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A Reexamination of Species Boundaries and Hypotheses of Hybridization Concerning *Solanum megistacrolobum* and *S. toralapanum* (*Solanum* sect. *Petota*, series *Megistacroloba*): Molecular Data

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ABSTRACT. *Solanum megistacrolobum* and *S. toralapanum* are two phenetically similar wild potato (*Solanum* sect. *Petota*) species, classified in series *Megistacroloba*, that together are distributed from southern Peru to northwestern Argentina. They have variously been synonymized, recognized as varieties of *S. megistacrolobum*, or recognized as distinct species. We used 22 single- to low-copy random genomic DNA probes from potato, hybridized to total DNA digested with *Dra*I, *Eco*RI, *Eco*RV, and *Hin*dIII, to investigate their taxonomic status. We also investigated the hybrid origin of *S. raphanifolium* and the hybrid origin of *S. acaule* subsp. *aemulans*. Our results are concordant with a separate morphological study, showing weak differentiation between *S. megistacrolobum* and *S. toralapanum*, but possible only with multivariate methods. These combined morphological and molecular results most closely fit the contemporary treatment of *S. megistacrolobum* and *S. toralapanum* at the varietal level. We propose the new combination **S. megistacrolobum** subsp. **toralapanum** in order to bring consistency to taxa within sect. *Petota*, where different authors recognize identical taxa as varieties or subspecies. Our results also show possible hybridization between *S. megistacrolobum* and *S. acaule* subsp. *aemulans* in Argentina, discount the hybridization hypothesis of *S. raphanifolium*, and show that the species-specific bands of *S. albicans* relative to *S. acaule* are shared with many other species in sect. *Petota*.

Recent advances in molecular biology have provided new tools for systematic questions. At the species level or below, restriction fragment length polymorphisms (RFLP's) have provided powerful new data to assess phenetic similarity. Combined with morphological data, RFLP studies are enabling researchers to answer questions not possible with previous methods. Beckman and Soller (1986a, 1986b), Tanksley (1983), and Whitkus et al. (in press) have described many of the useful properties of RFLP's. These include ubiquity, stability, Mendelian codominant inheritance, the presence of multiple alleles for a given locus, and freedom from pleiotropic effects. Additionally, RFLP's are detectable in all tissues at all developmental stages. This, coupled with the relatively long shelf life of DNA, makes RFLP analysis a convenient method for assessing genetic variation and lower-rank systematic questions.

This study employs single-to low-copy nuclear DNA probes to generate nuclear restriction fragment length polymorphisms (nRFLP's) to investigate hypotheses of species boundaries and hybridization involving *Solanum megistacrolobum* Bitter and *S. toralapanum* Cárd. and Hawkes. These are closely related and phenetically similar wild potato taxa (*Solanum* L. sect. *Petota* Dumort.) distributed from southern Peru to northwestern Argentina. Both are classified in series *Megistacroloba*, a group of diploid ($2n = 24$), self-incompatible, and largely interfertile species (Buck 1966; Hawkes 1990; Ochoa 1990; Pandey 1960). There is much intra-and interspecific morphological variability between *S. megistacrolobum* and *S. toralapanum*. These two taxa have been taxonomically treated as conspecific (Ochoa 1984), varieties of a single species (Ochoa 1990), or as distinct species (Correll 1962; Gorbatenko 1989; Hawkes 1990).

Previous studies have shown that nRFLP's can result from both structural changes (i.e., insertions, deletions, inversions) and site mutations. Data distinguishing these alternatives have come from inferences based on comparisons of numbers of nRFLP patterns and length of areas being probed, or on recurrent patterns of polymorphisms using the same probe with different enzymes (Neuhausen 1992; Wang and Tanksley 1989; Wang et al. 1992). The only way

to distinguish the genetic basis of nRFLP differences absolutely, however, would be to clone the fragment under consideration and map it with double digests, sequencing studies, or by segregation analyses.

If most polymorphisms are caused by structural changes, then combining data from more than one probe/enzyme combination would overweigh these differences relative to those produced from site mutations. The majority of intraspecific nRFLP's in rice (*Oryza* L.) are apparently structural changes (McCouch et al. 1988; Wang and Tanksley 1989). In *Cucumis* L., however, nRFLP's within some species (e.g., *C. melo* L.) are apparently mainly site mutations, but those between species are a combination of site changes and structural mutations (Neuhausen 1992). It is possible, therefore, that site mutations are more predominant in more recently evolved taxa, but we have no data to address recency of origin of *S. megistacrolobum* and *S. toralapanum*. While some studies have used a reduced data set of only one probe/enzyme combination for data analysis (e.g., Chase et al. 1991; Miller and Tanksley 1990), other studies have combined data from more than one probe/enzyme combination (e.g., Jarrett et al. 1992; Kochert et al. 1991), while others have eliminated a subset of the probe/enzyme combinations inferred to have resulted from structural mutations (e.g., Hosaka and Spooner 1992).

This molecular study complements a separate morphological study of *S. megistacrolobum* and *S. toralapanum* (Giannattasio and Spooner 1994). That study demonstrated that these two taxa could be weakly distinguished, but only with multivariate methods. Similar to the morphological study (Giannattasio and Spooner 1994), the primary objective here was to assess the extent and geographical partitioning of differences among *S. megistacrolobum*, *S. toralapanum*, and putative relatives in series *Megistacroloba* ($2n = 24$; *S. boliviense* Dunal, *S. raphanifolium* Cárd. and Hawkes, and *S. sogarandinum* Ochoa), and to use these data to assess the taxonomic boundaries of *S. megistacrolobum* and *S. toralapanum*.

Two secondary objectives of this study were to investigate separate hybridization hypotheses involving *S. megistacrolobum*, *S. albicans* Ochoa, and *S. raphanifolium*. Series *Acaulia* (containing *S. acaule* Bitter subsp. *acaule*, $2n = 4x = 48$; subsp. *aemulans* Bitter and Wittm., $2n = 48$; subsp. *punae* (Juz.) Hawkes and Hjerting, $2n = 48$; *S. albicans*, $2n = 6x = 72$) has been hypothesized to be related to series *Megistacroloba* by virtue of habit and leaf and peduncle morphology (Hawkes and Hjerting 1969, 1989; Ochoa 1990). *Solanum albicans* has been hypothesized to be a hybrid species with a distinct third genome (Matsubayashi 1991), but the identity of this genome is unknown. Hosaka and Spooner (1992) showed that 22 of 94 probe/enzyme combinations in *S. albicans* (combining data from different restriction endonucleases) revealed nRFLP's that were unique to this species relative to the three subspecies of *S. acaule*. We wanted to see whether our study could discover the source of this third genome in our examined members of series *Megistacroloba*. Separately, *S. canasense* Hawkes (series *Tuberosa*, $2n = 24$) and *S. raphanifolium* were examined because *S. raphanifolium* has been hypothesized to be a hybrid between *S. canasense* and *S. megistacrolobum*. Chloroplast and nuclear ribosomal DNA data failed to support this hypothesis (Spooner et al. 1991), and we wished to reexamine it further with data from nRFLP's.

We also examined *S. brevidens* Philippi (series *Etuberosa*, $2n = 24$) and *S. bulbocastanum* Dunal (series *Bulbocastana*, $2n = 24$). These two species are phenetically different and members of separate clades (based on chloroplast DNA) from South American members of sect. *Petota* (Hawkes 1990; Hosaka et al. 1984; Spooner and Sytsma 1992; Spooner et al. 1993). They were included to compare their relative degree of difference from our examined members of series *Acaulia* and *Megistacroloba*.

Materials and Methods

Plants. Seeds from 25 accessions of *S. megistacrolobum*, 19 accessions of *S. toralapanum*, three accessions of *S. acaule* (one representative of each of the three subspecies, see above), two accessions of *S. boliviense*, and one accession each of *S. albicans*, *S. brevidens*, *S. bulbocastanum*, *S. canasense*, *S. raphanifolium*, and *S. sogarandinum* were obtained from the National Research Support Program-6 (NRSP-6; formerly known as the Inter-Regional Potato Introduction Project, IR-1; Hanneman and Bamberg 1986). The accessions of *S. megistacrolobum* represent a subset of those chosen for the morphological study (see Table 2 of Giannattasio and Spooner 1994), and, as in that study, were chosen to represent the widest

possible geographical range of these species. In this paper, we refer to these accessions by two numbers, the first from column one of Table 1 of Giannattasio and Spooner (1994) that refers to our accession number, followed by a dash and the one or two digit map location from Figure 1 of that paper. The *S. toralapanum* accessions were the same as those used in the morphological study except for the elimination of tor 102-6, 112-21, and the addition of tor 111-21. Seeds from all accessions were grown in a greenhouse for approximately 60 days before extraction of DNA from young leaves.

Probes. A random genomic library of total DNA from *Solanum phureja* Juz. and Buk. was used as described by Hosaka and Spooner (1992). A total of 22 clones (P43, P82, P93, P101, P132, P135, P161, P209, P215, P247, P256, P265, P278, P279, P298, P332, P368, P374, P403, P417, P562 and P648) were chosen and used for RFLP analysis. We chose some of the clones to compare our results to that of a prior study of *S. acaule* (Hosaka and Spooner 1992). The clones were amplified by the Polymerase Chain Reaction and radiolabeled with ^{32}P-dCTP by the method of Feinberg and Vogelstein (1984).

DNA Isolation and Restriction Site Comparison. Five to ten gm of bulked leaf tissue from about 10 plants per accession were ground with a mortar and pestle in liquid nitrogen. The resulting powder was mixed with 10 ml of warm 2× CTAB buffer (Doyle and Doyle 1987) and incubated 1 hr at 60°C with gentle agitation at 15 min intervals. An equal volume of chloroform-isoamyl alcohol (24:1) was then added and the contents were gently mixed by inversion for 3 min. Centrifugation for 10 min at 10,000 rpm was carried out using a JA-13.1 rotor (Beckman). The aqueous supernatant was filtered through Miracloth (Calbiochem®) and precipitated in 20 ml cold isopropanol in the presence of a small piece of unsiliconized glasswool. The solution was decanted and the glasswool/DNA aggregate was washed twice using 75% ethanol with 10 mM ammonium acetate and 75% ethanol, each for 30 min. The DNA was resuspended using five ml of 50 mM Tris-HCl buffer pH 8.0 and 20 mM EDTA gently agitated overnight. The DNA was then precipitated in cold 100% ethanol, redissolved in Tris-HCl EDTA, to bring the DNA to final concentrations of approximately 0.3 μg per μl. Five μl of RNAse was added to each sample before digestion.

Five μg of each DNA sample were digested with *Dra*I, *Eco*RI, *Eco*RV, and *Hin*dIII restriction endonucleases according to manufacturer's instructions. Samples that showed only partial digestion were repurified by phenol-chloroform extraction as described by Sambrook et al. (1989). Gel electrophoresis was performed using 1% agarose gels containing 30 μl of a four mg/ml solution of ethidium bromide. Southern transfers were made by the alkaline method of Reed and Mann (1985) to nylon membranes (Zeta-Probe®, Bio-Rad). Hybridization was carried out using a buffer containing 0.25 M $NaHPO_4$ (pH 7.2), 0.25 M NaCl, 7% SDS, 10% polyethylene glycol 8000, 0.5% nonfat powdered milk, and 1mM EDTA (Amasino 1986). Four membranes, with the DNA binding surfaces outside, were placed in heat-sealable plastic bags sandwiched with sheets of Miracloth (Calbiochem®). Prehybridization was performed at 65°C for at least 3 hr after which a denatured probe was injected into each bag. Hybridization continued overnight at 65°C with a probe concentration of 100 ng/75 ml of hybridization solution. Hybridized membranes were washed for 15 min at room temperature, then for 30 min at 65°C in a solution of 1% SDS, 20× SSC (0.3 M NaCl, 0.03 M sodium citrate), and 0.1% tetrasodium pyrophosphate, followed by two washes at 65°C for 30 min each in a solution of 1% SDS, 0.1× SSC, and 0.1% tetrasodium pyrophosphate. The membranes were then placed in plastic wrap and autoradiography was performed with the aid of Lightning Plus (DuPont®) intensifying screens at −80°C or at room temperature with no intensifying screens for overnight to six days, depending on signal intensity.

Data Analysis. Polymorphic bands were converted to one (presence) and zero (absence) data. An alternative method of interpretation would have been an allele/locus model. Such interpretation, however, depends either on a knowledge of the genetic basis of the banding patterns (see above) or inferences of alleles based on these patterns. Such inferences can be most reliably made with simple patterns. Our banding patterns vary from relatively simple (Fig. 1A) to more complex (Fig. 1B). Because we lack knowledge of the genetic basis of these bands, and because we cannot reliably infer their allelic nature, we believe that the presence/absence scoring method is more appropriate here.

Because we bulked individuals within acces-

sions for DNA extractions, some bands that may have been in low frequency within accessions were light. Alternatively, light bands may have been caused by partial homology of probe to DNA sequence. Only clearly visible bands were scored (Fig. 1A, B). Two data sets were used, one containing data from all scorable probe/enzyme combinations, and a reduced set using data from only one enzyme per probe. Because we lack knowledge of the nature of the mutations detected in this study (i.e., site mutations vs. structural mutations, see above), our discussion will focus on the reduced data set, with a short discussion of the similarity of these results to those of the entire data set. For the reduced data set, the probe/enzyme combination showing the greatest number of bands was chosen for analysis. The data were analyzed phenetically with programs in NTSYS-pc® version 1.70 (Rohlf 1992). Similarity matrices were generated from all 11 options available for two-state data in SIMQUAL. Clustering was performed using the unweighted pair-group method (UPGMA) in SAHN. Cophenetic correlation coefficients (COPH, in MXCOMP) were used to assess distortion between the similarity matrices and the resultant 11 phenograms (Rohlf and Sokal 1981; Sokal 1986).

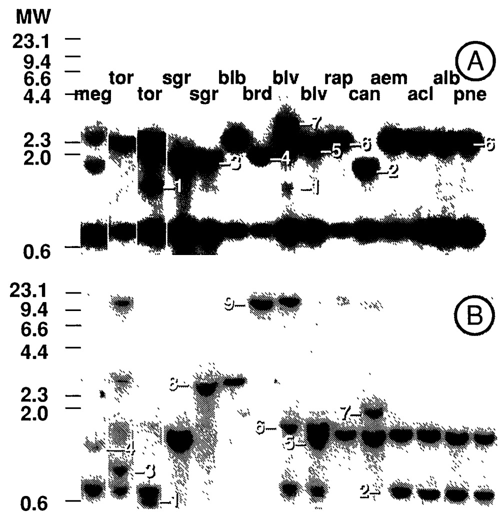

FIG. 1. Autoradiograms of selected lanes of probe/enzyme combinations. A. P93/*Dra*I. B. P209/*Hin*dIII. MW refers to molecular weights in kilobases. Numbers on the autoradiograms refer to different scored bands. acl = *Solanum acaule* subsp. *acaule*, aem = *S. acaule* subsp. *aemulans*, pne = *S. acaule* subsp. *punae*, alb = *S. albicans*, blb = *S. bulbocastanum*, blv = *S. boliviense*, brd = *S. brevidens*, can = *S. canasense*, meg = *S. megistacrolobum*, rap = *S. raphanifolium*, sgr = *S. sogarandinum*, and tor = *S. toralapanum*.

RESULTS

The 22 probes and four restriction endonucleases resulted in 60 probe/enzyme combinations that could be scored clearly. Of these 60 combinations, 11 were monomorphic across all accessions and were not used; the remaining 49 (82%) showed polymorphisms and resulted in 241 scored bands.

Reduced Data Set. Phenetic Analysis. For the reduced data set, we chose the 22 probe/enzyme combinations showing the greatest number of polymorphisms per probe. This resulted in 130 scored bands. Of the 11 similarity algorithms, K1 (Kulczynski's coefficient number 1) produced a phenogram with the highest cophenetic correlation coefficient (0.97). However, the K1 option produced a phenogram (not shown) in which all branching points were so compressed that phenetic structure was not clearly discernible. The remaining algorithms resulted in cophenetic correlation coefficients ranging from 0.84–0.90, and in topologies identical or nearly identical to K1's but with less compressed branch connections. Rohlf (1992) indicates that cophenetic values above 0.9 represent very good fits between the similarity matrix and the resultant phenogram, and values between 0.8–0.9 represent good fits. To show branch connections clearly, we present a phenogram with topology identical to K1's but with less compressed nodes [Fig. 2, based on the UN2 option (Unnamed coefficient 2), with a cophenetic correlation coefficient of 0.9], with the understanding that phenetic structure is best represented by a phenogram with the topology of Figure 2, but with greatly compressed nodes.

In general, accessions form taxon-specific clusters (Fig. 2). The only exceptions to taxon clustering were *S. megistacrolobum* (4-7, 83-30, 84-31, 86-31) and *S. toralapanum* (109-17 and 117-Z). The two accessions of *S. sogarandinum* clustered together, and these clustered with all four taxa within series *Acaulia*, that clustered with a "misplaced" accession of *S. megistacrolobum* (83-30) and the "misplaced" accession of *S. toralapanum* (109-17). *Solanum raphanifolium* and *S. canasense* clustered together, but *S. raphanifolium*

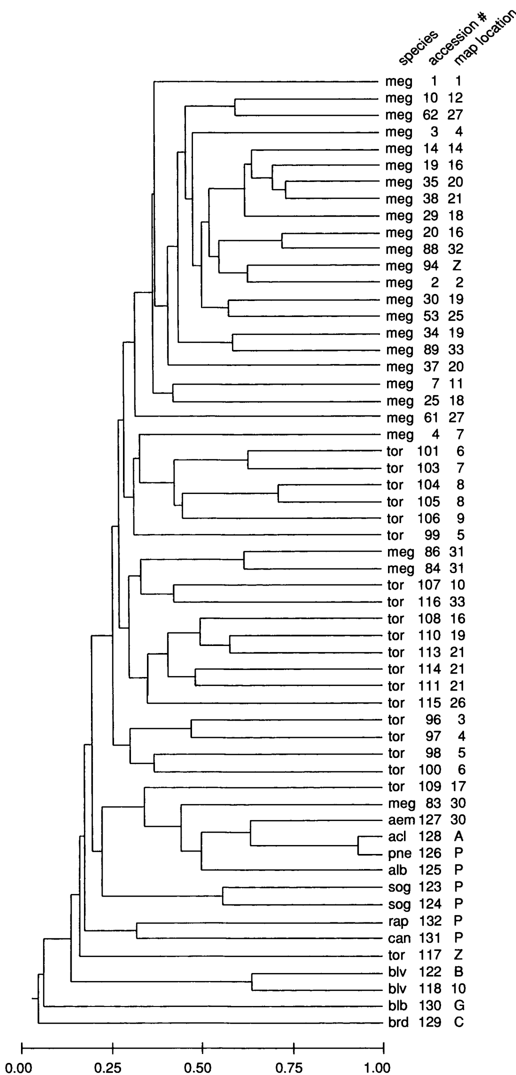

FIG. 2. UPGMA phenogram (UN2 similarity option) based on the 130 nRFLP bands of *Solanum acaule* subsp. *acaule* (acl), *S. acaule* subsp. *aemulans* (aem), *S. acaule* subsp. *punae* (pne), *S. albicans* (alb), *S. boliviense* (blv), *S. brevidens* (brd), *S. bulbocastanum* (blb), *S. canasense* (can), *S. megistacrolobum* (meg), *S. raphanifolium* (rap), *S. sogarandinum* (sgr), and *S. toralapanum* (tor). See Figure 1 and Table 2 of Giannattasio and Spooner (1994) for map locations. The letters refer to accessions not appearing on that map. A = Argentina, B = Bolivia, C = Colombia, G = Guatemala, P = Peru, and Z = unknown location.

showed no combination of bands that were additive profiles between *S. canasense* and *S. megistacrolobum* (Fig. 1A, B). The two accessions of *S. boliviense* clustered together, and were nearly as phenetically separated from the remaining species as were the very unrelated species, *S. brevidens* and *S. bulbocastanum*.

Entire Data Set. Phenetic Analysis. Similar to the results of the reduced data set, K1 resulted in a phenogram (not shown) with the highest cophenetic correlation coefficient (0.97), but with the nodes so highly compressed that branch points were not clearly discernible. The remaining algorithms resulted in lower cophenetic correlation coefficients. The topologies of these phenograms, as in Figure 2, generally retained taxon-specific clusters, but differed in the internal arrangements of accessions within the *S. megistacrolobum* and *S. toralapanum* clusters. The "misplaced" accessions are the same as in the phenogram of the reduced data set (Fig. 2), except that *S. megistacrolobum* 84-31 and 86-31 cluster with other accessions of *S. megistacrolobum*.

Eighteen of the 60 probe/enzyme combinations resulted in at least one band that was unique to *S. albicans* relative to the three subspecies of *S. acaule*, while the reduced data set had 7 of 22 probe/enzyme combinations with this pattern. The following discussion reports figures from the entire data set first, with those from the reduced data set in parentheses. Of these 18 (7) combinations, only 2 (1) had bands unique to *S. albicans* relative to all accessions here examined. The remaining 16 (6) probe/enzyme combinations showed bands that were shared by at least one accession of the following: *S. brevidens* 1 (1), *S. bulbocastanum* 1 (1), *S. canasense* 6 (2), *S. megistacrolobum* 6 (3), *S. raphanifolium* 6 (3), *S. sogarandinum* 6 (2), and *S. toralapanum* 13 (6).

DISCUSSION

The phenograms weakly separated *S. megistacrolobum* and *S. toralapanum*, but there were no taxon-specific probe/enzyme combinations, and multivariate techniques were necessary to distinguish these taxa. Also, there were some "misplaced" accessions of *S. megistacrolobum* (Fig. 2). Some of these "misplaced" accessions, however, can be accounted for by reasonable explanations. *Solanum megistacrolobum* (4-7) also clus-

tered with other accessions of *S. toralapanum* in the morphological analysis (Giannattasio and Spooner 1994), and was collected on the eastern slope of the Andes mountains where *S. toralapanum* is most common (Giannattasio and Spooner 1994), and near the type locality of this species. This accession is not listed in Hawkes and Hjerting (1989) or Ochoa (1990), and our only identification comes from Hanneman and Bamberg (1986). The NRSP-6 records indicate that it was identified as *S. megistacrolobum* by Carlos Ochoa from living plantings at NRSP-6 in 1983 and 1987. The concordance of morphological, molecular, and distributional data suggests that it has been misidentified.

Solanum megistacrolobum 83-30 clusters closely with *S. acaule* subsp. *aemulans* 127-30. *Solanum megistacrolobum* 83-30 was collected near the locality of *S. acaule* subsp. *aemulans* in Argentina, Province of Jujuy. This subspecies is known only from two localities in northwestern Argentina, one in the Province of Jujuy and the other in the Province of La Rioja. A separate nRFLP analysis grouped both populations of subsp. *aemulans* as distinct from the other subspecies of *S. acaule*, and further was able to distinguish the two populations from each other (Hosaka and Spooner 1992). The populations of *S. acaule* subsp. *aemulans* from the Province of Jujuy have been hypothesized to be of hybrid origin from *S. acaule* subsp. *acaule* and *S. megistacrolobum* that contributed $2n$ gametes (Okada and Clausen 1982). *Solanum acaule* subsp. *acaule*, subsp. *aemulans*, and *S. megistacrolobum* all occur in this area (Okada and Clausen 1982). Our data suggest one of three alternatives: 1) *S. megistacrolobum* 83-30 has introgressed with *S. acaule* subsp. *aemulans* from the Province of Jujuy, 2) The taxa are cladistically related and have shown incomplete divergence from this area, or 3) *S. megistacrolobum* 83-30 is misidentified and is *S. acaule* subsp. *aemulans*. The morphological analysis (Giannattasio and Spooner 1994) groups *S. megistacrolobum* 83-30 with other populations of *S. megistacrolobum*, but no accessions of *S. acaule* subsp. *aemulans* were analyzed there to show possible clustering with this taxon, so we cannot address the third alternative hypothesis with phenetic data. However, this accession looks to us like *S. megistacrolobum*. The phenogram from the reduced data set differs from that produced by the entire data set by the "misplacement" of two additional accessions of *S. megistacrolobum* (86-31, 84-31) within the *S. toralapanum* cluster. It is of interest that these two accessions were collected from the same geographic area (31; see Figure 1 of Giannattasio and Spooner 1994). An alternative explanation to hybridization for the "misplaced" accessions of *S. megistacrolobum* and *S. toralapanum* mentioned above is chance stochastic events leading to parallel mutations.

The results showing no additive bands in *S. raphanifolium* relative to *S. canasense* and *S. megistacrolobum* (or *S. toralapanum*) provide no support for Ugent's (1970) hybridization hypothesis of *S. raphanifolium*. These data are concordant with results from chloroplast DNA and nrDNA (Spooner et al. 1991) in discounting this hybridization hypothesis.

Separately, our results fail to identify a possible third genome donor for *S. albicans*. Our results, like those of Hosaka and Spooner (1992), showed many *S. albicans* specific bands relative to *S. acaule*, but these bands were shared with all other species examined here (see Results). It has been assumed that *S. albicans* has arisen from hybridization of *S. acaule* and an unidentified species. This hypothesis was generated by the morphological similarity of *S. albicans* to *S. acaule* (Hawkes 1963) and by meiotic analyses of trihaploid derivatives of *S. albicans* (Matsubayashi 1991). Our results (Fig. 2) support the relationship of *S. albicans* to *S. acaule*. The third genome hypothesis depends on pairing relationships not being under genetic control, but this assumption has been questioned by Lamm (1945) and Dvořák (1983) in *Solanum* sect. *Petota*. It is possible, therefore, that *S. albicans* is not of hybrid origin based on meiotic data. However, the large number of *S. albicans* specific bands relative to *S. acaule* supports a third genome donor, but this possible donor has not been identified here.

As in the morphological study (Giannattasio and Spooner 1994), the primary objective here was to examine the extent and geographical partitioning of differences between *S. megistacrolobum* and *S. toralapanum*, and to use these data to assess contemporary divergent hypotheses regarding their taxonomic circumscriptions and ranks [i.e., varieties (Ochoa 1990) or species (Hawkes 1990; Hawkes and Hjerting 1989)]. Similarities between the results of this molecular study and a separate morphological study (Giannattasio and Spooner 1994) show: 1) there is much intraspecific variation within these two taxa, 2) there are no individual species-spe-

cific characters (morphological or molecular) to distinguish them, 3) the taxa generally can be distinguished, but only by multivariate techniques, where accessions occasionally cluster with other taxa (these taxa are partitioned into contiguous and somewhat overlapping geographical areas, with *S. megistacrolobum* generally to the east of *S. toralapanum*), and 4) the branch lengths distinguishing taxa are small relative to the lengths of the ultimate branches, suggesting more intra-than interspecific variability, resulting in a weak separation of taxa.

These results, therefore, are concordant with treating *S. toralapanum* as a variety of *S. megistacrolobum* [as *S. megistacrolobum* var. *toralapanum* (Cárd. and Hawkes) Ochoa]. We agree with Hamilton and Reichard (1992) and Stuessy (1990) who suggest that subspecies should be used when only one category below species is needed. The use of subspecies and varieties for the same taxon by different authors has inflated the large number of names in *Solanum* sect. *Petota*. This group already has 531 validly published basionyms, plus 67 *nomina nuda* or *nomina dubia*, and subsequent transfers to other ranks raise the number of names to 664 (Spooner and van den Berg 1992). Despite our reluctance to add new names to this group, we propose the new combination **S. megistacrolobum** subsp. ***toralapanum*** in order to bring consistency to taxa within sect. *Petota* where different authors recognize identical taxa as varieties or subspecies. Our synonymy is in agreement with those provided by Hawkes and Hjerting (1989) and Ochoa (1990).

SOLANUM MEGISTACROLOBUM Bitter, Repert. Spec. Nov. Regni Veg., 10: 536. 1912.—TYPE: Bolivia, Dept. Tarija, Prov. Aviles, Puna Putanca, 3700 m, 8 Jan. 1904, *K. Fiebrig 2618 p.p.* (lectotype, Hawkes and Hjerting 1969, p. 289]: S [drawing: Hawkes and Hjerting 1989, p. 217!, photo: BM!]; isotypes: B [photos: G!, BM!, drawing: Hawkes and Hjerting 1989, p. 218!], G—2 sheets! [photos: BM!, LL!], GOET—2 sheets [photos: BM!, LL!, herb. of the Nat. Research Support Program-6!], LD [photos: BM!, LL!], M—3 sheets! [photos: BM!, LL!], NY [photos: BM!, LL!], S—2 sheets [photos: BM!, LL!], U [photos: BM!, LL!], W! [photos: BM!, LL!, herb. of the Natl. Research Support Program-6!], Z [photos: BM!, LL!]).

Solanum alticola Bitter, Repert. Spec. Nov. Regni Veg. 12: 5. 1913, as *'alticolum'*.—TYPE: Bolivia, Dept. La Paz, Prov. Larecaja, 1860, *G. Mandon 398* (holotype: P [photos: G!, LL!]).

Solanum alticola 'alticolum' var. *xanthotrichum* Hawkes, Bull. Imp. Bur. Pl. Breed. Genet., Cambridge: 42, 120. 1944.—TYPE: Argentina, Prov. Jujuy, Dept. Tilcara, San Gregorio, above Tilcara, 4000 m, 15 Feb 1939, *E. K. Balls 5986 p. p.* (lectotype, Hawkes and Hjerting 1969, p. 291: herb. of the Commonwealth Potato Collection, Scottish Crop Research Inst., Edinburgh, Great Britain [photos: BM!, LL!, drawing: Hawkes and Hjerting 1969, p. 291!; isotype: BOLV! [photo: PTIS!]).

Solanum tilcarense Hawkes, Bull. Imp. Bur. Pl. Breed. Genet., Cambridge: 41, 119. 1944.—TYPE: Argentina, Prov. Jujuy, Dept. Tilcara, San Gregorio, above Tilcara, 4000 m, 15 Feb 1939, *E. K. Balls 5986 p. p.* (lectotype, Hawkes and Hjerting 1969, p. 291: herb. of the Commonwealth Potato Collection, Scottish Crop Research Inst., Edinburgh, Great Britain, [drawing: Hawkes and Hjerting 1969, p. 291!, photos: BM!, LL!]; isotypes: K! [photos: BM!, LL!, herb. of the Natl. Research Support Program-6!], NA [photo: LL!], US [photo: BM!]).

Solanum megistacrolobum subsp. **toralapanum** (Cárd. and Hawkes) R. B. Giannattasio and D. M. Spooner, comb. et stat. nov.—*S. toralapanum* Cárd. and Hawkes, J. Linn. Soc., Bot. 53: 98. 1945.—*S. megistacrolobum* var. *toralapanum* (Cárd. and Hawkes) Ochoa, *The potatoes of South America: Bolivia.* 170. 1990 [actual release date 13 Jun 1991 (Spooner and van den Berg 1992)], Cambridge: Cambridge Univ. Press.—TYPE: Bolivia, Dept. Cochabamba, Prov. Arani, Tiraque, Hacienda Toralapa, 3700 m, Feb 1944, *M. Cárdenas 3504* (lectotype, Hawkes and Hjerting 1989, p. 230: K! [drawing: Hawkes and Hjerting 1989, p. 227!, photo: herb. of the Natl. Research Support Program-6!]; isotype: herb. of the Commonwealth Potato Collection, Scottish Crop Research Inst., Edinburgh, Great Britain [photos: BM!, LL!]).

Solanum decurrentilobulum Cárd. and Hawkes. J. Linn. Soc., Bot. 53: 97. 1945.—TYPE: Bolivia, Dept. Cochabamba, Prov. Arani, near Tiraque, Hacienda Toralapa, 3700 m, Feb 1944, *M. Cárdenas 3503* (lectotype, Hawkes and

Hjerting 1989, p. 230: K! [photo: herb. of the Natl. Research Support Program-6!]; isotype: herb. of the Commonwealth Potato Collection, Scottish Research Inst., Edinburgh, Great Britain [photos: BM!, LL!]).

Solanum toralapanum var. *subintegrifolium* Cárd. and Hawkes, J. Linn. Soc., Bot. 53: 99. 1945.—TYPE: Bolivia, Dept. Cochabamba, Prov. Arani, near Tiraque, Hacienda Toralapa, 3700 m, Feb 1944, *M. Cárdenas 3505 p. p.* (holotype: K! [photo: herb. of the Natl. Research Support Program-6!]; isotypes: herb. of the Commonwealth Potato Collection, Scottish Crop Research Inst., Edinburgh, Great Britain—2 sheets [photos: BM!, LL!]).

Solanum ellipsifolium Cárd. and Hawkes, J. Linn. Soc., Bot. 53: 100. 1945.—TYPE: Bolivia, Dept. Cochabamba, Prov. Arani, near Tiraque, Hacienda Toralapa, 3700 m, Feb 1944, *M. Cárdenas 3505 p. p.* (holotype: K! [photo: herb. of the Natl. Research Support Program-6!]; isotype: herb. of the Commonwealth Potato Collection, Scottish Crop Research Inst., Edinburgh, Great Britain [photo: BM!]).

Solanum ureyi Cárd., Bol. Soc. Peruana Bot. 5: 32, pls. III-B, IV-D. 1956.—TYPE: Bolivia, Cochabamba, Prov. Ayopaya, Hacienda Sailapata, 3600 m, Nov 1935 (on herbarium sheet, but Nov 1936 in publication), *M. Cárdenas 3262* (holotype: herbarium of the Departamento de Fitotécnia, Facultad de Ciencias Agrícolas y Pecuarias, Universidad de San Simón, Cochabamba, Bolivia! [photo: herb. of the Natl. Research Support Program-6!]; isotype: US [photos: BM!, LL!]).

The results from this study build upon a growing body of evidence demonstrating the utility of nRFLP's to address systematic questions at low taxonomic levels. Some of these studies, such as that done in lettuce (Landry et al. 1987) have not correlated with isozyme data. More commonly, data from nRFLP's do correlate with isozyme and/or morphological data, and the methods detect more variability. This generally has been the case in bamboo (Friar and Kochert 1991), banana (Jarret et al. 1992), beans (Chase et al. 1991), mustards (Figdore et al. 1988), lentil (Havey and Muehlbauer 1989), maize (Evola et al. 1986; Helentjaris et al. 1985; Melchinger et al. 1991; Rivin et al. 1983), melons (Neuhausen 1992), peanut (Halward et al. 1991; Kochert et al. 1991), potato (Debener et al. 1990, 1991), rice (McCouch et al. 1988), sorghum (Aldrich and Doebley 1992), soybean (Menancio et al. 1990), and tomato (Helentjaris et al. 1985). Many questions remain regarding species boundaries and hypotheses of hybridization in potato (Spooner and van den Berg 1992) that will benefit from continuing morphological and nRFLP studies.

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