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A MOLECULAR REEXAMINATION OF DIPLOID HYBRID SPECIATION OF *SOLANUM RAPHANIFOLIUM*[1]

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Interspecific hybridization has long been the focus of intensive study in many groups of plants. Since the pioneering work of Anderson (1949), hybridization has been implicated in the formation of sporadic, nonviable, "accidental" hybrids, introgressive hybridization, and the production of new species (Raven, 1976, 1980; Grant, 1981). Hybrid speciation at the diploid level presents a special case where the immediate and potentially strong isolating mechanism provided by allopolyploidy that separates the newly derived hybrid from its parents is absent. Diploid hybridization hypotheses have invoked a number of isolating mechanisms to maintain the integrity of the newly formed species such as ecogeographical, genetic, or mechanical isolation mechanisms (Levin, 1978; Grant, 1981). Most of these hybridization hypotheses were initially inferred on the basis of intermediate morphology and distributional data, but many have been further tested by artificial crosses, cytogenetic data, and secondary chemistry. The strengths and weaknesses of these methods have been pointed out by various workers (Gottlieb, 1972; Gallez and Gottlieb, 1982; Crawford, 1985; Rieseberg et al., 1988). The major problems with morphological, and to a lesser extent with cytogenetic and secondary chemical data, are our present ignorance of their genetic basis and our resulting inability to reliably distinguish between competing hypotheses of hybridization, convergent evolution, retention of ancestral characters, and phenotypic plasticity (Rieseberg et al., 1988; Sytsma, 1989).

Recent work with genetically more precise isozyme and DNA characters have provided new insights into hybridization hypotheses, with some supported (Roose and Gottlieb, 1976; Gallez and Gottlieb, 1982; Palmer et al., 1983; Soltis and Soltis, 1989; Warwick et al., 1989; Rieseberg et al., 1990) and some questioned or rejected (Rieseberg et al., 1988, 1990; Crawford and Ornduff, 1989). Molecular data also have indicated hybridization where it previously has not been suspected (Palmer et al., 1983; Smith, 1988; Doebley, 1989; Smith and Sytsma, 1990). Additionally, molecular data have been useful in documenting that natural hybridization does not always lead to speciation (Rick, 1974; Levin, 1975; Bell and Lester, 1978; Doebley et al., 1984; Doyle et al., 1985; Palmer et al., 1985; Soltis and Soltis, 1986; Doyle and Doyle, 1988; Doyle and Brown, 1989).

Solanum sect. *Petota*, the potato and its wild relatives, is a group where natural hybridization and hybrid speciation has been hypothesized to be prevalent (Hawkes, 1958, 1962; Ugent, 1970*a*; Hawkes, 1990). Hawkes (1990) includes 232 species in the group, which he divides into 21 taxonomic series. The species are widely distributed throughout the Americas from southwestern Nebraska to southern Chile. Natural hybrids are believed to be quite common in some areas. For example, Hawkes and Hjerting (1969) state that 9.5% of the collections they examined from Argentina, Brazil, Paraguay, and Uruguay represented hybrids. Although there are well-developed sterility barriers between some species (Grun, 1961; Grun et al., 1962, 1977; Hawkes, 1958, 1981; Johnston and Hanneman, 1982), the majority of species can cross naturally or artificially. These hybrids are highly fertile, at least in the F_1 generation, even in many interseries crosses (Hawkes and Hjerting, 1969, 1989).

Solanum raphanifolium (ser. *Megistacroloba*) was hypothesized by Ugent (1970*b*) to be a recent stabilized diploid hybrid species between *S. canasense* (ser. *Tuberosa*) and *S. megistacrolobum* (ser. *Megistacroloba*). This hypothesis was supported on the basis of a number of features: 1) The morphology of *S. raphanifolium* is strikingly intermediate between its two putative parents, especially in the features of habit, leaves (Fig. 1), and flower shape (Table 1); 2) *Solanum raphanifolium* occurs in weedy disturbed habitats unlike the generally undisturbed habitats of its putative parents; 3) *S. raphanifolium* occurs in a restricted overlap zone between the two species in southern Peru (Fig. 1); and 4) Ugent (1970*b*) found many intermediate individuals in this overlap zone that he believed were F_1 and later generation hybrids between *S. canasense* and *S. megistacrolobum* suggesting that hybridization was an ongoing process. This combination of morphological, ecological, distributional, and field evidence made this a well-documented a very reasonable hypothesis.

This study was initiated to test the *S. raphanifolium* hybridization origin hypothesis with chloroplast DNA (cpDNA) and 18S-25S nuclear ribosomal DNA (nrDNA) characters. Assuming relatively recent or even ongoing hybridization between the two putative parental species, the expectation is for the hybrid to possess an identical, or nearly identical maternally inherited cpDNA pattern to one parent (Hosaka et al., 1984;

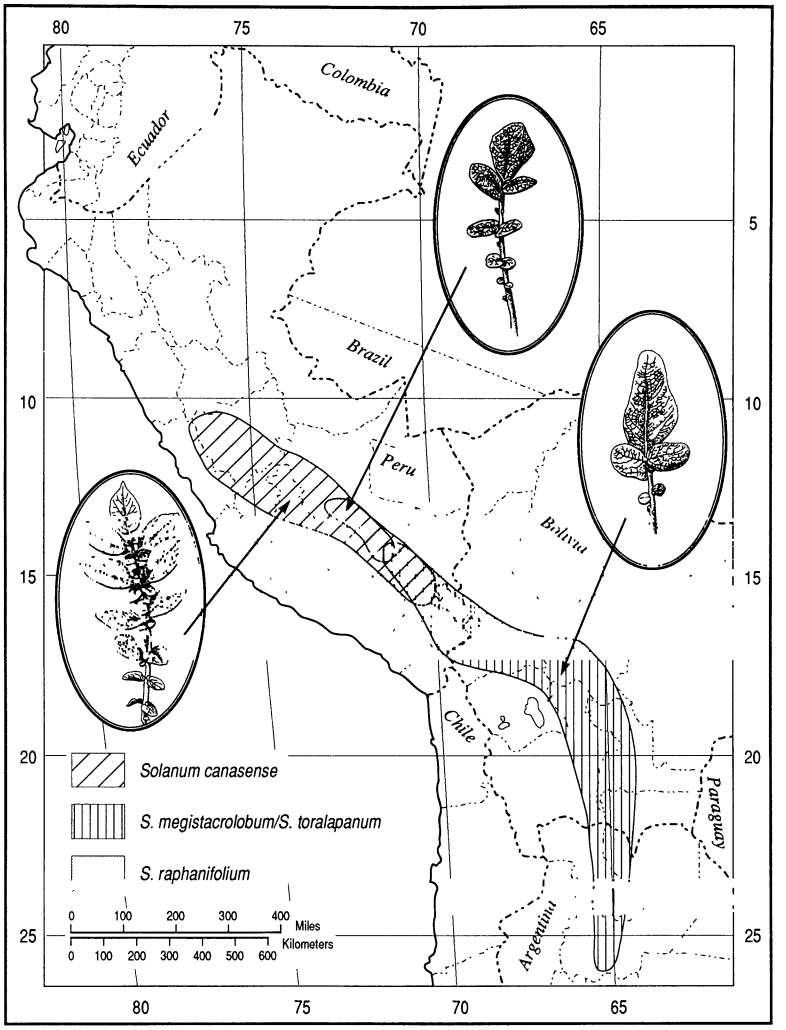

FIG. 1. Distribution of *Solanum canasense, S. megistacrolobum/S. toralapanum,* and *S. raphanifolium* in Peru, Bolivia, and Argentina. The intermediacy of leaf morphology for *S. raphanifolium* is shown relative to its putative parental species.

Corriveau and Coleman, 1988) and an additive biparentally inherited nrDNA pattern from both parents (Doyle et al., 1985; Doyle and Doyle, 1988; Rieseberg et al., 1988). If *S. raphanifolium* were not derived from recent diploid hybridization, a cpDNA analysis would place it with either of the other two species but exhibiting a number of autapomorphies, or perhaps even place it as a sister group to the other two species; the nrDNA pattern would not be expected to exhibit additivity.

MATERIALS AND METHODS

Plants.—Seeds of *S. canasense* (USDA Plant Inventory, PI, numbers 210035, 230511, 246533, 265863, 265864, 265875, 283074, 283080, 283084, 310940, 458375, 458377, 442696, 473345, 473346, 473347, 473355), *S. megistacrolobum* (PI 210034, 310978, 435072, 458346, 458348, 473109, 473118, 473121, 473128, 473137, 473146, 473159, 473356, 473360, 473361, 498257, 498258, 498259, 498263, 500031), *S. raphanifolium* (PI 265862, 310953, 310998, 458382, 458406, 473370, 473465, 473466, 473502, 473526; intraspecific hybrids, female first, 246539 × 210048, 290944 × 246539) and *S. toralapanum* (195210, 458396, 472804, 472806, 472807, 472808, 473389, 498144, 498145, 498146), respectively (ingroup species); and of one accession each of *S. bulbocastanum* (PI 275184), *S. ochranthum* (PI 365919), and *S. pinnatisectum* (PI 275320) (outgroup species) were obtained from the Inter-Regional Potato Introduction Project (IR-1; Hanneman and Bamberg, 1986). The *S. canasense* accessions were from Peru, Departments of Ayacucho, Cuzco and Puno; *S. megistacrolobum* from Argentina, Departments of Jujuy and Salta, and Bolivia, Departments of Cochabamba, La Paz, and Potosí; *S. raphanifolium* from Peru, Department of Cuzco; and *S. toralapanum* from Argentina, Department of Salta, and from Bolivia, Department of Chuquisaca

TABLE 1. Comparison of *Solanum canasense, S. raphanifolium,* and *S. megistacrolobum* (from Ochoa, 1962; Ugent, 1970*a*; Hawkes, 1990).

	S. canasense	*S. raphanifolium*	*S. megistacrolobum*
Habit	Tall, erect	Decumbent	Low, rosette
Leaves	Highly dissected, with 8–12 laterals and 4–25 interjecteds; laterals sessile or short-petiolate, no decurrency on petiole.	Intermediate in dissection with 4–8 laterals and 0–6 interjecteds; laterals sessile, often decurrent on petiole.	Simple to poorly dissected with 0–4 laterals and no interjecteds; laterals highly decurrent on petiole.
Corolla shape, color	Broadly rotate, light blue-purple.	Pentagonal to semi-rotate, purple or violet.	Stellate to pentagonal-rotate, purple to blue-lilac.
Habitat	Steep mountain slopes, in rocky or shrubby places, occasionally in field borders and roadsides. 2,900–4,100 m elev.	Growing in disturbed areas with native and introduced weeds, among rocks, grass, or shrubs, often in cultivated fields. 2,800–3,800 m elev.	In grassy areas in the altiplano. 3,500–4,450 m elev.
Distribution	Central to southern Peru.	Southern Peru (Cuzco), peripatrically distributed in the overlap zone of *S. canasense* and *S. megistacrolobum.*	Southern Peru, Bolivia, northern Argentina.

and Cochabamba; *S. bulbocastanum* from Mexico; *S. ochranthum* from Ecuador and *S. pinnatisectum* from Mexico. *Solanum raphanifolium,* PI 473506 is from Peru, not Bolivia as it appears in Hanneman and Bamberg (1986). *Solanum toralapanum* was included in this analysis because of its very close relationship to *S. megistacrolobum. Solanum toralapanum* has been considered to be conspecific with *S. megistacrolobum* (Ochoa, 1984), informally referred to as a variety of it (Johns et al., 1987), or as a closely related species (Hawkes and Hjerting, 1989; Hawkes, 1990). For the purposes of this paper, *S. megistacrolobum* will be discussed to include *S. toralapanum.*

DNA Isolation and Restriction-Site Comparison.—Five grams of fresh leaf tissue were collected from pooled leaf samples of one to eight plants per accession from two-month old plants. Preparations of total DNA were made following the procedure of Doyle and Doyle (1987) and purified over CsCl/ethidium bromide gradients. Restriction endonuclease digestions, agarose-gel electrophoresis, bidirectional transfer of DNA fragments from agarose gels to nylon filters, labeling of recombinant probes by nick-translation, filter hybridization, and autoradiography were conducted following the methods of Palmer (1986). Twenty endonucleases were used to examine cpDNA and nrDNA variation in *Solanum* (*Bam* HI, *Ban* I, *Ban* II, *Bcl* I, *Bgl* II, *Bst* NI, *Bst* XI, *Cla* I, *Dra* I, *Eco* RI, *Eco* RV, *Hae* II, *Hinc* II, *Hind* III, *Hph* I, *Nci* I, *Nsi* I, *Sst* I, *Ssp* I, and *Xba* I). Membrane filters were probed with 12 *Pst* I and two *Sal* I clones representing nearly the entire chloroplast genome of *Petunia* (Sytsma and Gottlieb, 1986*a*). One clone of *Lactuca* was used, representing the small single-copy region between the inverted repeats of the cpDNA molecule (Jansen and Palmer, 1987). For nrDNA restriction site variation, the 18S-25S nrDNA repeat from *Glycine max* (courtesy of E. Zimmer) was used to reprobe the filters used in the cpDNA analysis.

Data Analysis.—Restriction site divergence was quantified by the *p* values of Nei and Li (1979), where p = proportion of base substitutions per nucleotide position. Restriction site data were analyzed using two parsimony approaches. Wagner parsimony, which weights equally convergent site gains and convergent site losses was performed using PAUP (version 2.4.1, D. Swofford, Illinois Natural History Survey, Urbana). The most parsimonious trees were sought (MULPARS option) with global branch swapping (SWAP = GLOBAL) and a consensus tree was constructed of equally parsimonius trees (CONTREE). Dollo parsimony (LeQuesne, 1974; DeBry and Slade, 1985), which discriminates against convergent site gains or loss/gains and invokes instead additional convergent site losses or gain/losses, also was performed (PHYLIP, version 2.7). The trees were rooted by outgroup comparison with *S. ochranthum.* Although the outgroup relationships of *Solanum* sect. *Petota* are not fully resolved, Hawkes (1990) includes *S. ochranthum* in sect. *Petota* but in a distinct subsection (subsection *Estolonifera* Hawkes) separated from all of the other species here examined (subsection *Potatoe* G. Don). To provide further insights into outgroup structure, two additional species from subsection *Potatoe* were included in the analysis: *S. bulbocastanum* (ser. *Bulbocastana*) and *S. pinnatisectum* (ser. *Pinnatisecta*). These two diploid Mexican species are isolated by strong crossability barriers from the South American ingroup species and also are apparently relatively distantly related to them (Hawkes, 1958; Johnston and Hanneman, 1982; Hosaka et al., 1984).

RESULTS

Chloroplast DNA Analysis.—Approximately 650 restriction sites were identified in each *Solanum* accession for a total of 3,770 nucleotide base pairs in each chloroplast genome with an average size of 155 kb. About 2.4% of the *Solanum* chloroplast genome was sampled. The greatest genetic distance among the species was between *S. ochranthum* and the ingroup species ($p = 0.0095$). The greatest amount of interspecific

divergence within the ingroup species is found between *S. megistacrolobum* and *S. raphanifolium* ($p = 0.0013$, range = 0.0008–0.0021). *Solanum canasense* and *S. megistacrolobum* showed the lowest amount of interspecific cpDNA divergence within the ingroup species (mean $p = 0.0007$, range 0.0–0.0015). Considerable intraspecific site variation was seen in both *S. canasense* (0.0013, maximum values) and *S. megistacrolobum* (0.0015), the highest values of which approximated the average divergence between the ingroup species. *Solanum raphanifolium,* however, exhibited insignificant levels of intraspecific variation (mean = 0.0003). Some deletions or insertions were seen in *S. ochranthum* relative to the other species. Because of uncertainty as to the nature and homology of these deletions or insertions (Palmer et al., 1985; Sytsma and Gottlieb, 1986*a*), these were not included in the phylogenetic analysis.

Out of a total of 67 site mutations, 46 occurred in more than one accession and thus were phylogenetically informative. A full list of these mutations is available from the authors. No site mutations were observed with *Bgl* II and *Bst* XI. Wagner parsimony analysis produced over 100 equally parsimonius 87-step trees with a consistency index of 0.77. The large number of equally parsimonius trees resulted both from zero-length branches (unresolved nodes) and homoplasy within the ingroup.

A strict consensus tree (by PAUP) of 100 Wagner trees (Fig. 2) clearly distinguishes *S. bulbocastanum, S. pinnatisectum,* and *S. ochranthum.* This tree also unites all populations of *S. canasense, S. megistacrolobum,* and *S. raphanifolium* in an unresolved polychotomy, but *S. raphanifolium* is the only ingroup species distinguished as a monophyletic lineage, and is defined by four cpDNA mutations. Two of these four mutations, numbers in kilobases (*Dra* I, probe P8, 2.1 = 2.0 + [0.1]; *Hae* II, probes P8/P10, 9.5 = 7.0 + 2.5) are possessed exclusively by all populations of *S. raphanifolium,* the other two (*Dra* I, probe P3, 4.8 + 1.0 = 5.8, *Eco* RI, probes P16/S6, 5.8 = 4.1 + 1.7) are shared by all populations of *S. raphanifolium, S. ochranthum,* and two populations of *S. megistacrolobum* (PI 473356, 473360).

Nuclear Ribosomal-DNA Analysis.—Restriction site variation was screened for 16 of the 20 enzymes used in the cpDNA analysis. The *Dra* I, *Hae* II, *Hinc* II, and *Nci* I variations were too difficult to interpret due to poor cutting or complex banding patterns and were not used. The *Bam* HI, *Bcl* I, *Cla* I, *Eco* RI, *Hind* III, *Hph* I, *Nsi* I, and *Xba* I patterns showed no intra- or interspecific variability. Interspecific, but no intraspecific variability was observed in the eight remaining enzymes, specifying 13 site mutations (list available from the authors). Fourteen equally parsimonius Wagner trees with a length of 14 steps and a consistency index of 0.93 were generated. A strict consensus tree (not shown) exhibits an unresolved node for all species except *S. ochranthum,* due to incompatability involving two characters (*Bgl* II, site gain and *Bst* XI, site loss). Invoking Dollo parsimony generates only three equally parsimonious trees of length 14. These three Dollo trees produce a consensus tree with an unresolved trichotomy (Fig. 3). This tree thus is the best representation of relationships in the species based on nrDNA using both Wagner and Dollo parsimony.

Discussion

Intraspecies Variation.—The intraspecific cpDNA polymorphisms present within *S. canasense, S. megistacrolobum,* and *S. toralapanum* parallels that found in other wild potatoes (Hosaka and Hanneman, 1988) and in other groups of plants (see Soltis and Soltis, 1989, for a summary). However, unlike the results reported in Soltis and Soltis (1989), there is no geographical component to the cpDNA variation that would indicate its utility to elucidate phylogenetic relationships in this group. Some individual polymorphic mutations in these three species show a geographical component, but the sum total of the cpDNA site mutations shows no intraspecific geographical partitioning. Three explanations could account for this pattern: 1) The ancestral populations of these three species were highly polymorphic in cpDNA types; 2) Chance convergent cpDNA mutations occurred among geographically separated populations; and 3) Matriarchal lineage sorting (Neigel and Avise, 1986) where recently evolved species may share a maternal chloroplast lineage with another species and not with other conspecific populations. No examples of this phenomenon have yet been fully documented in plants (Sytsma, 1990). It is noteworthy that each species exhibits a single and distinctive nrDNA site pattern. Thus, accessions can be unambiguously placed to species based on nrDNA, but not cpDNA. The homogeneity within species of nrDNA site mutations, but not length mutations, as opposed to cpDNA, suggest that concerted nrDNA evolution might have occurred within species (Zimmer et al., 1980). Moreover, because morphology and nrDNA are consistent indicators of species boundaries but cpDNA is not, matriarchal lineage sorting of cpDNA may well be responsible for these results.

Origin of Solanum raphanifolium.—Restriction fragment analysis of cpDNA and nrDNA provide concordant results in reexamining the putative hybrid origin of *S. raphanifolium.* The cpDNA phylogeny (Fig. 2) indicates that *S. raphanifolium* is not of recent hybrid origin from the two putative parental species. Four synapomorphies support the *S. raphanifolium* clade distinct from the other two species. There are more site differences between *S. raphanifolium* and either putative parental species than there are between the two putative parents. Genetic distances based on cpDNA (*p* values) also support the highly divergent status of *S. raphanifolium,* as *S. canasense* and *S. megistacrolobum* are closer to each other than either is to *S. raphanifolium.* The two shared mutations among all populations of *S. raphanifolium* and two populations, respectively, of *S. megistacrolobum* must be viewed as convergent in light of their rare occurrence in *S. megistacrolobum* and the absence in *S. megistacrolobum* of the other mutations distinguishing *S. raphanifolium.*

The nrDNA tree (Fig. 3) also provides no support for the hybridization hypothesis. The combination of Wagner and Dollo parsimony indicates that *S. canasense* and *S. megistacrolobum* (with *S. toralapanum*) form a clade defined by a *Bgl* II site gain. *Solanum raphanifolium* represents a sister taxon to this clade and is further defined by an autopomorphic *Bam* HI site gain. Both the lack of the additivity in, and the divergent nature of *S. raphanifolium* nrDNA, argue

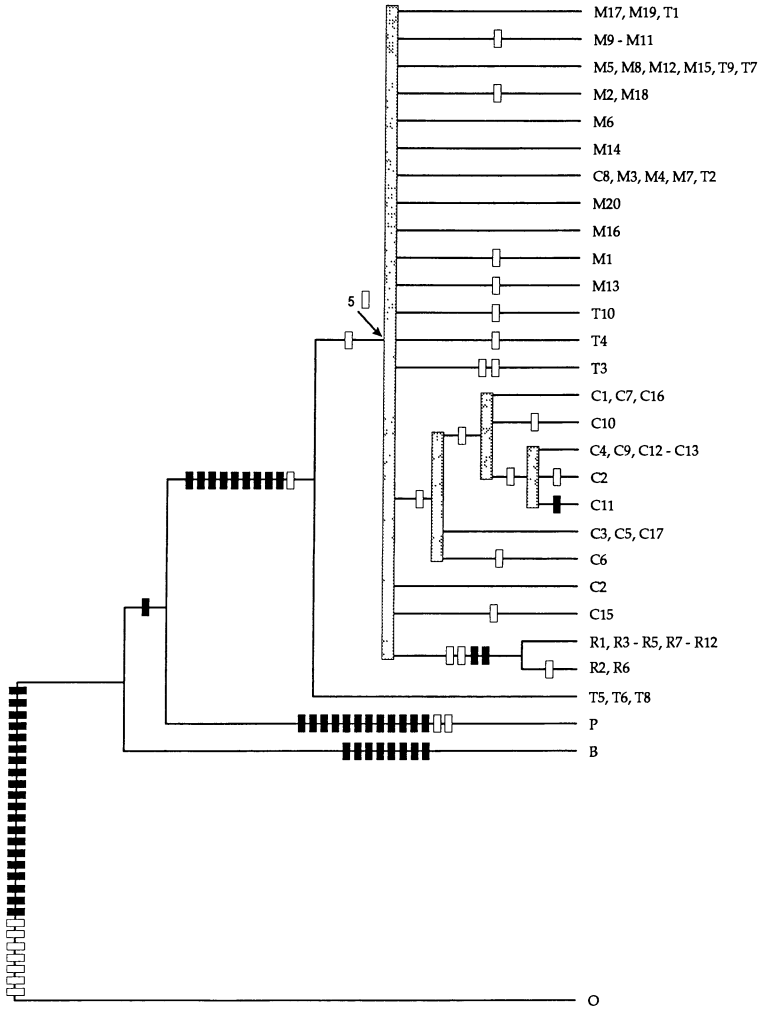

FIG. 2. Consensus Wagner tree for cpDNA restriction site variation among *Solanum canasense* (C), *S. megistacrolobum* (M)/*S. toralapanum* (T), *S, raphanifolium* (R) and three outgroup species (*S. ochranthum* [O], *S. bulbocastanum* [B], and *S. pinnatisectum* ([P]). The tree is derived from 100 equally most parsimonious Wagner trees of 87 steps (Consistency Index = 0.77). Solid bars indicate site changes occurring only once, and open bars indicate convergent mutations. The wide gray bar indicates a polychotomous node. An additional five mutations could only be placed in fully resolved trees and are indicated by the arrow at the polychotomous node.

against the recent diploid hybridization hypothesis of *S. raphanifolium*.

Discounting hybridization as the cause for the morphological intermediacy of *S. raphanifolium* necessitates other explanations for the origin of *S. raphanifolium*. The results in this study are unexpected not only because they fail to support hybridization, but also because *S. megistacrolobum* and *S. raphanifolium* are grouped in ser. *Megistacroloba* and *S. canasense* in ser. *Tuberosa*. One alternative hypothesis is that *S. raphanifolium* represents the sister species to either *S. canasense* of *S. megistacrolobum* but exhibits morphological convergence to the other species. The divergent cpDNA and nrDNA seen in *S. raphanifolium* would suggest that this would be an unlikely scenario. A more likely hypothesis for the origin of *S. raphanifolium* is that it is related to other *Solanum* species not here examined. In this case, the intermediate morphological traits (Table 1) of *S. raphanifolium* relative to its putative parents would be superficial and misleading. Of interest is that independent glycoalkaloid data (Johns and Osman, 1986) also suggest that *S. raphanifolium* may not be related to other members of *Solanum* ser. *Megistacroloba*.

It is possible, but unlikely, that *S. raphanifolium* is an ancient hybrid between *S. canasense* and *S. megistacrolobum*. This analysis fails to resolve the relationships of these ingroup species to other *Solanum* species.

The relationship of *S. raphanifolium* to other *Solanum* species will require a much wider phylogenetic analysis. Because one-half of all of the potato species are believed to occur in Peru, with a concentration in southern Peru, it was beyond the scope of this study to conduct these analyses. If *S. raphanifolium* resulted from ancient hybridization, however, it is curious why its cpDNA would have evolved at a much more rapid rate relative to its parents. Additional evidence on the hybrid origin of *S. raphanifolium* could be provided by the construction of artificial interspecific hybrids and a comparison of morphology and nuclear DNA. Hawkes (1990) alludes to unpublished evidence concerning a morphological comparison of artificial hybrids that fails to support this hybridization hypothesis. Wider cpDNA surveys of additional *Solanum* species, artificial hybridizations, and additional nuclear DNA analyses are in progress.

Hybridization and its various effects have long been accepted to be a dominant evolutionary force in *Solanum* sect. *Petota* (Hawkes, 1958, 1962; Ugent, 1970*a*). These hypotheses often have been supported by extensive and carefully gathered data from field observations, reconstruction of artificial hybrids (e.g., Astley and Hawkes, 1979; Cribb and Hawkes, 1986), and extensive morphometric analyses, utilizing both morphological and chemical characters (e.g., Johns et al., 1987). No phylogenetic study in this group has benefited, however, from detailed cladistic studies such as that of Whalen et al. (1981) in *Solanum* sect. *Lasiocarpa.* In view of the results obtained here with *S. raphanifolium,* the other hybridization hypotheses should be subjected to reinvestigation by both molecular and morphological cladistic analyses. The striking morphological intermediacy, plus the distributional and ecological data (Table 1, Fig. 1), made the diploid hybridization origin hypothesis of *S. raphanifolium* most reasonable. The myriad of other hybridization hypotheses in *Solanum* sect. *Petota* may very well hold up under the rigors of molecular reexamination, as they have in other plant groups, but must currently be suspect in light of these surprising results from *S. raphanifolium.*

The results obtained in this study build upon a growing body of evidence for plants that certain analyses using morphology sometimes can yield estimates of evolutionary relationships differing from those based on molecular studies (Sytsma, 1990). Molecular phylogenetic analyses, which can generate large data sets, have shown that concordance between morphological and molecular data sets is the rule. However, a number of examples of discordant phylogenies have been discovered (Palmer et al., 1988; Sytsma and Smith, 1988; Sytsma, 1990). In the Asteraceae, Jansen and Palmer (1988) pointed out the paraphyletic status of some currently accepted tribes; Rieseberg et al. (1988) demonstrated the nonhybrid status of some races of *Helianthus bolanderi* previously thought to be of hybrid origin; and Schilling and Jansen (1989) demonstrated the paraphyletic status of the large genus *Viguiera.* In the Onagraceae, Sytsma and Gottlieb (1986*b*) demonstrated the quite unexpected inclusion of the distinctive *Heterogaura* within *Clarkia.* In the Orchidaceae, Palmer et al. (1988) discovered the paraphyletic status of *Oncidium.* Potatoes, especially, represent a group with extensive phenotypic plasticity (Correll, 1962; Hawkes, 1990) that may make the utility of morphological characters for phylogenetic and systematic inference difficult, at best.

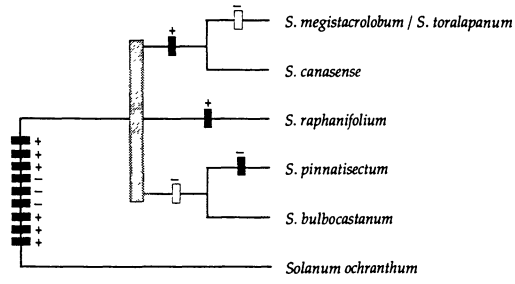

FIG. 3. Consensus Dollo tree for nrDNA restriction site variation among *Solanum canasense, S. megistacrolobum/S. toralapanum, S. raphanifolium, S. ochranthum, S. bulbocastanum,* and *S. pinnatisectum.* The tree is derived from three equally most parsimonious Dollo trees of 14 steps. Eleven additional Wagner trees of equal length (14 steps) exhibit an unlikely convergent gain rather than a more likely convergent loss, and are not included in this consensus tree. Solid bars indicate site change occurring only once, and open bars indicate convergent mutations. The symbol + indicates site gain, and the symbol − indicates site loss.

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IS SINGLE-GENE SPECIATION POSSIBLE?

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It is often claimed that speciation cannot result from changes at a single gene (Dobzhansky, 1937 pp. 255–256; Muller, 1940, 1942). The reason is that any mutation causing complete reproductive isolation from the wild type is strongly selected against: if a mutation causes postmating isolation then the first mutant individual represents an inviable or sterile hybrid genotype, and the mutant allele is immediately lost. Mutations causing premating isolation also have a trivially small chance of becoming fixed: a dominant mutation will be lost in a single generation because the first mutant individual has no compatible mate. Although a recessive mutation causing premating isolation might rarely drift to some intermediate frequency, the mutant allele will suffer a tremendous selective disadvantage once the first homozygote appears: these homozygotes will be very rare in the population and thus frequently will go unmated.

For these reasons, Dobzhansky (1937) and Muller (1940, 1942) concluded that speciation must involve two or more loci. Both introduced two-locus, epistatic models of speciation, showing how the mutations that ultimately cause reproductive isolation could be easily fixed within geographically isolated populations of initial genotype *aa bb*: mutant allele *A* sweeps through

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