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Comparison of four molecular markers in measuring relationships among the wild potato relatives *Solanum* section *Etuberosum* (subgenus *Potatoe*)

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Abstract We evaluated chloroplast DNA (cpDNA), isozymes, single to low-copy nuclear DNA (RFLPs), and random amplified polymorphic DNAs (RAPDs) in terms of concordance for genetic distance of 15 accessions each of *Solanum etuberosum* and *S. palustre*, and 4 accessions of *S. fernandezianum*. These self-compatible, diploid (2n=24), and morphologically very similar taxa constitute all species in *Solanum* sect. *Etuberosum*, a group of non-tuber-bearing species closely related to *Solanum* sect. *Petota* (the potato and its wild relatives). Genetic distance and multidimentional scaling results show general concordance of isozymes, RFLPs and RAPDs between all three taxa; cpDNA shows *S. etuberosum* and *S. palustre* to be more similar to each other than to *S. fernandezianum*. Interspecific sampling variance shows a gradation of resolution from allozyme (low) to RAPD to RFLP (high); while intraspecific comparisons graded from RFLPs (low) to RAPDs (high; lack of sufficient allozyme variability within species precluded comparisons for allozymes). Experimental error was low in RFLPs and RAPDs.

Key words Genetic distance · Potato · RAPD · RFLP · *Solanum* section *Etuberosum*

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Introduction

Solanum L. section *Etuberosum* (Juz.) A. Child is comprised of morphologically very similar species. According to Hawkes (1990), the group contains five species: *S. brevidens* Phil. (with two subspecies), *S. etuberosum* Lindl., *S. fernandezianum* Phil., *S. palustre* Schltdl., and *S. subandinum* F. Meigen. The latter has been considered to be rare or extinct by Correll (1962), Hawkes (1990), and Brücher (1966). The latest revision of section *Etuberosum* (Contreras and Spooner 1996 in press) recognizes three species, *S. etuberosum* (including *S. subandinum*), *S. fernandezianum*, and *S. palustre* (including *S. brevidens*). *Solanum palustre* and *S. etuberosum* occur in southern Chile with *S. palustre* generally further south and in adjacent southern Argentina, while *S. fernandezianum* is endemic to Masatierra Island in the Juan Fernández Archipelago, 650 km west of continental Chile (Fig. 1).

All members of section *Etuberosum* lack tubers, but otherwise are very similar morphologically to members of section *Petota* Dumort. All of the species are self-compatible and set abundant seed in nature. Despite strong crossing barriers between members of section *Etuberosum* and section *Petota*, fertile sexual hybrids can be obtained between stions via bridging species and polyploidization (Ehlenfeldt and Hanneman 1984; Hermsen and Taylor 1979; Hermsen et al. 1981).

Based on morphological and crossing data, Correll (1962) and Hawkes (1990) classified *S. etuberosum*, *S. fernandezianum*, and *S. palustre* into *Solanum* section *Petota*, series *Etuberosa* Juz. A chloroplast DNA (cpDNA) cladistic analysis (Spooner et al. 1993) examined 1 accession each of these three species in a wider examination of relationships within *Solanum*, *Cyphomandra* Sendtn., and *Lycopersicon* Mill. This analysis supported an alternate classification (Child 1990) that removed series *Etuberosa* from stion *Petota* and recognized it at the higher sectional rank. Thirty-five synapomorphies (shared derived characters) united all three species. Three synapomorphies united *S. palustre* and *S. etuberosum*, while *S. fernandezianum*

was established as a sister clade, distinguished by three autapomorphies (derived characters supporting only 1 accession). No difference was detected between *S. palustre* and *S. etuberosum*.

An allozyme study, evaluating 32 accessions, showed close relationships between all three species, with *S. palustre* and *S. etuberosum* more closely related than either to *S. fernandezianum* (Spooner et al. 1992). The study further showed lower within-population diversity for all three species compared to the diversity observed between populations.

Classification and organization of genotypes into systematic relationships is based on a wide array of morphological, biochemical, and molecular descriptors. Regardless of the type of characters chosen for analysis, all are assumed to have a genetic basis and to be appropriate. No character can be viewed in a vacuum, and its true value can only be evaluated in comparison to independent datasets (Crawford 1990). To measure genetic relationships accurately, the ideal descriptor would provide an unbiased estimate of total genome variation and be sufficiently abundant to minimize errors due to sampling variance.

In the study presented here we expanded on a prior cpDNA study (Spooner et al. 1993) by characterizing more accessions of *S. palustre*, *S. etuberosum*, and *S. fernandezianum*. We use data from a prior allozyme study (Spooner et al. 1992) and use single to low-copy nuclear DNA probes to generate nuclear restriction fragment length polymorphisms (RFLPs), and random amplified polymorphic DNAs (RAPDs). These are four very different marker systems. Chloroplast DNA is usually maternally inherited as a single linkage block of functional genes. Isozymes are codominant secondary functional gene products. Single-copy genomic RFLPs generally are non-functional codominant DNA sequences, while RAPDs generally are non-functional dominant DNA sequences. Our objective was to evaluate the concordance of these four marker systems in terms of genetic distance among these three species.

Materials and methods

Germ plasm

We analyzed 15 accessions each of *S. palustre* and *S. etuberosum*, and 4 accessions of *S. fernandezianum* (Table 1). Most of these accessions came from recent germ plasm-collecting expeditions to Argentina (Spooner and Clausen 1993) and Chile (Spooner et al. 1991), and were evaluated by Spooner et al. (1992) using allozymes. Accessions and vouchers are deposited at the National Research Support Program-6 [NRSP-6 (Bamberg and Martin 1993; Bamberg and Spooner 1994) formerly called the Inter-Regional Potato Introduction Project, IR-1]. The accessions occur in 24 generalized areas (Fig. 1), and we chose them to represent the maximum geographic distribution available from the collection. Complete locality data can be obtained from Contreras and Spooner (1996). The low numbers of *S. fernendezianum* accessions examined represent the true rarity of this species.

Table 1 Examined populations of *Solanum fernandezianum* (frn), *S. etuberosum* (etb), and *S. palustre* (pls) (see Fig. 1 for map localities). Species numbers correspond to Spooner et al. (1992) except for the addition of frn x,y

Species, number	Map locality[a]	Study[b]	PI[c]	Collector[d]
frn 1	1	a,i	320270	*C. Skottsberg s.n.*
frn 2	1	a,c,f,i	473463	*C. Ochoa 13069*
frn x	1	a	566752	*G. Anderson 1569*
frn y	1	a	566755	*Co 88*
etb 3	2	a,f,i	558286	*SCo 4325*
etb 4	3	a,c,f,i	558285	*SCo 4324*
etb 5	4	a,c,f,i	558303	*S 4473*
etb 6	4	a,i	558304	*S 4474*
etb 7	5	a,i	558287	*S Co 4326*
etb 8	7	a,c,f,i	558288	*S Co 4328*
etb 9	8	a,i	558290	*S Co 4331*
etb 10	9	a,c,f,i	498311	*Co 1322*
etb 11	10	a,c,f,i	245924	*D. Correll C143*
etb 12	10	a,c,f,i	245939	*D. Correll C134*
etb 13	10	a,i	498412	*A. Montaldo s.n.*
etb 14	10	a,c,f,i	558294	*S Co 4338*
etb 15	10	a,c,f,i	558295	*S Co 4340*
etb 16	11	a,c,f,i	558311	*S 4490*
etb 17	13	a,c,f,i	558297[g]	*S Co 4349*
pls 18	6	a,c,f,i	558233	*S Co 4329*
pls 19	12	a,c,f,i	558280	*S 4484*
pls 20	14	a,c,f,i	245763	*D. Correll C14*
pls 21	15	a,c,f,i	558241	*S Co 4392*
pls 22	16	a,c,f,i	558276	*S 4469*
pls 23	17	a,c,f,i	245764	*D. Correll C15*
pls 24	18	a,c,f,i	558246	*S Co 4398*
pls 25	19	a,c,f,i	558161	*S Cl 4518*
pls 26	20	a,c,f,i	558253	*S Co 4406*
pls 27	21	a,c,f,i	473401	*Diem s.n.*
pls 28	22	a,i	558180	*S Cl 4539*
pls 29	23	a,i	558257	*S 4450*
pls 30	24	a,c,f,i	558258	*S 4451*
pls 31	–[e]	a,c,f,i	218228	*EBS 338*
pls 32	–[f]	a,c,f,i	498415	*H. Brücher 81-8*

[a] See Fig. 1
[b] a=RAPD, c=cpDNA, f=nRFLP, i=allozyme
[c] USDA plant introduction numbers (see Bamberg and Martin 1993)
[d] Cl=Andrea Clausen, Co=Andrés Contreras, EBS=Erwin Baur Sortiment Genebank, Germany, S=David Spooner
[e] This collection was sent to the U.S. Potato Station by the Erwin Baur Sortiment Genebank without collector or locality data
[f] H. Brücher's locality data (Chile, Quetrihue) is confusing. The Peninsula Quetrihue is in the range of *S. palustre* in Argentina, as are three separate localities spelled Quetrahue in Chile
[g] Misidentified in Bamberg and Martin (1993) as *S. palustre*

DNA isolation and restriction site comparison, cpDNA, RFLPs

DNA was obtained from 5 to 10 g of bulked fresh leaf tissue of 5 plants per accession from 2-month old plants. Preparations of total DNA followed the procedure of Doyle and Doyle (1987) and were purified over CsCl gradients. Restriction endonuclease digestions, agarose gel electrophoresis, and the unidirectional transfer of DNA fragments from agarose gels to nylon filters (Biotrans™ , ICN for cpDNA; Zeta-Probe™ , Bio-Rad for RFLPs) followed the methods of Palmer (1986). For cpDNA variation, 2 μg of each DNA sample was digested with 14 endonucleases (*Ava*I, *Bam*HI, *Bgl*I, *Bst*NI, *Cla*I, *Dra*I, *Eco*RI, *Eco*RV, *Hin*cII, *Hin*dIII, *Hph*I, *Nci*I, *Sst*I, *Xba*I). For RFLP variation, 5 μg of each DNA sample was digested separately with 4 endonucleases (*Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII) according to

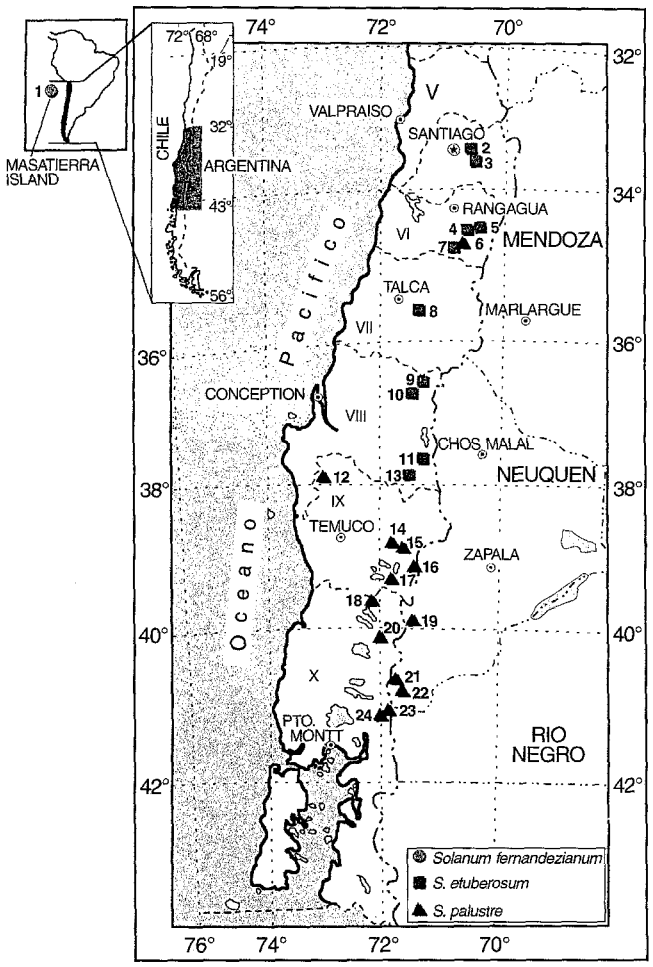

Fig. 1 Distributions of the 34 populations of *Solanum fernandezianum* (*triangles*), *S. etuberosum* (*squares*), and *S. palustre* (*circles*) examined in this study, divided into 24 geographic areas (see Table 1)

manufacturer's instructions (BRL). Hybridization protocols followed those of Giannattasio and Spooner (1994).

Chloroplast DNA

Nylon filters were probed with 12 *Pst*I and 2 *Sal*I clones, representing the entire cpDNA genome of *Petunia* except for the small single-copy region (Sytsma and Gottlieb 1986). Five clones of *Nicotiana* covering the small single-copy region (Olmstead and Palmer 1992) were used to complete the coverage for the entire cpDNA molecule (approximately 155 kb long).

Nuclear RFLPs

A genomic library of total DNA from *Solanum phureja* Juz. and Buk. was used as described by Hosaka and Spooner (1992). A total of 25 unmapped probes (P43, P82, P93, P101, P132, P140, P148, P158, P161, P204, P209, P263, P279, P307, P352, P368, P374, P392, P398, P470, P473, P562, P648, P778, P872) was used for RFLP analysis. To minimize the collection of potentially synonymous data, the enzyme yielding the greatest number of clearly discernable bands per probe was visually scored (Giannattasio and Spooner 1994).

RAPD primer selection and scoring

We evaluated an arbitrary set of 8 accessions that represent two of the three species for polymorphisms with 96 Operon technology (Alameda, Calif.) 10-mer primers. Twenty-one of these primers (Operon Technologies, Almeda, California) were selected on the basis of clearly discernable polymorphic bands: opa4, opa5, opa15, opa16, opa18, opd3, ope1, ope3, opf5, opr7, opr8, opr9, opr12, opr13, ops3, ops7, ops9, ops11, ops17, ops19, opt1. Fragments ranging from 0.3 kb to 2.2 kb were scored visually for presence and absence.

RAPD amplification

RAPD reactions were performed in an Idaho Technology Air Thermal Cycler™, Model 1605, programmed for 40 cycles. The cycling protocol for the first 2 cycles was denaturation at 91°C for 60 s, annealing at 42°C for 7 s, elongation at 72°C for 70 s. The subsequent 38 cycles were identical except for a denaturation time of 1 s (dos Santos et al. 1994).

Reactions were performed in thin-walled glass-capillary tubes. The reaction buffer was composed of 50 m*M* Tris, pH 8.5, 2 m*M* $MgCl_2$, 20 m*M* KCl, 500 µg/ml BSA, 2.5% Ficoll 400, 0.02% xylene cylanol. Reaction concentrations were 100 µ*M* DNTPs, 2 ng/µl DNA template, 0.4 µ*M* RAPD primer, and 0.6 units *Taq* DNA polymerase.

RAPD profiles were resolved by electrophoresis (5 V/cm) for 3 h in 1.5% Ultrapure™ (BRL) agarose gels. Gels were stained with ethidium bromide and photographed under UV light (270 nm) with Polaroid 667 film.

Chloroplast DNA analysis

The cpDNA data was examined with PAUP, version 3.1 (Swofford 1993). The tree was unrooted. The data were analyzed by Wagner parsimony, which gives equal weight to site gains and site losses. The most parsimonious trees were sought using an exhaustive search strategy.

Allozyme, RFLP, and RAPD analysis

The genetic distances (GD) among accessions based on allozyme, RFLP, and RAPD data were calculated using Nei's I (Nei 1972), the coefficient of Nei-Li (Nei and Li 1979), and the complement to the simple matching coefficient (Sneath and Sokal 1973). The simple matching coefficient measures associations among an accession's band scores that are „alike in state," and includes the concordant presence (11) or absence (00) of molecular marker bands (Dudley 1994; Tivang et al. 1994). These genetic distances are expressed as $GD(xy)=\Sigma[(x\neq y)+\Sigma(x\neq y)]$, where $\Sigma(x\neq y)$ represents the sum of all discordant observations while $\Sigma(x=y)$ is the sum of all concordant observations between accessions and y. The resulting genetic distance matrices generated by allozymes (32×32), RFLPs (25×25) and RAPDs (34×34) were reduced to two dimensions using Kruskos monotonic multidimensional scaling (MDS) analysis with Systat 5.2 (Wilkinson 1992).

To compare the sampling variance of genetic relationships based on allozyme, RFLP, and RAPD data, we drew 1000 bootstrap samples, each of size *n* [*n*=4, 6, 7, 9, 12 (stopped for allozymes), 16, 20, 26, 33, 43 (for RFLPs), 55, 70, 90, 116, 148, 178 (for RAPDs)], independently from each of the three molecular marker datasets (Tivang et al. 1994). The GD between all pairs of genotypes was calculated for each bootstrap sample. The variance among the 1000 bootstrap samples for each pair of genotypes was standardized to the coefficient of variation (CV) by dividing the standard deviation by the bootstrap sample mean. Natural log transformations were used to linearize the relationship between mean CVs and the sample size. Regression analysis was used to evaluate slopes and intercepts for the allozyme, RFLP and RAPD datasets.

Pairwise geographic distances between all 24 generalized geographic areas was obtained by converting latitude and longitude data

to distance. Concordance among marker systems and geographic distance were evaluated with the Spearman-rank correlation statistics using JMP software version 3.02 (SAS Institute, 1994).

Experimental errors

Duplication of 2 accessions (pls 21 and pls 30) was included in the RFLP dataset; similarly, 3 accessions (etb 5, pls 18, pls 22) were included in the RAPD dataset. We scored both datasets blindly. Error was estimated as the failure of bands (either RFLP or RAPD) to be scored identically over duplicated accessions.

Results and discussion

Chloroplast DNA cladistic analysis

Cladistic analysis transforms a taxon by character state data matrix into a tree of relationships based on minimizing character state changes in the tree, but direction is given only with use of an outgroup. Analysis of the entire chloroplast genome with 14 restriction enzymes and 14 clones revealed the presence of eight restriction variants among the 25 accessions examined. Three of these variants were shared by more than one taxon, the other five were unique to *S. fernandezianum* (Table 2). Four identical, 9-step, most parsimonious trees (consistency index of 0.67, excluding autapomorphies) were generated with Wagner parsimony. A strict consensus tree of these four trees was completely unresolved. Accessions etb 4, etb 16, and pls 21 shared one or two restriction site variants (etb 4) with *S. fernandezianum*. This result was in concordance with the results of Spooner et al. (1993), with the detection of two additional autapomorphies for *S. fernandezianum*, and the discovery of the three shared restriction site variants in some accessions of *S. etuberosum* and *S. palustre* with *S. fernandezianum*. The cpDNA data were not included in the sampling variance analysis (Fig. 2) because of insufficient numbers of markers and are not appropriate for phenetic analysis (Fig. 3).

Table 2 Chloroplast DNA restriction site mutations within *Solanum* sect. *Etuberosum*

No.	Enzyme	Region	Size (kb)	Species[a]
1	*Bst*NI	P3	2.0 + 1.6 = 3.6	fnd 2
2	*Dra*I	P10	2.8 + 0.5 = 3.3	fnd 2
3	*Dra*I	P6	15 + 3.5 = 18.5	etb 4, fnd 2
4	*Dra* I	T39/T40	3.0 + 2.2 = 5.2	fnd 2
5	*Eco*RV	P18/P19	1.0 = 0.7 + 0.3	etb 4, pls 21
6	*Hinc*II	P3	2.7 + 0.8 = 3.5	fnd 2
7	*Hph*I	T37	1.7 + 0.1 = 1.8	fnd 2
8	*Nci*I	T37/T38/T39	9.0 + 1.1 = 10.1	etb 4, etb 16, pls 21, fnd 2

[a] See Table 1 for definition of abbreviations

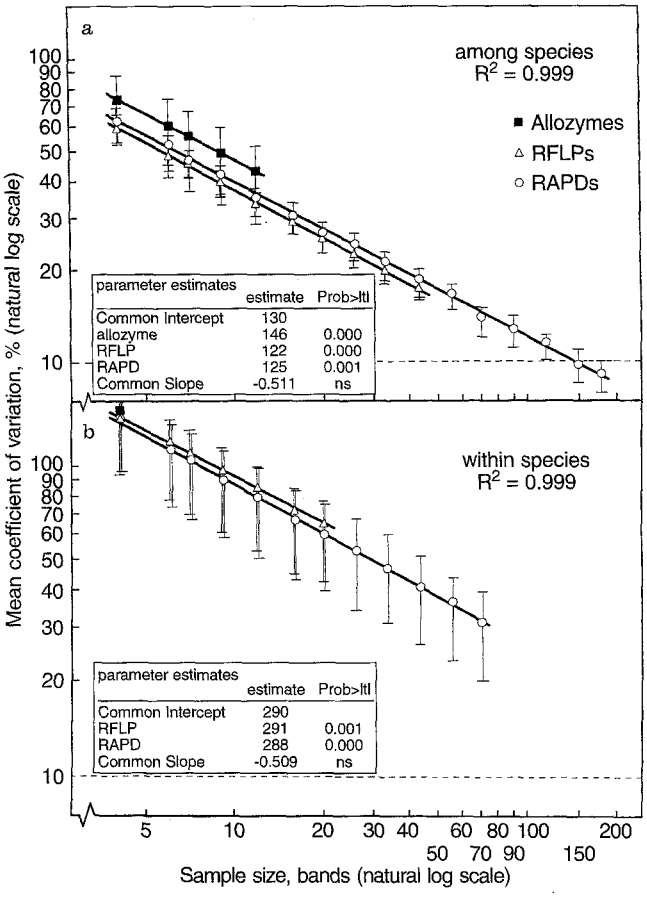

Fig. 2a, b Linear regressions of coefficients of variation on bootstrap sample size for allozymes, RFLPs, and RAPDs. The upper and lower quartile are indicated by *bars*

Allozyme, RFLP, RAPD datasets

The allozyme study of Spooner et al. (1992) evaluated seven enzyme systems, encoding 12 enzyme loci, and distinguished 19 presumptive alleles. Five enzyme systems were polymorphic, encoding 5 enzyme loci, resulting in 12 polymorphic alleles. A total of 25 RFLP probes were evaluated using any one of the four enzymes; 17 probe-enzyme combinations were polymorphic resulting in 50 polymorphic bands. A total of 21 RAPD primers were evaluated, resulting in 179 polymorphic bands. The RFLP and RAPD data are available from the corresponding author.

Experimental errors

The failure of duplicated genotypes to be scored identically constitutes experimental error. This error is confounded with the germ plasm sampling error in this study, since 5 individual plants per accession was bulked into one DNA sample, but it can be minimized by bulking many individuals. The mean experimental errors observed in this experiment were 3.93% and 1.38% for RFLPs and RAPDs, respectively. These percentages are within the range of those reported for *Brassica* by dos Santos et al. (1994).

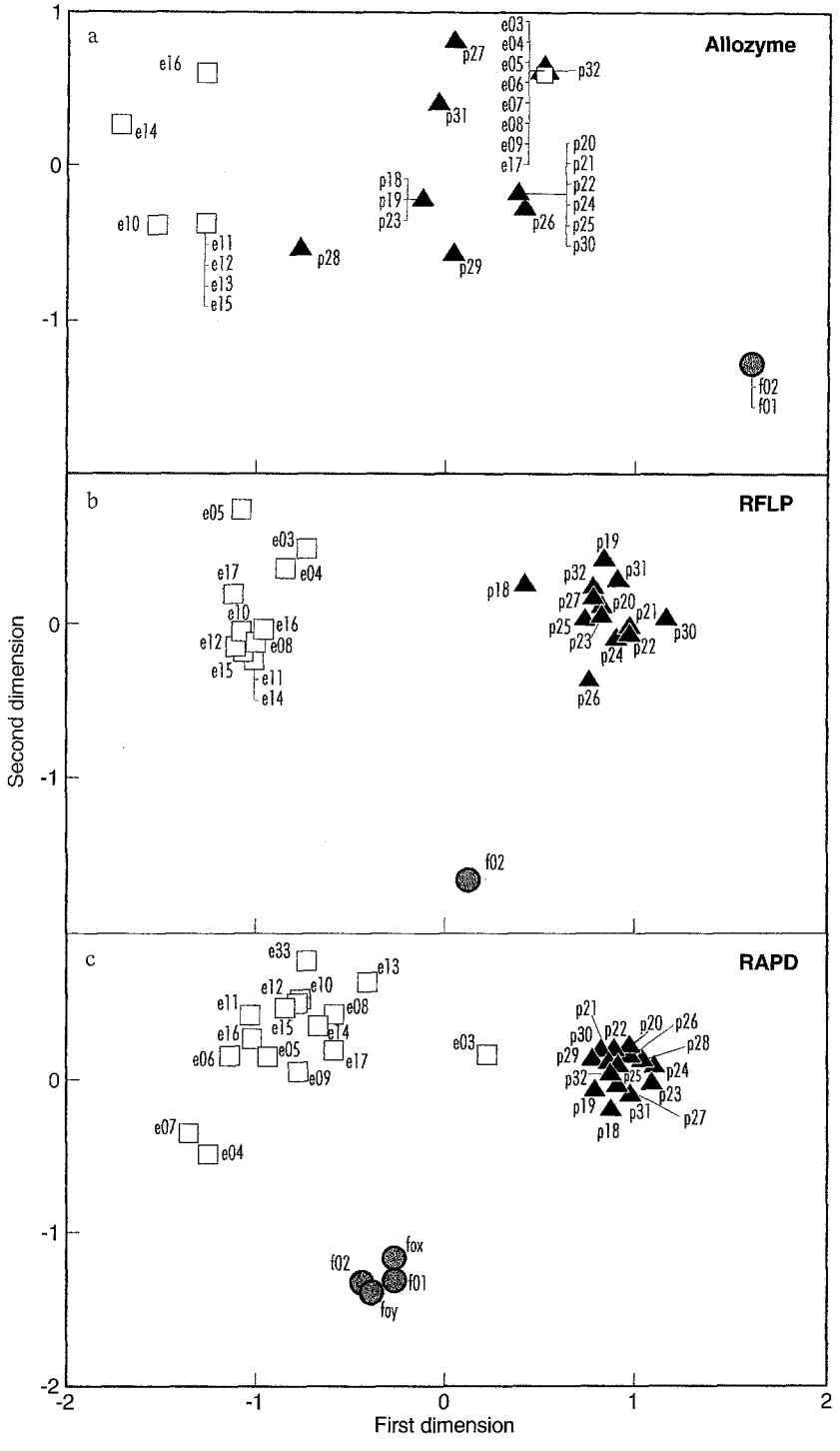

Fig. 3a–c Multidimentional scaling (MDS) plots of genetic distances using **a** allozymes, **b** RFLPs, and **c** RAPDs

Correlations among genetic distance estimators

Correlations among the three genetic distance estimators (Nei's I, Nei-Li, GD) were evaluated within the three marker systems (allozymes, RFLPs, RAPDs); they ranged from 0.986 to 0.999. The lowest correlation (0.986) among estimators was observed within allozymes between Nei's I (that uses allelic ratios) and GD (discarding allelic ratios). Because of a 97.9% fixation rate, allelic information with allozymes has limited impact on genetic relationship estimates. The RFLP and RAPD data were collected using bulked tissue; thus the allelic frequency was not obtained. In light of the high correlations among the estimators, we elected to use GD for all subsequent comparisons due to its simplicity.

Table 3 Geographic and genetic distances within and among species

Comparative statistics	Mean geographic distance (km)	Mean genetic distance					
		Allozymes		RFLP		RAPD	
		n[a]	Distance	n	Distance	n	Distance
Sect. *Etuberosum*	Total	496	0.298	300	0.310	595	0.359
Among species[b]							
pls – etb	396	225	0.307	143	0.441	240	0.463
pls – frn	1115	30	0.450	13	0.442	60	0.482
etb – frn	981	30	0.629	11	0.493	64	0.444
Within species							
pls	241	105	0.168	78	0.124	105	0.106
etb	225	105	0.281	55	0.163	120	0.280
frn	0	1	0.000	0	NA[c]	6	0.074

[a] Total comparisons
[b] See Table 1 for definitions
[c] Only one accession available

Sampling variance for isozymes, RFLPs, and RAPDs

Sampling variance associated with genetic relationships occur when discrepancies are detected between a random subset of molecular marker bands and all available bands (Tivang et al. 1994). Sampling variance among species showed a gradation of intercepts (resolution) from allozyme (low) to RAPD to RFLP (high; Fig. 2a). All of the marker systems can be observed to share a common slope, indicating that the rate of information addition per band will remain constant for each system. Despite the greater resolution of RFLPs in determining interspecific relationships, RAPDs yielded a better estimate (9.6% CV) because of their greater numbers.

Sampling variance within species likewise indicated a gradation of resolution from RFLPs (low) to RAPDs (high; Fig. 2b). Both marker systems likewise share a common slope. Because of the lower number of polymorphic bands observed for the within-species comparisons (mean of 24 bands for RFLP, with *S. etuberosum* and *S. palustre*, and a mean of 70 bands for RAPDs for all three species), the precision of these comparisons was greatly compromised. This problem had the greatest impact on allozymes, which had only a mean of 4 polymorphic bands within each of the three species. Because of this low level of polymorphism, the allozymes could not be included in the regression analysis.

Even though RAPDs appear to be the most efficient system for intraspecific analysis in section *Etuberosum*, the lack of polymorphic bands suggests that the phenetic relationships within each species as displayed in Fig. 3 are unreliable. Using the regression parameters for RAPDs, we estimated that a mean of 650 polymorphic bands are required for an arbitrary precision of a 10% CV. This means that an additional 55 equally polymorphic primers must be located.

The screening results indicated that an additional 200 primers must be evaluated to discover these 55 primers.

Multidimensional scaling (MDS) and geographic and genetic distances

MDS is a multivariate procedure that is related to factor and principle component analysis. It has the advantage over these latter procedures in that it frequently accounts for most of the variation in two dimensions (Nienhuis et al. 1993; Wilkinson 1992). The percentage of variance in the GD matrices explained by the first two dimensions of MDS (Fig. 3) accounted for 98.8%, 98.9%, and 95.9% of the variance associated with the GD matrices for the allozyme, RFLP, and RAPD datasets, respectively. Each of the MDS (Fig. 3) plots separate *S. palustre*, *S. etuberosum*, and *S. fernandezianum* into three groups and appear to have a similar relative positioning among them. However, the allozyme data show considerable overlap between *S. palustre* and *S. etuberosum*.

Quantification of visual observations based on the MDS analysis (Table 3) confirmed the general trend of greater distance between species than within species. The pooled average distance between species is 0.459±0.077 among all marker systems. Only allozyme observations exceeded values greater than one standard deviation of the mean. This is in concordance with the large sampling variance obtained with allozymes and supports the visual observation of approximately equal nuclear genomic distance among the three species. A less apparent visual clue, quantified in Table 3, showed that a consistently greater mean distance among the marker systems is observed within *S. etuberosum* (0.257) than within *S. palustre* (0.134). *Solanum fernandezianum* showed the smallest mean distance among accessions, but this could be an artifact of the few

Table 4 Spearman rank correlation statistics among geographic, allozyme, RFLP, and RAPD distances

	Geography	Allozymes	RFLP	RAPD
Geography	1			
Allozymes	0.263	1		
RFLP	0.527	0.410	1	
RAPD	0.436	0.494	0.750	1

accessions available. A preliminary conclusion suggests that *S. etuberosum* has the greatest diversity of all three species.

Concordance of datasets

Concordance of the three marker systems was evaluated using Spearman-rank correlations. Only accessions included in all of the three marker systems were considered (Table 1). The selection of a non-parametric correlation of pairwise association was justified by our interest in the preservation of relative ranks, not magnitude of distance. Geographic distance was included only to maximize the information content of our study. The concordance of results from isozymes, RFLPs, and RAPDs as displayed in the MDS plots (Fig. 3) shows the ability of these markers to measure genetic distance in *S. etuberosum*, *S. fernandezianum*, and *S. palustre*. The concordance among molecular marker systems shows an increasing correlation between isozymes to RFLPs to RAPDs (Table 4). This trend is in agreement with the sampling variance increasing with greater numbers of bands and the discrimination ability among the marker systems. A positive correlation among all three methods to geography was observed; i.e., there is a greater similarity among closely spaced accessions than among distantly located accessions. The data do not allow more refined investigations into possible clinal patterns because of the lack of frequency data within accessions.

Systematic relationships within section *Etuberosum*

Solanum fernandezianum is endemic to Masatierra Island (Contreras and Spooner 1996). This island, 650 km west of continental Chile, is 4 million years old (Stuessy et al. 1984) and forms part of the Juan Fernández Archipelago. Isozyme data suggests that *S. fernandezianum* is 1.1 million years old (Spooner et al. 1992), and distributional data suggest that *S. fernandezianum* is derived from *S. palustre*, *S. etuberosum*, or their ancestor.

A cpDNA cladistic analysis (Spooner et al. 1993), using appropriate outgroups for series *Etuberosum* placed *S. etuberosum* and *S. palustre* on one clade (supported by 3 synapomorphies) and *S. fernandezianum* on another clade (distinguished by three autapomorphies) but provided no conclusions of progenitor derivative relationships. Our new cpDNA data simply point out previously undiscovered polymorphisms in *S. palustre* and *S. etuberosum* while failing to resolve their interspecific relationships. The low levels of diversity of cpDNA compared to that of nuclear genes was expected (Wolfe et al. 1987).

Our isozyme, RFLP, and RAPD results, unlike the cpDNA data, show approximately an equal distance between all three species. The interspecific relationships of these species, therefore, remains unresolved with these data as well. The discordance between the more distant relationship of *S. fernandezianum* relative to *S. etuberosum* and *S. palustre* by cpDNA data, and their approximately equal separation with the other nuclear markers is unresolved. A possible explanation is an ancestral "chloroplast capture" phenomenon where *S. etuberosum* and *S. palustre* could have hybridized with backcrossing to one or the other species to share chloroplast types. This phenomenon has been documented in other plant groups (Rieseberg and Wendel 1993).

The greater variability in *S. etuberosum* (0.257) than in *S. palustre* (0.134) suggests that the former is ancestral and has had time to accumulate more variability.

Conclusion

Our study is in agreement with most intraspecific studies that have compared marker systems either statistically (Aldrich and Doebley 1992; dos Santos et al. 1994; Beer et al. 1993; Gerdes and Tracy 1994; Halldén et al. 1994; Heun et al. 1994; Messmer et al. 1992; Smith and Smith 1992; Thorman et al. 1994) or by other methods (Chase et al. 1991; Havey and Muehnenbauer 1989; Prince et al. 1992; Wang et al. 1992; Wang and Tanksley 1989; Yang and Quiros 1993) in showing a general concordance of results at low taxonomic levels. Thorman et al. (1994) investigated six cultivated *Brassica* species and one species of *Raphanus*. They showed good RFLP/RAPD intraspecific concordance, but poor interspecific concordance. Our study, in contrast, showed good interspecific RFLP/RAPD concordance and inferred good intraspecific concordance. Other studies claiming good concordance of RAPDs and various other datasets are those of Halward et al. (1992) van Coppenolle et al. (1993), Wilkie et al. (1993), and Williams and St. Clair (1993). Our intraspecific concordance is tentative, however, since relatively few RFLP and RAPD markers yielded low levels of precision.

Questions regarding the reliability of RAPDs have been raised by Smith et al. (1994), Thorman et al. (1994), and Williams and St. Clair (1993). These studies used RAPD fragments as probes to show non-homology of some comigrating RAPDs from different species, suggesting the need for similar homology studies with other taxa. Additionally, Smith et al. (1994) showed fragment absences to be low-resolution presences, and the RAPD reaction to be dependent on the synthesis of RAPD products from unrelated loci. Our study does not provide experimental data for homology, neither by labelling fragments as probes nor by inheritance studies investigating the allelic nature of

bands. The concordance of our independent RFLP and RAPD results (Fig. 3, Table 4) suggests that most of the RAPDs have biological relevance in elucidating genetic distances among species in sect. *Etuberosum*.

The sampling variance of RAPDs in comparison to that of RFLPs in our study is greater in interspecific comparisons (Fig. 2a) than in intraspecific comparisons (Fig. 2b). These results suggest that the magnitude of non-homology (noise) increases with increasing taxonomic levels. Despite this noise, the MDS plots of RFLPs and RAPDs (Fig. 3b,c) show similar associations among species, suggesting that the noise levels in this study have reduced impact on biologically relevant results. Additional support for the limited impact of noise is confirmed by the relatively low experimental error for RFLPs and RAPDs. In contrast to interspecific comparisons, intraspecific comparisons show greater noise with RFLPs. This may be caused simply by the lower resolution of this marker within these genetically similar species.

Our study showed the applicability of RAPDs for both intra- and interspecific studies in *Solanum* section *Etuberosum*. These species, however, are three morphologically very similar diploid inbreeders. The applicability of RAPDs and concordance of these three marker systems have not been tested at different levels of genetic divergence and taxonomy, in various breeding systems, and at different ploidy levels of sect. *Petota*.

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