The Enigma of *Solanum maglia* in the Origin of the Chilean Cultivated Potato, *Solanum tuberosum* Chilotanum Group[1]

David Spooner*[,2,3], Shelley Jansky[2,3], Andrea Clausen[4], Maria del Rosario Herrera[5], and Marc Ghislain[5]

[2]U.S. Department of Agriculture, Agricultural Research Service, Vegetable Crops Research Unit, 1575 Linden Drive, Madison, WI 53706-1590, USA
[3]Department of Horticulture, University of Wisconsin, 1575 Linden Drive, Madison, WI 53706-1590, USA
[4]Estación Experimental Agropecuaria Balcarce, Instituto Nacional de Tecnología Agropecuaria (INTA), C.C. 276, 7620 Balcarce Argentina
[5]International Potato Center (CIP), P.O. Box 1558, Lima 12 Peru
*Corresponding author; e-mail: david.spooner@ars.usda.gov

The Enigma of *Solanum maglia* in the Origin of the Chilean Cultivated Potato, *Solanum tuberosum* Chilotanum Group. Landrace potato cultivars occur in two broad geographic regions: the high Andes from western Venezuela south to northern Argentina (*Solanum tuberosum* Andigenum Group, "Andigenum"), and lowland south-central Chile (*S. tuberosum* Chilotanum Group, "Chilotanum"), with a coastal desert and 560 km between southernmost populations of Andigenum and Chilotanum. Unlike Andigenum landraces, Chilotanum landraces are adapted to long days and carry a 241 base pair plastid DNA deletion. However, Andigenum and Chilotanum landraces are morphologically similar. We investigated a hypothesis that Chilotanum landraces arose from *Solanum maglia*, a rare tuber-bearing species found in Chile and Argentina. This hypothesis was formulated first based on morphological analyses of starch grains of extant and preserved (12,500 years before present) *S. maglia*, and on putative sympatry of extant *S. maglia* and Chilotanum landraces. Our new starch grain analyses fail to support this hypothesis; we could find no evidence of current sympatric distributions, and *S. maglia* lacks the 241-bp plastid deletion. However, microsatellite data group all accessions of *S. maglia* exclusively with Chilotanum, which is supported by our previous observation at the single locus of the waxy gene. These results could be interpreted in various ways, but all explanations have problems. One explanation is that *S. maglia* is a progenitor of Chilotanum. However, the plastid deletion in Chilotanum but not *S. maglia* cannot be easily explained. Another explanation is that Chilotanum was formed by hybridization between *S. maglia* and pre-Chilotanum, but this conflicts with prior cladistic analyses. These new data shed light on aspects of this question and highlight various evolutionary scenarios, but the origin of Chilotanum and the involvement of *S. maglia* in its origin remain an enigma.

Key Words: Microsatellites, potato, *Solanum maglia*, *Solanum tuberosum* Andigenum Group, *Solanum tuberosum* Chilotanum Group.

Introduction

Landrace potato cultivars occur in two broad geographic regions: the high Andes from western Venezuela south to northern Argentina, at 3,000–4,000 m (*Solanum tuberosum* Andigenum Group, "Andigenum," containing diploids, 2n = 24, triploids, 2n = 36, and tetraploids, 2n = 48), and in south-central Chile in the Chonos and Guaitecas Archipelagos and nearby mainland, from sea level to 500 m (*S. tuberosum* Chilotanum Group, "Chilotanum," containing mostly tetraploids). There is a 560 km break in distribution between

[1]Received 26 September 2011; accepted 6 February 2012; published online 21 February 2012.

© 2012, by The New York Botanical Garden Press, Bronx, NY 10458-5126 U.S.A.

them, incorporating a coastal desert, and the high Andean cordillera, which is unsuitable for potato cultivation (Spooner et al. 2010), and we can only speculate how the high Andean populations got to the lowlands of Chile. It is possible that this distributional difference was much less in paleoecological times. The landraces of both cultivar groups are highly diverse, with a great variety of shapes and skin and tuber colors (Castronovo 1949; Ochoa 1990). Andigenum had its origin in southern Peru from wild species in the *Solanum brevicaule* complex (Miller and Spooner 1999; Spooner et al. 2005; Ugent 1970; Van den Berg et al. 1998). The three rarer cultivated species (*S. ajanhuiri* Juz. and Bukasov, diploid; *S. juzepczukii* Bukasov, triploid; and *S. curtilobum* Juz. and Bukasov, pentaploid) are products of hybridization of Andigenum with wild species (Rodríguez et al. 2010). The above classification of cultivated potatoes follows Spooner et al. (2007b) and Ovchinnikova et al. (2011), differing considerably from most other classifications that recognize more cultivated species, especially Andigenum, which recognizes each ploidy variant as a separate species. Although Chilotanum landraces are considered by some (e.g., Hawkes 1990) to be exclusively tetraploid, there are a few reports of diploid and triploid cultivated potatoes that have been collected in lowland Chile (Brücher 1963, 1965; Contreras 1987; Sykin 1971).

There are various theories for the origin of Chilotanum landraces. The dominant set of hypotheses proposes that they evolved from Andigenum landraces, after human-mediated transport to Chile, with subsequent minor morphological differentiation and long-day adaptation (Andigenum landraces are short-day adapted) (Hawkes 1990). However, the theory in support of long-day adaptation from Andigenum germplasm has been challenged recently (Ghislain et al. 2009).

Grun (1990) hypothesized that the Chilotanum landraces arose from a cross between Andigenum landraces and an unidentified wild species, perhaps *S. chacoense*. Hosaka (2002) documented a 241-bp deletion in a subset of the Bolivian and Argentinean wild species *S. berthaultii* (that we here consider to include *S. tarijense*; Spooner et al. 2007a) and *S. neorossii* Hawkes and Hjert. Hosaka (2003) used additional plastid markers to document relationships of some *S. berthaultii* accessions (but not *S. neorossii*) from southern Bolivia and northern Argentina to Chilotanum. Hosaka (2004) expanded this survey to include additional cultivated accessions, showing that these plastid similarities were shared with nine tetraploid Andigenum accessions, seven of them from northern Argentina. He concluded that some populations of *S. berthaultii* with these plastid genotypes crossed naturally as females with Andigenum from which Chilotanum was naturally selected, and these migrated to Chile, similar to a prior hypothesis of Grun (1990), who did not identify *S. tarijense* as this wild species. Supporting this idea, some accessions of *S. berthaultii* serve as superior parents to crosses with cultivated Chilotanum potato, producing progeny with long-day tuberization (Hermundstad and Peloquin 1985).

Alternatively, Ugent et al. (1987) proposed that Chilotanum landraces had an independent origin from *S. maglia* Schltdl. not involving Andigenum landraces. The bases of their hypothesis follow: 1) Potato tuber skins were found in a bowl from Monte Verde, a 12,500 BP archaeological site in south-central Chile in a region where potatoes today are the principal crop (Dillehay 1989). 2) Starch grains from this fossil tuber were examined and compared to extant grains of *S. maglia*, but from Chilean populations 1,000 km farther north. They claimed that both the fossil and extant *S. maglia* produces starch grains with four shapes, but modern *S. tuberosum* has only one shape (oval), probably due to selection of the *S. maglia* progenitor for culinary traits. Tuber starch grain morphology has been used as a taxonomic trait in potato (Verdun 1982) and includes grain size, shape, and the arm lengths and angles of a birefringence "cross" as seen under polarized light, produced by the highly structured molecular configuration of the starch grain (Liu 1997). 3) They claimed that they found extant populations of *S. maglia* in south-central Chile, reflecting supposed distributions 12,500 BP. 4) They proposed that diploid forms of *S. maglia* underwent spontaneous somatic doubling to produce a tetraploid form of *S. tuberosum* in Chile.

Solanum maglia, the sole tuber-bearing wild potato of the southern temperate region of the South American continent, is distinguished morphologically from *S. tuberosum* by its relatively broad leaflets, especially the terminal leaflet, and by its anthers with the tissue grading into the filaments. In central and southern Chile in the geographic Regions V, VII, VIII, IX, and X, it grows in coastal humid valleys, from sea level to about 1,200 m. The closest extant population of *S.*

maglia to Monte Verde is about 1,000 km. away (herbarium data from a monograph in revision of sections *Etuberosum* and *Petota* in the Southern Cone of South America by Spooner and collaborators). In Argentina, *S. maglia* is known from a single locality, in a gorge in Mendoza Province, Argentina, Quebrada de Alvarado, with an elevation of 1,400–1,820 m. This gorge, unlike others in that area, is deep and narrow with habitats shaded throughout the day (Spooner and Clausen 1993). The known Chilean populations are triploid (Hawkes and Hjerting 1969), and the multiple populations in Argentina are diploid (Spooner and Clausen 1993). Significant impact of climate change has taken place since the earliest human settlements in Monte Verde, more than 12,500 BP, which could explain the current rare distribution of *S. maglia* (Dillehay et al. 2008).

Raker and Spooner (2002) investigated the differentiation of the Andigenum and Chilotanum landraces with nuclear microsatellites and showed that the single analyzed accession of *S. maglia* Schltdl. from Chile clustered with Chilotanum landraces. More recently, Rodríguez et al. (2010) found that two of three Chilotanum accessions had the same waxy gene alleles as *S. maglia* (Rodríguez et al. 2010).

The report of extant populations of *S. maglia* in southern Chile sympatric with *S. tuberosum* by Ugent et al. (1987) are not supported by voucher specimens and likely are misidentifications of *S. tuberosum*. We base this statement on reports of others who have collected extensively in south-central Chile (Contreras 1987; Contreras et al. 1993; Spooner et al. 1991), and on a letter from Carlos Ramírez to Spooner, indicating that these were likely misidentifications of *S. tuberosum* as *S. maglia* (see Spooner et al. 1991).

The purpose of our study is to reinvestigate the hypothesis that *S. maglia* was the progenitor of Chilotanum, using a comprehensive microsatellite analysis, plastid deletion data, and starch grain analysis, and to place these data in the context of prior data. Because the world's cultivated potato crop had its origin from Chilotanum (Ames and Spooner 2008), this question is of interest to phylogenists and plant breeders alike.

Materials and Methods

Plant Material

Eight accessions of *Solanum maglia* were examined from the germplasm banks of CIP (CIP prefix), NRSP-6 (PI prefix), and Balcarce Argentina (ClM prefix). All accessions of *S. maglia* accessible as germplasm collections and used here come from two generalized areas. The four CIP and NRSP-6 accessions come from the area of Valparaiso Chile: 1) CIP 761484, Viña Reñaca (on N side of Viña del Mar); 2) CIP 761485, near Costa Brava (12 km from Viña del Mar); 3) PI 245087, between Viña del Mar and Concon, on a steep slope just above the ocean; and 4) PI 558316, Petorca, ca. 500 m S of beach at Zapallar. The four Argentinean accessions (ClM 870-2; ClM 868-3; ClM 865-4; ClM 871-8) all come from one narrow canyon, the Cañón del Alvarado in Mendoza Argentina (Spooner and Clausen 1993). We added these accessions to the preexisting microsatellite and plastid deletion data from Spooner et al. (2007b) of 742 potato landraces of all cultivated potato species and eight accessions of wild species that included seven diploid wild species accessions in the northern *S. brevicaule* complex and one accession of tetraploid *S. acaule*. Starch grain data were based on tubers produced by eight wild species and 23 landrace accessions obtained from the US Potato Genebank, NRSP-6.

Starch Grain Analysis

Tubers were generated in a greenhouse using controlled lighting to induce tuberization in short day plants. After tubers were harvested, they were stored at 4°C until they were processed by removing a thin slice perpendicular to the long axis and through the center of the tuber. Slices were placed in a −80°C freezer and then lyophilized. Each sample was prepared by scraping dried tissue from the slice, placing it on a microscope slide with a drop of water, and adding a cover slip. A polarizing filter was used to observe interference patterns. Samples were observed at 400× magnification.

Nuclear SSR and Plastid DNA Marker Analyses

This study obtained new SSR and plastid deletion data from the eight populations of *S. maglia* and added them to the data set from Spooner et al. (2007b). All procedures for genomic DNA isolation and purification, DNA quantification, SSR and plastid marker analyses, and data analysis follow that study. SSR marker data and plastid deletion are available online from the bioinformatics portal of the Generation

Challenge Program web site (www.generationcp.org) and of CIP (http://research.cip.cgiar.org/confluence/display/IPD/SSR+Marker).

Results

Starch Grains

In *S. maglia*, the arms of the interference pattern cross in the center of round and rectangular grains, and toward one end of oval and triangular grains as in Fig. 1 and as described by Ugent et al. (1987). However, these authors do not describe how this differs from the patterns in other *Solanum* species. The hilum (the point of origin of the starch granule and the center of the cross) is off-center in *S. maglia*, also as described by Ugent et al. (1987), but the same is true for both cultivar groups of *S. tuberosum* and wild species (Fig. 1), and as documented in Liu (1997). Ugent et al. (1987) claimed that *S. maglia* starch grains are unusual because they are found in four shapes—oval, round, triangular, and rectangular—while the starch grains of *S. tuberosum* consist are only oval (Ugent et al. 1987). Our data, however, document considerable variation in the proportion of oval-shaped grains both within and among wild and cultivated species. All four types of starch grains occur in *S. tuberosum* (both Andigenum and Chilotanum) and other wild and cultivated *Solanum* species (Fig. 1). Small starch grains are round, while larger ones have expanded into oval shapes. Space constraints due to tight packing in tuber parenchyma tissue likely led to the production of starch grains with rectangular, triangular, and other irregular shapes (Singh et al. 2009). Verdun (1982) and Ugent and Verdun (1983) evaluated only five starch grains per sample in their taxonomic analysis, which was subsequently cited by Ugent et al. (1987).

Our observations of thousands of starch grains reveal large variability in appearance due to differences in both their shape and, because they are three-dimensional objects, their orientation when viewed under a microscope. For example, oval starch grains observed on end appear to be round. Ugent et al. (1987) claimed that in *S. maglia*, starch grain size varies from 10–20 μ for round grains and from 35–80 μ for other types. In *S. tuberosum*, starch grains range from 1–110 um (Singh et al. 2009). Consequently, there is considerable overlap in starch granule size among species. Even within species, starch grains are highly variable in size as in Fig. 1 and as reported in Geddes et al. (1965). Hence, neither the original observations by Ugent et al. (1987) nor our observations lend support to the idea that starch grains of *S. maglia* are useful to infer an independent origin of Chilotanum landraces from *S. maglia* not involving Andigenum landraces.

Nuclear SSR and Plastid DNA Marker

Four of the 50 SSRs amplified in Spooner et al. (2007b), STM0030, STM0031, STM017, and STWAX-2, did not amplify in *S. maglia*. Because they amplified in control samples, we considered these as true data "0." STM1031 failed to amplify *S. maglia* and the control samples due to unknown technical reasons, and we left these data in the original database and scored all accessions of *S. maglia* as missing data "9." In total, the database consisted of 760 taxa and 556 alleles. Missing data for the entire data set was 2.3% and for *S. maglia* 2.2%. Neighbor-joining analysis (Fig. 2) firmly placed all eight accessions *S. maglia* with Chilotanum, but not the other closely related wild species relatives of *S. tuberosum*.

Since the potato genetic identity kit (Ghislain et al. 2004) was developed using single locus markers, the presence of 2 or 3 alleles is indicative of presence of diploid and triploid *S. maglia* accessions. All of the Argentinean accessions had two alleles, and three of the four Chilean accessions had three alleles. PI 245087 from Chile had two alleles. Of the remaining 27 SSR markers, 18 were monomorphic, 10 with one allele and the other 8 with 2 alleles. Neighbor-joining analysis grouped *S. maglia* into two separate groups but adjacent to each other, all diploid Argentinean accessions and Chilean putative diploid PI 245087 in one group, and all remaining putative triploid Chilean accessions in another group. The topology of the remaining accessions is similar to Spooner et al. (2007b). Therefore, there is no area-specific differentiation of these accessions. The four accessions from the INTA genebank, collected at the same location, were all identical with one exception for the CLM 862-2, which did not amplify the SSR locus STI001. This polymorphism was repeatedly tested and found reliable. Hence, this accession might be mutated at this precise microsatellite marker locus.

The 241-bp deletion marker was absent from all DNA samples of *S. maglia*, whereas DNA from the majority of the Chilotanum accessions displayed the expected deletion. Ames and

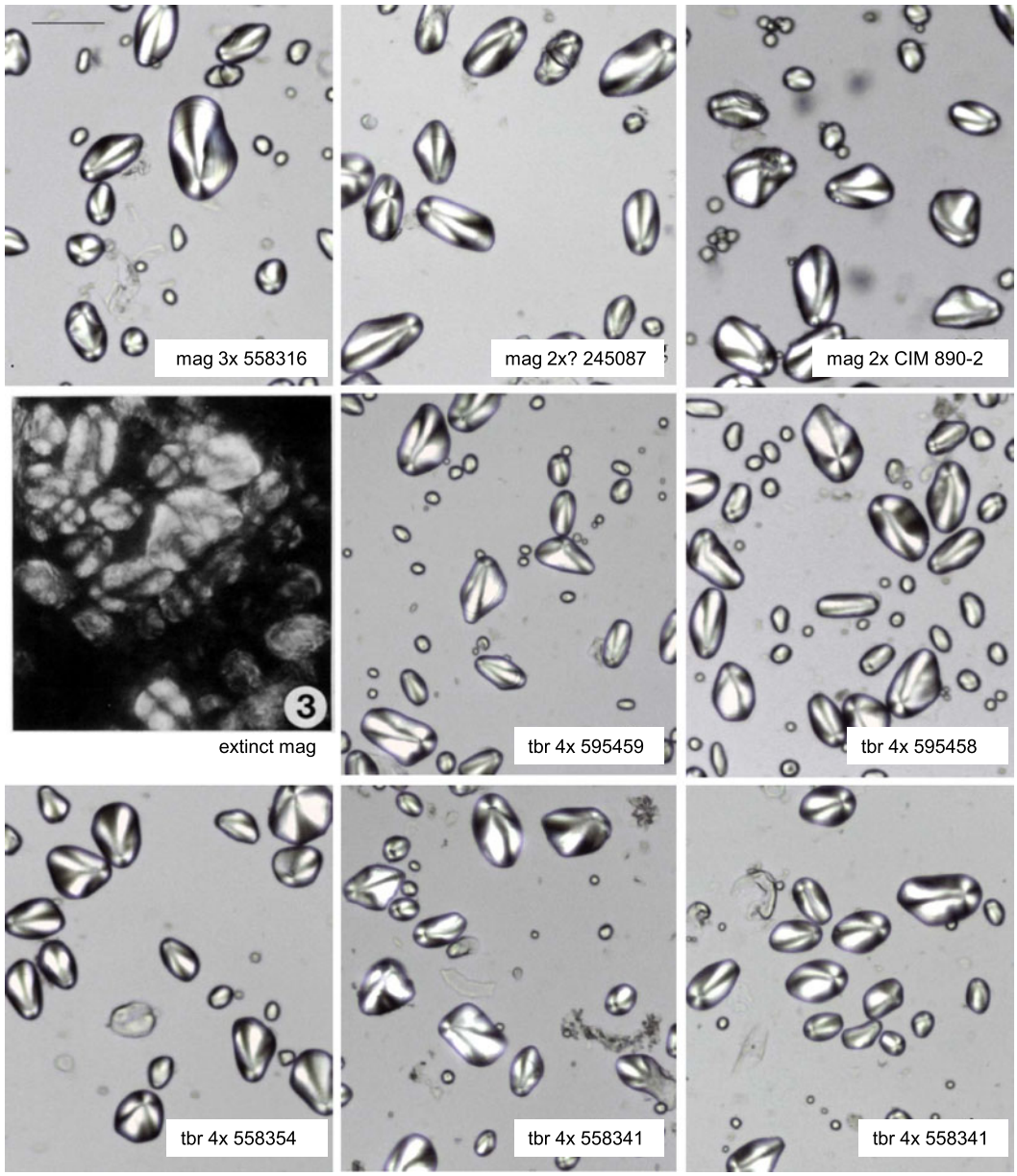

Fig. 1. Starch grains of landrace populations of *S. curtilobum* (cur), *S. juzepczukii* (juz), *S. tuberosum* Andigenum Group (adg), *S. tuberosum* Chilotanum Group (tbr), and wild species *Solanum maglia* (mag) and *S. bukasovii* (buk); ploidy is listed after name.

Spooner calculated that the 241-bp deletion in the *trn* V-UAC/ *ndh* C intergenic region of the plastid DNA molecule is absent in 94% (or 95%) of the Andean tetraploid landraces and present in 86% (or 81%) of the tetraploid Chilean landraces, depending on the studies of Hosaka (2004) or Spooner et al. (2007b).

Discussion

Starch Grains

Starch grains increase in size as tubers expand, so the size of starch grains varies with the time at which tubers are collected (Geddes et al. 1965; Noda et al. 2004). It is impossible to know the

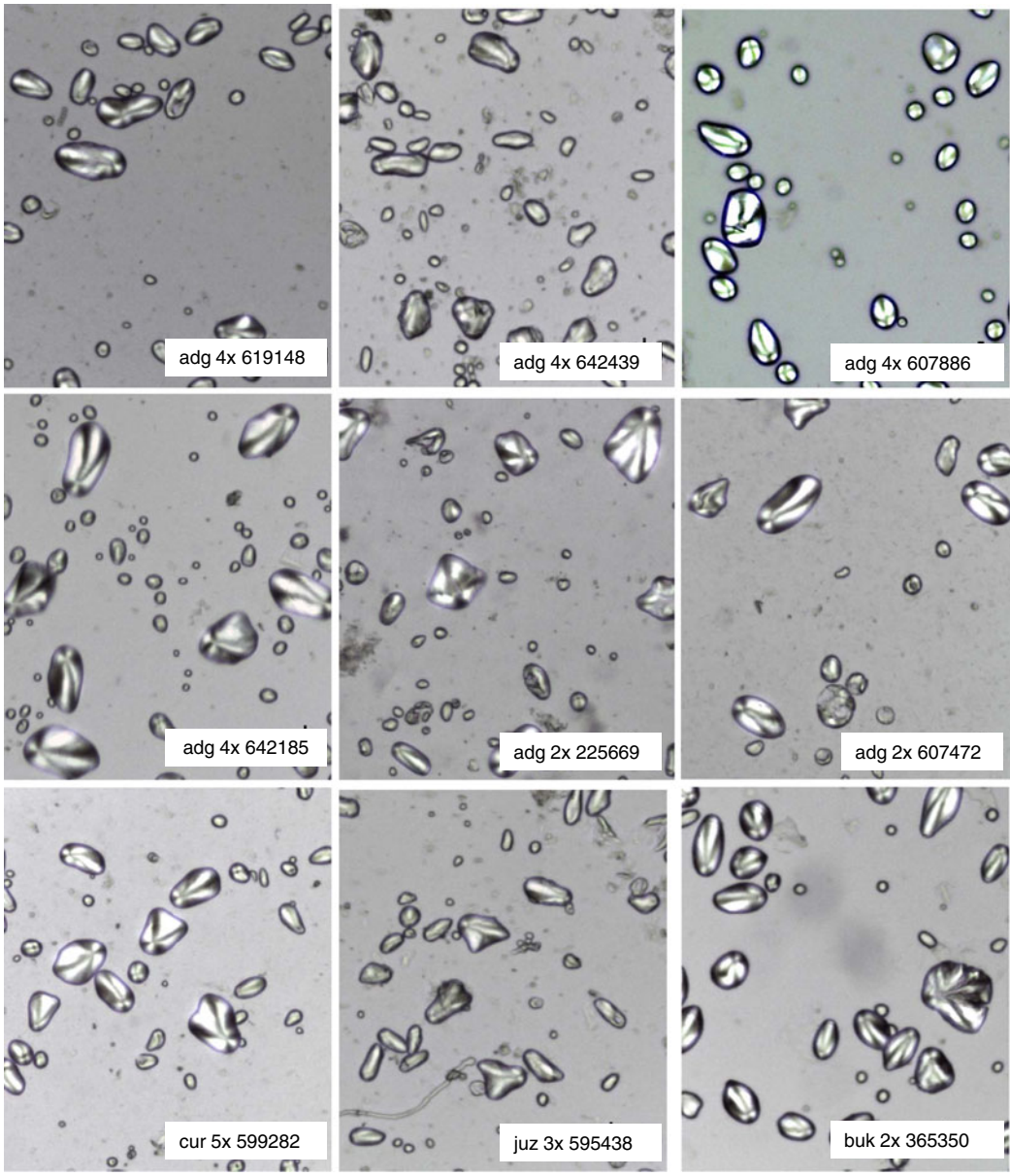

Fig. 1. (continued).

developmental stage in which the tubers from the Monte Verde site were collected, confounding comparisons between archaeological specimens and tubers produced for comparison in modern studies. Post-harvest conditions also affect starch grain size. We do not know whether the tubers from the Monte Verde site were freshly harvested or had been stored. During storage, the number of small grains in a tuber increases (Chung and Hadziyev 1980) and the number of large grains decreases (Mica 1975), as starch is converted into sugars.

Environmental conditions during tuber growth and storage also affect the size of starch grains (Shannon et al. 2009). The climate in which the tubers from Monte Verde were formed was likely slightly warmer than present-day conditions (Dillehay 1989). Tubers formed in a warm environment (10–15°C) contain starch grains

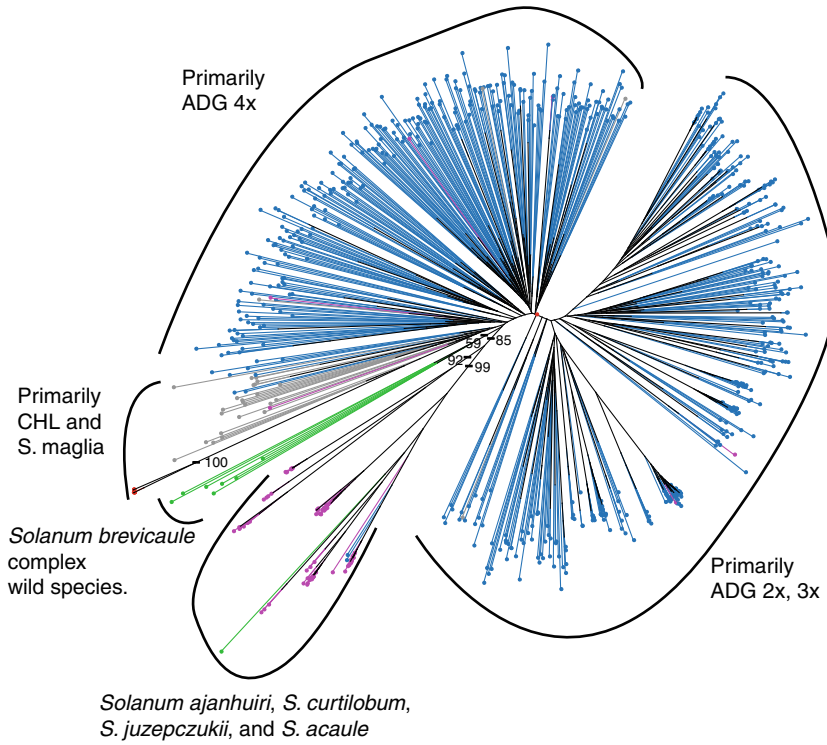

Fig. 2. Neighbor-joining tree using SSR data of cultivated potatoes and putative ancestors, adding eight accessions of *Solanum maglia* to the study of Spooner et al. (2007b). Bootstrap values are only placed in the basal branches or on the interior branches defining the wild species and the "bitter" hybrid potatoes and at the base of the branch containing all seven accessions of *S. maglia* (only two dots can be seen for *S. maglia* because of extreme similarity and overlapping dots). Blue (colors on online version) is ADG, pink are the "Bitter" hybrid potatoes, green are wild species, gray is CHL, and red (nested on the lower end of CHL) is *S. maglia*.

with a smaller length to width ratio than those formed at warmer temperatures (20–25°C) (Hizukuri 1969). The long-term conditions under which the tubers at the Monte Verde site were preserved for 12,000 years likely affected starch parameters. Small starch grains are more susceptible to biotic degradation than large ones; introducing bias due to differential decomposition (Haslam 2004).

Microsatellites

The potato genetic identity kit amplified well all *S. maglia* accessions but one of the 50 nuclear SSR markers. We did not eliminate this SSR marker to be able to use the large data set obtained previously; instead *S. maglia* accession were coded as missing data. Three SSR markers did not amplify DNA from *S. maglia*. The lack of amplification is most likely due to single nucleotide polymorphism(s) in at least one of the primer sequence as lowering the annealing temperature increased the amplification product. This can be expected for DNA from wild species as we have previously observed higher null allele with higher genetic distance between wild species and *S. tuberosum*. More than half of the SSR markers for *S. maglia* were monomorphic, suggesting that this species is self-compatible. The neighbor-joining grouping of *S. maglia* with Chilotanum is similar to the accession of *S. acaule* grouping with *S. curtilobum* and *S. juzepczukii* (Fig. 1; Spooner et al. 2007b) forming distinct clusters from the large Andigenum cluster. *Solanum acaule* is supported as a parent of both of these hybrid cultivated potatoes (Hawkes 1962; Rodríguez et al. 2010; Schmiedische et al. 1980). Similarly, our microsatellite data provide support to *S. maglia* as a parent or introgressant in the hybrid origin of Chilotanum. The hybrid hypothesis is further supported by GBSSI sequencing data of Rodríguez et al. (2010), showing two of

the three examined accessions of Chilotanum sharing alleles with both *S. maglia* and Andigenum.

Conclusions

Solanum maglia groups with Chilotanum based on microsatellite data (present results) and with some accessions based on GBSSI sequence data (Rodríguez et al. 2010). This prompts a reconsideration of its involvement in the origin of Chilotanum, either as a wild species progenitor or as an introgressant species. However, this hypothesis has three problems: (1) the starch grain data that built this hypothesis fail to distinguish these species; (2) current distributional data of extant *S. maglia* are not sympatric with Chilotanum; and (3) the shared occurrence of plastid DNA of Chilotanum and some accessions *S. berthaultii* wild species occurring in southern Bolivia and adjacent northern Argentina are lacking in *S. maglia*.

The starch grain data are neutral and address no competing hypotheses. The current distributional data may not reflect those 12,500 BP, when *S. maglia* and Chilotanum (or its initial populations) may have been sympatric. The plastid DNA data provide the biggest problem to *S. maglia* being a parent of introgressant to Chilotanum. One hypothetical explanation for these discontinuities follows: Andigenum populations in southern Bolivia or northern Argentina hybridized in that region with populations of *S. berthautii* with the plastid deletion type. We assume that this hybridization has happened as we have found Andigenum landrace accessions with the 241-bp deletion in northern Argentina. These could have been transported to Chile and crossed as females to long-day adapted potatoes initially domesticated entirely from *S. maglia*, resulting in new local landraces combining nuclear DNA alleles of *S. maglia* and Andigenum.

Dillehay (1997) suggested that *S. maglia* was likely a staple food for the prehistoric inhabitants of south-central Chile. However, tubers of many wild relatives of potato contain high levels of bitter, toxic glycoalkaloids (Valkonen et al. 1996). Glycoalkaloids are stable up to 300°C, so they would not degrade due to cooking (Friedman and Dao 1992). Consequently, consumption of these tubers, especially as a staple food source, would be problematic. Ugent et al. (1987) suggested that *S. maglia* tubers are not bitter, but other reports support the contrary (Correll 1962; Johns and Alonso 1990). In fact, tubers of extant *S. maglia* were found to contain 56 mg glycoalkaloids per 100 g fresh tuber tissue, which likely confer a bitter taste and could lead to poisoning, even after cooking.

In conclusion, our data do not support one or the other hypothesis of the origin of Chilotanum and the involvement of *S. maglia* in its origin remains an enigma. They do, however, revive the hypothesis that Chilotanum is indigenous to southern Chile as proposed by many authors in the beginning of the 20th century (cited in Ugent et al. 1987). As a potato genome has been sequenced, the sequencing of multiple potato genomes, cultivated and wild (to include *S. maglia*), combined with the identification of domestication alleles, will provide new insight into this century-old debate over the origin of the cultivated potato.

Acknowledgments

We thank Juan Daniel Montenegro and Jorge Núñez for technical support in the amplification of SSR markers and the analysis of genetic data, respectively. We acknowledge the contributions of Kelly McMillan in the collection of starch granule data.

Literature Cited

Ames, M. and D. M. Spooner. 2008. DNA from herbarium specimens settles a controversy about origins of the European potato. American Journal of Botany 95:252–257.

Brücher, H. 1963. Das südlichste Vorkommen diploider Kulturkartoffeln in Südamerika auf der Insel Chiloé. Qualitas Plantarum et Materiae Vegetabiles 9:187–202.

——— 1965. Über ein "maritime" Wildkartoffel (*Solanum maglia* Molina) im argentinischen Anden-Gebirge. Berichte der Deutschen Botanischen Gesellschaft 78:492–498.

Castronovo, A. 1949. Papas chilotas, descripciones y clave para el reconocimiento de muestras de papas recogidas en una excursión al sur de Chile. Revista de Investigaciones Agrícolas 3:209–245 + 8 pl.

Chung, I. and D. Hadziyev. 1980. Tuber and starch characteristics of Alberta grown potatoes. Canadian Institute of Food Science and Technology 13:143–153.

Contreras, M. A. 1987. Germoplasma chileno de papas (*Solanum spp*). Anales Simposio Recursos Fitogenéticos. Valdivia 1984. Universidad Austral de Chile, International Board for Plant Genetic Resources, 43–75.

Contreras, A., L. Ciampi, S. Padulosi, and D. M. Spooner. 1993. Potato germplasm collecting expedition to the Guaitecas and Chonos Archipelagos, Chile, 1990. Potato Research 36:309–316.

Correll, D. S. 1962. The potato and its wild relatives. Contributions from the Texas Research Foundation. Botanical Studies 4:1–606.

Dillehay, T. D. 1989. Monte Verde: A late Pleistocene settlement in Chile. Volume 1. Palaeoenvironment and site context. Washington and London: Smithsonian Institution Press.

———. 1997. Monte Verde: A late Pleistocene settlement in Chile. Volume 2. The archaeological context and interpretation. Washington and London: Smithsonian Institution Press.

Dillehay, T. D., C. Ramírez, M. Pino, M. B. Collins, J. Rossen, and J. D. Pino-Navarro. 2008. Monte Verde: Seaweed, food, medicine, and the peopling of South America. Science 320:784–786.

Friedman, M. and M. Dao. 1992. Distribution of glycoalkaloids in potato plants and commercial potato products. Journal of Agricultural and Food Chemistry 40:419–423.

Geddes, R., C. T. Greenwood, and S. MacKenzie. 1965. Studies on the biosynthesis of starch granules. Part III. The properties of the components of starches from the growing potato tuber. Carbohydrate Research 1:71–82.

Ghislain, M., J. Núñez, M. del Rosario Herrera, and D. M. Spooner. 2009. The single Andigenum origin of Neo-Tuberosum materials is not supported by microsatellite and plastid marker analyses. Theoretical and Applied Genetics 118:963–969.

———, D. M. Spooner, F. Rodríguez, F. Villamón, J. Núñez, C. Vásquez, R. Waugh, and M. Bonierbale. 2004. Selection of highly informative and user-friendly microsatellites (SSRs) for genotyping of cultivated potato. Theoretical and Applied Genetics 108:881–890.

Grun, P. 1990. The evolution of cultivated potatoes. In: New perspectives on the origin and evolution of New World domesticated plants, ed. P. K. Bretting. Economic Botany (3 Suppl.) 44:39–55.

Haslam, M. 2004. The decomposition of starch grains in soils: Implications for archaeological residue analysis. Journal of Archaeological Science 31:1715–1734.

Hawkes, J. G. 1962. The origin of *Solanum juzepczukii* Buk. and *S. curtilobum* Juz. et Buk. Zeitschrift fur Planzenzucht 47:1–14.

——— 1990. The potato: Evolution, biodiversity and genetic resources. Belhaven Press, Oxford.

——— and J. P. Hjerting. 1969. The potatoes of Argentina, Brazil, Paraguay and Uruguay: A biosystematic study. Oxford University Press, Oxford.

Hermundstad, S. A. and S. J. Peloquin. 1985. Germplasm enhancement with potato haploids. Journal of Heredity 76:463–467.

Hizukuri, S. 1969. The effect of environment temperature of plants on the physicochemical properties of their starches. Journal of the Japanese Society of Starch Science 17:73–88.

Hosaka, K. 2002. Distribution of the 241 bp deletion of chloroplast DNA in wild potato species. American Journal of Potato Research 79:119–123.

——— 2003. T-type chloroplast DNA in *Solanum tuberosum* L. ssp. *tuberosum* was conferred from some populations of *S. tarijense* Hawkes. American Journal of Potato Research 80:21–32.

——— 2004. Evolutionary pathway of T-type chloroplast DNA in potato. American Journal of Potato Research 81:153–158.

Johns, T. and J. G. Alonso. 1990. Glycoalkaloid change during the domestication of the potato, *Solanum* Section *Petota*. Euphytica 50:203–210.

Liu, Q. 1997. Characterization of physic-chemical properties of starch from various potato and other sources. Ph.D. thesis, Universite Laval, Quebec, Canada.

Mica, B. 1975. Characteristics of the starches of selected potato varieties. 1. Changes in starch content and starch granule size during storage. Starch 27:181–186.

Miller, J. T. and D. M. Spooner. 1999. Collapse of species boundaries in the wild potato *Solanum brevicaule* complex (Solanaceae, *S.* sect. *Petota*): Molecular data. Plant Systematics and Evolution 214:103–130.

Noda, T., S. Tsuda, M. Mori, S. Takigawa, C. Matsuura-Endo, K. Saito, W. H. A. Mangalika, A. Hanaoka, Y. Suzuki, and H. Yamauchi. 2004. The effect of harvest dates on the starch properties of various potato cultivars. Food Chemistry 86:119–125.

Ochoa, C. M. 1990. The potatoes of South America: Bolivia. Cambridge University Press, Cambridge.

Ovchinnikova, A., E. Krylova, T. Gavrilenko, T. Smekalova, M. Zhuk, S. Knapp, and D. M. Spooner. 2011. Taxonomy of cultivated potatoes (*Solanum* section *Petota*: Solanaceae). The Botanical Journal of the Linnean Society 165:107–155.

Raker, C. and D. M. Spooner. 2002. The Chilean tetraploid cultivated potato, *Solanum tuberosum*, is distinct from the Andean populations: Microsatellite data. Crop Science 42:1451–1458.

Rodríguez, F., M. Ghislain, A. M. Clausen, S. H. Jansky, and D. M. Spooner. 2010. Hybrid origins of cultivated potatoes. Theoretical and Applied Genetics 121:1187–1198.

Schmiedische, P. E., J. G. Hawkes, and C. M. Ochoa. 1980. Breeding of the cultivated potato species *Solanum* x *juzepczukii* Buk. and *Solanum* x *curtilobum* Juz. et Buk. I. Euphytica 29:685–704.

Shannon, J. C., D. L. Garwood, and C. D. Boyer. 2009. Physiology of starch development. Page 894 in J. N. BeMiller and R. L. Whistler, eds., Starch: Chemistry and Technology. Elsevier, Amsterdam.

Singh, J., L. Kaur, and O. J. McCarthy. 2009. Potato starch and its modification. Pages 273–318 in J. Singh and L. Kaur, eds., Advances in Potato Chemistry and Technology. Elsevier, San Diego.

Spooner, D. M. and A. M. Clausen. 1993. Wild potato (*Solanum* sect. *Petota*) germplasm collecting expedition to Argentina in 1990, and status of Argentinian potato germplasm resources. Potato Research 36:3–12.

———, A. Contreras, and J. B. Bamberg. 1991. Potato germplasm collecting expedition to Chile, 1989, and utility of the Chilean species. American Potato Journal 68:681–690.

———, K. McLean, G. Ramsay, R. Waugh, and G. J. Bryan. 2005. A single domestication for potato based on multilocus AFLP genotyping. Proceedings of the National Academy of Sciences USA 102:14694–14699.

———, D. Fajardo, and G. J. Bryan. 2007a. Species limits of *Solanum berthaultii* Hawkes and *S. tarijense* Hawkes and the implications for species boundaries in *Solanum* sect. *Petota*. Taxon 56:987–999.

———, J. Núñez, G. Trujillo, M. Herrera, F. Guzmán, and M. Ghislain. 2007b. Extensive simple sequence repeat genotyping of potato landraces supports a major reevaluation of their gene pool structure and classification. Proceedings of the National Academy of Sciences USA 104:19398–19403.

———, T. Gavrilenko, S. H. Jansky, A. Ovchinnikova, E. Krylova, S. Knapp, and R. Simon. 2010. Ecogeography of ploidy variation in cultivated potato (*Solanum* sect. *Petota*). American Journal of Botany 97:2049–2060.

Sykin, A. G. 1971. Zur Frage der Abstammung und der wildwachsenden Vorfahren chilenischer Kulturkartoffeln. Zeitschrijt fur Pflanzenzücht 65:1–14.

Ugent, D. 1970. The potato: What is the origin of this important crop plant, and how did it first become domesticated? Science 170:1161–1166.

——— and M. P. Verdun. 1983. Starch grains of the wild and cultivated Mexican species of *Solanum*, subsection *Potatoe*. Phytologia 53:351–363.

———, T. Dillehay, and C. Ramirez. 1987. Potato remains from a late Pleistocene settlement in Southcentral Chile. Economic Botany 41:17–27.

Valkonen, J. P. T., M. Keskitalo, T. Vasara, and L. Pietila. 1996. Potato glycoalkaloids: A burden or a blessing? Critical Reviews in Plant Sciences 15:1–20.

Van den Berg, R. G., J. T. Miller, M. L. Ugarte, J. P. Kardolus, J. Villand, J. Nienhuis, and D. M. Spooner. 1998. Collapse of morphological species in the wild potato *Solanum brevicaule* complex (Solanaceae: sect. *Petota*). American Journal of Botany 85:92–109.

Verdun, M. P. 1982. Starch grains: A taxonomic character in *Solanum* (Tourn.) L., section *Tuberarium*. M.S. thesis, Southern Illinois University, Carbondale, Illinois.