European cultivated potatoes based on the SSR results from this study. But who first gave them the potato, and where did this one originate? "Ozette," "Kasaan," and "Maria's" potatoes originated from sources other than the old and modern European, and North American, and Central Andean cultivars. Originating from Mexican and Chilean sources is not difficult to explain considering the trade along the Pacific Coast of South and North America that was carried on for centuries. However, Spanish explorers did not succeed in going directly north and safely returning until the latter half of the eighteenth century due to prevailing northerly winds in the boreal summers. SSR data identified certain links; however, there are still gaps between them. To answer these questions, additional data and more powerful molecular analysis are needed.

In conclusion, three Native American and Native Alaskan potatoes (Ozette, Kasaan and Maria's) appear to be closely related to Mexican and Chilean cultivars, and less aligned with Central Andean, or various groups of cultivars bred outside of South America. Among the five possible routes (*dash lines in* Fig. 4), Mexico and Chile were the most plausible sources for the "Ozette" and "Maria's" and the "Kasaan" cultivars based on this phylogenetic study and other historical evidence. "To-Le-Ak" falls into the *Tuberosum* cluster and does not show a definitive affinity for a particular origin. This does not exclude it from a Chilean origin, however, having a T-cytoplasm as do the other three. These Native potato cultivars present a possible second route of diffusion distinct from the South America to Europe transfer which has been assumed to the sole means by which potato was spread out of South America.

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