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RESEARCH ARTICLE

Genetic diversity and conservation of two endangered eggplant relatives (*Solanum vespertilio* Aiton and *Solanum lidii* Sunding) endemic to the Canary Islands

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Abstract *Solanum vespertilio* Aiton and *Solanum lidii* Sunding are endemic, endangered wild species from the Canary Islands. These species are of potential value for eggplant (*S. melongena*) breeding, given that they are part of the secondary genepool of this crop. We study genetic diversity with amplified fragment length polymorphisms (AFLPs) markers from 5 populations of *S. vespertilio* (47 samples) and 3 of *S. lidii* (26 samples). Five related African species (*S. dasyphyllum* Schumach. et Thonn., *S. delagoense* Dunal, *S. campylacanthum* Hochst., *S. panduriforme* E. Mey, *S. aff. violaceum* Ortega) were also included in the analysis. A total of 235 AFLP markers included 178 and 156 that were polymorphic in *S. vespertilio* and *S. lidii*, respectively. Analysis of genetic distance, phenograms, and principal component plots showed that these rare Canarian species are differentiated (G_{ST} = 0.412) from the continental materials and that *Solanum vespertilio* is more distinct to its African congeners than is *S. lidii*. There is a relatively high level of differentiation between the two species (G_{ST} = 0.373), that presumably reflects geographic restrictions (*S. lidii* to Gran Canaria; *S. vespertilio* essentially to Tenerife). However, both species have similar levels of total diversity. We speculate that the combination of the many unusual reproductive features (andromonoecy, zygomorphy, heteranthery and weak enantiostyly in *S. vespertilio*) help explain genetic diversity that is high for self compatible species. The high genetic diversity may also indicate populations were larger in the past. A decrease in population size could contribute to the relatively low genetic differentiation

This paper is dedicated to the memory of Dr. Richard N. Lester, who made significant contributions to the taxonomy, biosystematics and conservation of genetic resources of African species of *Solanum*.

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among the populations. The data presented herein provide the foundation for initiation of ex situ and in situ conservation programs for these wild relatives of eggplant.

Keywords AFLP analysis · Canary Islands · Conservation · Population genetic structure · *Solanum lidii* · *Solanum vespertilio*

Introduction

The Canary Islands (Spain) are an Atlantic Ocean archipelago of seven islands of volcanic origin off the northwestern coast of Africa that belong to the Macaronesian region (Fernández-Palacios and Esquivel 2001). They have a rich endemic flora that reflects their geological age, perhaps 70–80 million years old (Anguita and Hernan 2000). The islands have around 570 endemic angiosperms, this constituting at least 40% of the approximately 2000 native angiosperms of the archipelago (Santos-Guerra 2001). It is estimated that around 20% of the endemics are endangered, with about 120 included in the Spanish 'Red Book' of rare and endangered species (Bañares et al. 2004).

Among the endangered endemic taxa, there are two closely related species of *Solanum* (both in subgenus *Leptostemonum*): *S. lidii* Sunding—endemic to Gran Canaria Island with six small populations distributed in the south central area (Bañares et al. 2004)—and *S. vespertilio* Aiton—endemic to Tenerife Island with several populations, mostly in the northeast Anaga region (and a recently described subspecies restricted to the northern part of Gran Canaria Island with only one population with 2–3 individuals; Marrero and Gonzalez Martin 1998; Bañares et al. 2004). Both species have been included in molecular-based phylogenetic studies and have been shown to be most closely linked to African species also in the 'spiny *Solanum*' group (Anderson et al. 2005), and in particular to the lineage of an economically important crop of this group, the eggplant (*Solanum melongena* L.) (Bohs and Olmstead 2001). Interspecific hybrids between one of these island endemics, *S. lidii*, and *S. melongena* have been reported to be vigorous and partially fertile (Hassan 1989; Collonnier et al. 2001). To the best of our knowledge, there are no reports of attempts to cross the other species, *S. vespertilio*, with *S. melongena*, but there is a great genetic affinity between these materials (Bohs and Olmstead 2001). Therefore, because these Canarian solanums can be considered part of the secondary genepool of eggplant, they constitute genetic resources of potential interest for the present or future genetic improvement of this crop. These two uncommon Canarian species deserve special attention as well because of their inordinate combination of highly unusual reproductive characters (Anderson et al. 2005). Typical *Solanum* flowers are hermaphroditic, five parted, actinomorphic, with five equal-sized anthers, and the styles are straight, and not deflected to one side of the flower. In contrast, both *S. lidii* and *S. vespertilio* are andromonoecious, self compatible, heterandrous with 3–4 short ''reward'' anthers and one long ''pollination'' anther, and have zygomorphic corollas; in addition, *S. vespertilio* flowers are tetramerous and a significant percentage of its hermaphroditic flowers display incipient enantiostyly (Anderson et al. 2005).

The knowledge of the genetic diversity of species, obviously, is essential for designing effective conservation programs (Holsinger 1991; Hamrick and Godt 1996). Understanding the level and apportionment of genetic diversity within and among populations of island endemics is perhaps even more important for conservation than for continental species because island plants often have few populations with few individuals that may make them even more susceptible to extinction (Frankham 1997, 1998).

Molecular markers can provide useful information for formulating conservation strategies (Hamrick and Godt 1996; Neel and Ellstrand 2003). Thus, molecular markers can provide valuable information about the genetic diversity and structure of natural plant populations (Nybom 2004). Amplified fragment length polymorphisms (AFLP) are notably polymorphic in some groups, including the eggplant and wild relatives (e.g., Mace et al. 1999; Prohens et al. 2005). In addition, AFLPs are useful, because one does not need previous genomic information from the target species to employ them (Vos et al.

1995), they allow a large number of loci to be scored in a single reaction, and finally, they have much better repeatability among laboratories than other markers such as random amplified polymorphisms (RAPDs) of DNA (Jones et al. 1997; Bussell et al. 2005).

Here we study the diversity within and among populations of *S. vespertilio* and *S. lidii* as well as their genetic relationships. The information obtained will be relevant for designing conservation strategies and can be useful in assessing the potential value of these species for eggplant breeding.

Materials and methods

Plant materials

Plants used (see Table 1) came from five populations of *S. vespertilio* (V1, V2, V3, V4, V5) and three populations of *S. lidii* (L1, L3, L4) plus five accessions representing five continental African species of *Solanum*. The *S. lidii* accessions are all from Gran Canaria Island, the only place it grows naturally. The *S. vespertilio* plants were all sampled from Tenerife. However, historically, *S. vespertilio* is known from Gran Canaria, in very limited numbers (Webb and Berthelot 1845), and has recently been recognized from there as a distinct subspecies (*S. vespertilio* ssp. *doramae* A. Marrero et M. Gonzalez Martin). However, there are only two or three plants of *S. vespertilio* reported left on Gran Canaria, so no samples were taken. The five African species represent a diversity of African species, with emphasis on andromonoecious species. They were: two from section *Melongena* (*S. dasyphyllum* Schumach. et Thonn., *S. delagoense* Dunal) and three from the highly polymorphic ''*S. indicum*'' group of section *Oliganthes* (*S. campylacanthum* Hochst., *S. panduriforme* E. Mey., *S. aff. violaceum* Ortega).

Populations of *S. vespertilio* were collected on Tenerife and those of *S. lidii* on Gran Canaria (Fig. 1). Due to the small size of some of these populations in nature (especially so for *S. lidii)*, the number of individuals that could be sampled was limited and varied for each population (Table 1) between 6 (L1) and 11 (V1).

DNA extraction and generation of AFLP

Young leaves of *S. vespertilio* and *S. lidii* were collected from individual plants in the field and immediately placed in a bag with silica-gel and sealed, yielding rapid dehydration of the plant material. Material from the African continental species was collected from one plant per accession grown in the greenhouses of the Department of Ecology and Evolutionary Biology of the University of Connecticut and dehydrated in a similar manner.

Genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) following the protocol of the manufacturer. DNA was quantified on agarose, and a 0.125 μg DNA sample was digested by the enzyme combination EcoRI and MseI at 37°C for 2.5 h. Ligation was performed with the AFLP Core Reagent Kit (Invitrogen, Carlsbad, CA, USA) following the instructions of the manufacturer. After ligation, the reaction mixture was diluted 1:10 in Tris–EDTA (TE) buffer.

For the pre-selective amplification, a 5 μl aliquot from the DNA dilution was added to a 25 μl solution containing 2.5 μl of 10× buffer, 0.5 μl of primer EcoA (10 μM), 0.5 μl of primer MseC (10 μM), 1.0 μl of dNTPs (10 mM), and 0.8 units of Taq polymerase (Roche, Basel, Switzerland). After pre-amplification, DNA was diluted again 1:10 in TE buffer. The selective amplification was performed on 2-μl aliquots using four combinations of primers (Table 2). DNA fragments were separated in an ABI Prism 310 genetic analyzer (Applied Biosystems, Foster City, CA, USA). Resulting fragments were scored as binary traits (1 = present, 0 = absent) using Genographer 1.6 software (Montana State University, Bozeman, MT, USA).

Data analysis

Pairwise genetic similarities were estimated with the Dice (Sorensen) similarity coefficient $S_{ij} = 2a/(2a + b + c)$, where a is the number of bands shared by i and j, b is the number of bands present in i and absent in j, and c is the number of bands present in j and absent in i. The resulting genetic similarity matrix was used to generate

Table 1 Material examined of *Solanum lidii* and *S. vespertilio* (Canary Islands, Spain) and of five *Solanum* species from continental Africa, obtained from seeds from the Botanical Garden of the Radboud University of Nijmegen (The Netherlands), except *S. panduriforme*. Collection numbers are from G. J. Anderson, except when noted; voucher specimens deposited at CONN

Species and population codes	Collection numbers	Locality	GPS	Elevation (m)	Estimated population size
S. lidii L1	4784, 4786, 4787, 4790, 4791, 4792	1 km before Temisas on road 550, Gran Canaria	27°54′ N 15°30′ W	550	7–9 plants
S. lidii L3	4793, 4794, 4795, 4796, 4797, 4798, 4799, 4800, 4801, 4803	E end of village of Temisas, Gran Canaria	27°54′ N 15°30′ W	500	16 plants
S. lidii L4	4804, 4805, 4806, 4807, 4808, 4809, 4810, 4811, 4812, 4813	Fortaleza de Ansite, Gran Canaria	27°54′ N 15°33′ W	475	10 plants
S. vespertilio V1	4617, 4618, 4619, 4620, 4621, 4622, 4623, 4624, 4625, 4626, 4627	Teno: Los Cochinos, above Los Silos, Tenerife	28°21′ N 16°44′ W	500	25 plants
S. vespertilio V2	4629, 4630, 4631, 4633, 4634, 4635, 4636, 4638	Anaga: La Cumbrilla, Tenerife	28°34′ N 16°10′ W	600	50 plants
S. vespertilio V3	4640, 4641, 4642, 4643, 4644, 4645, 4646, 4648, 4649	Anaga: km 7 on Carretera Teresitas, below Bailadero, Tenerife	28°32′ N 16°11′ W	360	25 plants
S. vespertilio V4	4651, 4652, 4653, 4654, 4655, 4656, 4657, 4658, 4659, 4660	Anaga: Valle Brosque, east of Santa Cruz, Tenerife	28°31′ N 16°13′ W	220	20 plants
S. vespertilio V5	MB-1, MB-2, MB-3, MB-4, MB-5, MB-6, MB-7, MB-8, MB-9	5–7 km before Chinamada on road to Las Carboneras, Tenerife	28°33′ N 16°16′ W	630	8–10 plants
S. campylacanthum	C. Martine 475	Uganda, Africa			
S. dasyphyllum	C. Martine 298	Cameroon, Africa			
S. delagoense	C. Martine 1088	Africa*			
S. panduriforme	C. Martine 443	South Africa			
S. aff. violaceum	C. Martine 627	Togo, Africa			

* Our seed of this African species come from plants apparently cultivated in Burma

UPGMA (unweighted pair group method using arithmetic means) phenograms using the NTSYSpc 2.0 software package (Rohlf 1996). Supports for groups on the phenograms were tested by bootstrap analysis with 1000 replications. Principal coordinate analysis (PCoA) based on genetic similarity matrices was performed using the DCENTER and EIGEN algorithms of the NTSYSpc 2.0 software package.

Genetic diversity was estimated with the proportion of polymorphic fragments (P) and the total diversity (H_T) (Nei 1973). Total diversity was partitioned into diversity among (D_{ST}) and within (H_S) groups. The relative magnitude of gene differentiation among groups (G_{ST}) was calculated as the ratio D_{ST}/H_T (Nei 1973). The estimates of genetic identity (J) and standard genetic distance (D) were calculated as indicated in Nei (1974).

Results

AFLP analysis

A total of 235 AFLP fragments were scored, of which 230 (98%) were polymorphic (Table 3). Some 150 of the 230 polymorphic loci were

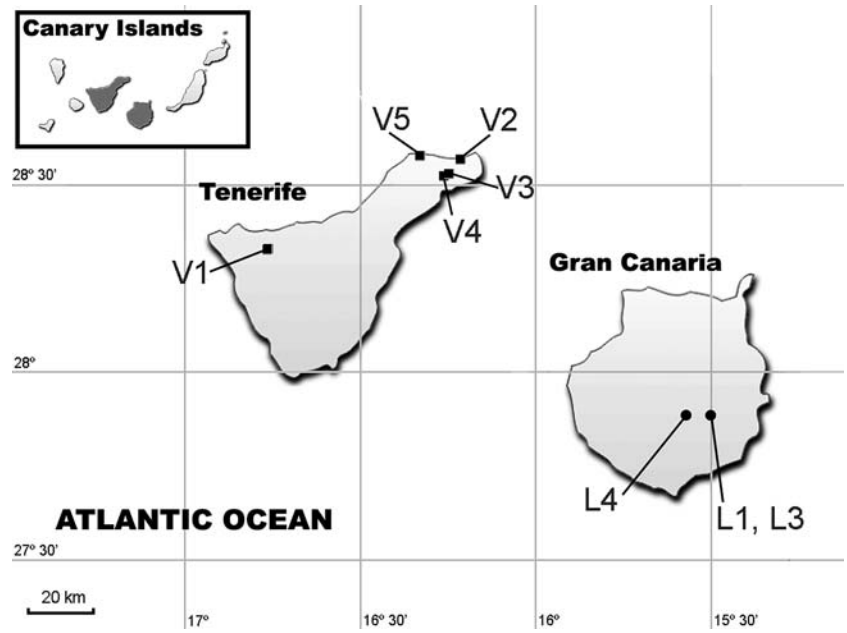

Fig. 1 Geographical location of the Canarian samples studied

polymorphic in the five accessions of the continental African species, and 203 in the 73 samples studied of the two Canarian species. Among the 47 *S. vespertilio* and 26 *S. lidii* samples, there were 178 and 156 polymorphic loci, respectively (Table 3). The number of polymorphic loci within populations varied between 124 (population L3; 10 individuals) and 78 (population V5; 9 individuals).

The total diversity of the samples studied was $H_T = 0.276$. The combined diversity among the 73 individuals of *S. vespertilio* and *S. lidii* was similar to that of the five individuals combined of the continental African species studied (Table 3). The genetic differentiation between the continental and the Canarian materials is quite high ($G_{ST} = 0.412$), i.e., there is a considerable divergence between them.

Solanum vespertilio and *S. lidii* display similar levels of total diversity ($H_T = 0.205$ and $H_T = 0.207$, respectively) and have a relatively high level of genetic differentiation between them ($G_{ST} = 0.373$). Mean diversity within populations is somewhat higher in *S. lidii* ($H_S = 0.167$) than in *S. vespertilio* ($H_S = 0.147$). Also, the mean number of polymorphic loci in *S. lidii* populations is 109, while in *S. vespertilio* it is 90.4. Genetic differentiation among populations of *S. vespertilio* ($G_{ST} = 0.284$) is greater than among populations of *S. lidii* ($G_{ST} = 0.194$). When studying the relationships between the geographic distance

Table 2 Oligonucleotide adaptors and primers used for the AFLP analysis

Adaptor	Restriction enzyme	Sequence
E-0 Adaptor	EcoRI	5′-CTCGTAGACTGCGTACC-3′ 3′-CTGACGCATGGTTAA-5′
M-0 Adaptor	MseI	5′-GACGATGAGTCCTGAG-3′ 3′-TACTCAGGACTCAT-5′
E-A	EcoRI	5′-AGACTGCGTACCAATTCA-3′
M-C	MseI	5′-GATGAGTCCTGAGTAAC-3′
E-ACC + M-CAT	EcoRI	5′-AGACTGCGTACCAATTCACC-3′
	MseI	5′-GATGAGTCCTGAGTAACAT-3′
E-AGG + M-CCG	EcoRI	5′-AGACTGCGTACCAATTCAGG-3′
	MseI	5′-GATGAGTCCTGAGTAACCG-3′
E-ACG + M-CTA	EcoRI	5′-AGACTGCGTACCAATTCACG-3′
	MseI	5′-GATGAGTCCTGAGTAACTA-3′

Table 3 Gene diversity statistics (Nei 1973) estimated from AFLP data for the African continental and Canarian *S. vespertilio* and *S. lidii* materials studied (see Table 1)

	Number of individuals	Number of polymorphic loci (%)	H_T	D_{ST}	H_S	G_{ST}
Total	78	230 (97.9)	0.276	0.114	0.162	0.412
Continental	5	150 (63.8)	0.223			
Canarian	73	203 (86.4)	0.246			
Canarian	73	203 (86.4)	0.246	0.092	0.155	0.373
S. vespertilio	47	178 (75.7)	0.205			
S. lidii	26	156 (66.4)	0.207			
S. vespertilio	47	178 (75.7)	0.205	0.058	0.147	0.284
Population V1	11	102 (43.4)	0.203			
Population V2	8	90 (38.3)	0.171			
Population V3	9	83 (35.3)	0.162			
Population V4	10	99 (42.1)	0.185			
Population V5	9	78 (33.2)	0.152			
S. lidii	26	156 (66.4)	0.207	0.040	0.167	0.194
Population L1	6	87 (37.0)	0.167			
Population L3	10	124 (52.8)	0.242			
Population L4	10	116 (49.4)	0.217			

H_T = total gene diversity; D_{ST} = among groups gene diversity; H_S = within groups gene diversity; G_{ST} = relative magnitude of gene differentiation among groups (D_{ST}/H_T)

(GPS coordinates; Fig. 1) and the genetic distance in the *S. vespertilio* or *S. lidii* populations, the only clear pattern found is within *S. vespertilio* ($r = 0.68$), in which the most geographically distant population (V1) is also the most distinct genetically (Fig. 2).

Analysis of AFLP fragments exclusive to each species and population, showed that *S. vespertilio* and *S. lidii* have 21 (8.9%) and 19 (8.1%) fragments, respectively, restricted to each species (Table 4). Among the restricted fragments, there are two in each species that are exclusive and universal (i.e., present in all individuals of a species but absent in the other species). The number of fragments exclusive to a population is: *S. vespertilio* from none (population V5, the smallest) to 4 fragments (population V2, the largest), and in *S. lidii* from 1 (population L4) to 7 (population L1). No fragment exclusive and universal to a population was found (Table 4).

The greatest values for the genetic distances (GD) and lowest genetic identities are found when comparing the continental African species to the Canarian species in aggregate (Table 5). Of the two Canarian solanums, there is a greater genetic distance from the continental species to *S. vespertilio* populations (GD range = 0.268–0.335, mean = 0.315) than from the continental species to *S. lidii* (GD range = 0.221–0.310, mean = 0.262). The genetic distances among the various *S. vespertilio* and *S. lidii* populations are much lower, ranging between 0.158 and 0.201, with a mean of 0.175 (Table 5). The GD among *S. vespertilio* populations per se range between 0.061 and 0.128, with a mean of 0.089, and the GD among *S. lidii* populations range from 0.064 to 0.096, with 0.075 as the mean value.

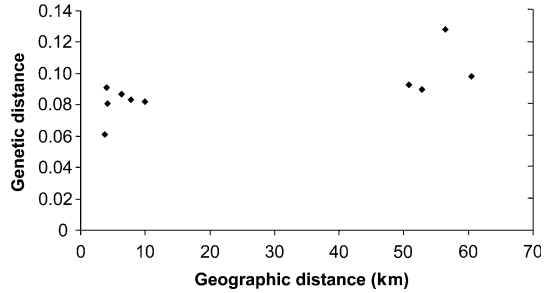

Fig. 2 Relationship of geographic and genetic distances among the five populations (thus 10 combinations) of *S. vespertilio*. The group of four points at the upper right corresponds to the comparison among the most geographically isolated population (V1) and the rest of populations (V2–V5)

Cluster analysis

The phenogram constructed with the AFLP data (Fig. 3) shows that the continental species are

Table 4 AFLP fragments exclusive, and exclusive and universal to *S. vespertilio* and *S. lidii* and their respective populations from a total of 235 fragments scored

Group	Exclusive	Exclusive and universal
S. vespertilio	21	2
Population V1	2	0
Population V2	4	0
Population V3	2	0
Population V4	1	0
Population V5	0	0
S. lidii	19	2
Population L1	7	0
Population L3	3	0
Population L4	1	0

clearly separated from *S. vespertilio* and *S. lidii* with a bootstrap value of 100%. All *S. vespertilio* and *S. lidii* accessions group together in a sub-cluster. However, the sub-cluster for each species is supported by a bootstrap of less than 50% (38% for *S. vespertilio*, 30% for *S. lidii*). This lack of strong support for the sub-clusters is attributable to two odd individuals of *S. vespertilio* (plants 4617 and 4618 from population V1, pointed out with arrows in Fig. 3). The remaining *S. vespertilio* samples group together in a sub-cluster with a bootstrap value of 95% (Fig. 3). Furthermore, when these two odd *S. vespertilio* plants are removed (that are however, genetically, as well as morphologically, clearly *S. vespertilio*), *S. lidii* and *S. vespertilio* are separated from each other with a bootstrap support of 100%.

The bootstrap values between different populations and individuals within each species are generally very low (Fig. 3); there are no high bootstrap values that support sub-clusters or different populations. The only high bootstrap values are those that link small groups (generally pairs) of individuals from the same population. As a consequence, the infra-specific relationships plotted in the phenogram have low consistency. Phenograms restricted to only *S. vespertilio* or *S. lidii* accessions (not shown) yield similar results to those obtained in the general phenogram. Therefore, we examined the relationships among *S. vespertilio* and *S. lidii* individuals and populations through principal coordinates analysis.

Principal coordinates analysis

When performing a PCoA with all samples included in the study, the first two coordinates account for 16.8% and 9.0% of the variation observed. However, this analysis (not shown) basically separates three groups, that correspond to the (i) continental African species, (ii) *S. vespertilio* (including plants 4617 and 4618), and (iii) *S. lidii*. Therefore, in order to study the relationships among the different populations, we performed two additional analyses, one each for *S. vespertilio* and *S. lidii* (Fig. 4).

In the *S. vespertilio* analysis, the two first coordinates explain 12.3% and 9.5% of the variation. Individuals from populations V1, V3, and V4 (except an odd individual of population V4) fall together, as does population V2 combined with population V5, but in different areas of the graph (Fig. 4). In the *S. lidii* analysis, the

Table 5 Nei (1974) estimates of genetic identities (above the diagonal) and standard genetic distances (below the diagonal) between *S. vespertilio* populations, *S. lidii* populations, and the African continental species of *Solanum* studied (see Table 1)

Population	*S. vespertilio*					*S. lidii*			Continental
	V1	V2	V3	V4	V5	L1	L2	L4	
S. vespertilio V1	–	0.907	0.880	0.914	0.911	0.841	0.852	0.847	0.765
S. vespertilio V2	0.098	–	0.913	0.920	0.921	0.845	0.854	0.850	0.716
S. vespertilio V3	0.128	0.091	–	0.941	0.917	0.830	0.823	0.818	0.717
S. vespertilio V4	0.090	0.083	0.061	–	0.922	0.844	0.843	0.832	0.732
S. vespertilio V5	0.093	0.082	0.087	0.081	–	0.844	0.840	0.834	0.719
S. lidii L1	0.173	0.168	0.186	0.170	0.170	–	0.938	0.909	0.734
S. lidii L2	0.160	0.158	0.195	0.170	0.175	0.064	–	0.938	0.775
S. lidii L4	0.166	0.163	0.201	0.184	0.182	0.096	0.064	–	0.802
Continental	0.268	0.335	0.333	0.312	0.330	0.310	0.255	0.221	–

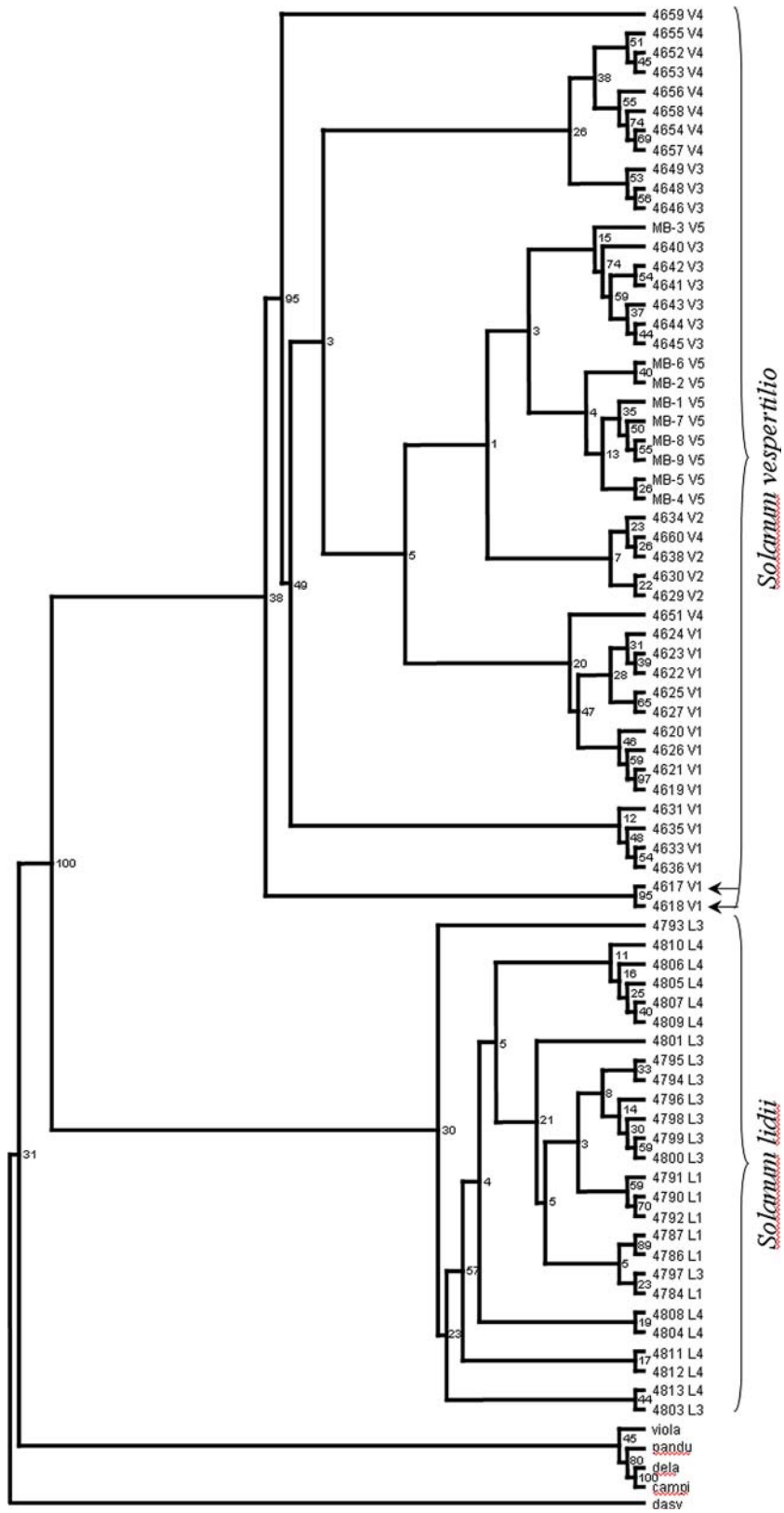

Fig. 3 UPGMA phenogram of 46 *S. vespertilio* individuals corresponding to five populations (V1–V5), 26 individuals of *S. lidii* corresponding to 3 populations (L1, L2, L4) (see Table 1), and to five individuals of continental species: *S. dasyphyllum* (dasy), *S. panduriforme* (pandu), *S. delagoense* (dela), *S. aff. violaceum* (viola), and *S. campylacanthum* (campi) based on AFLPs. Bootstrap values (percentage over a total of 1000 replications) are indicated at each node. Arrows indicate two odd individuals of *S. vespertilio* (see more explanation in Results)

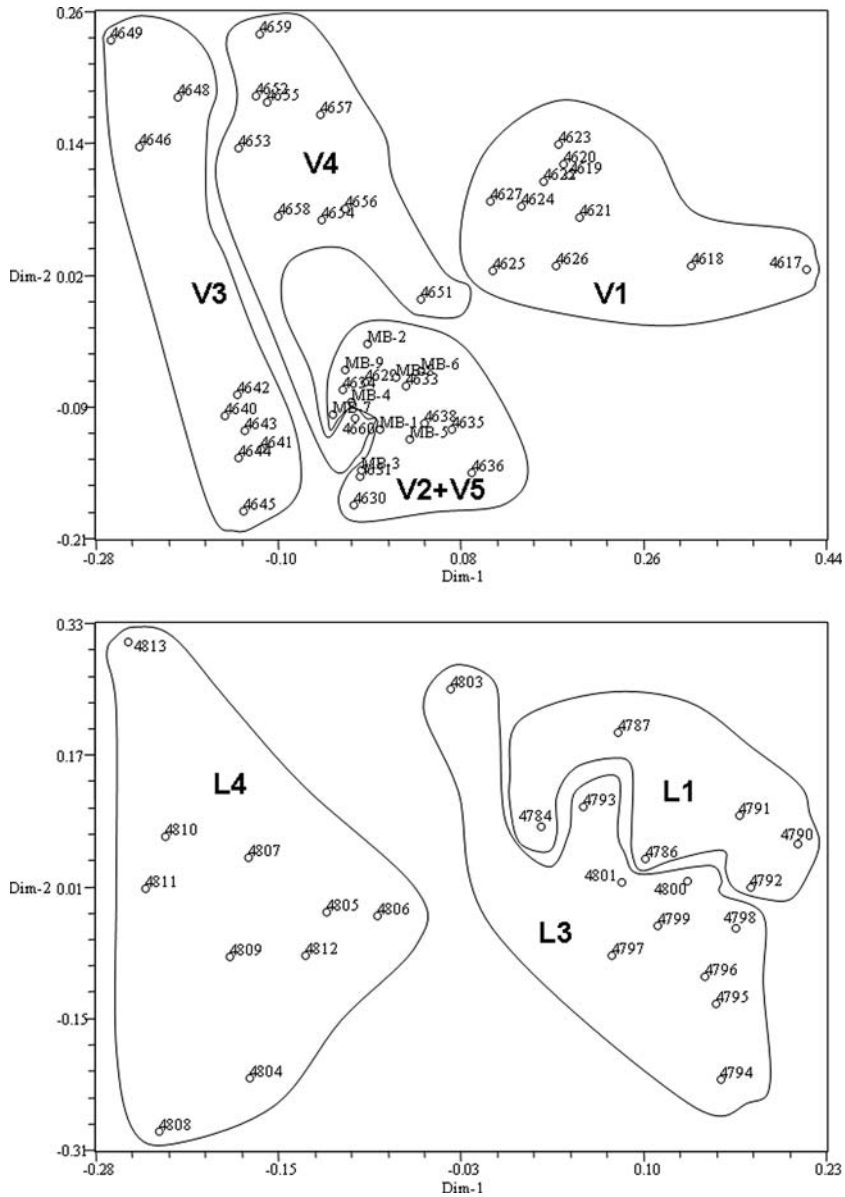

Fig. 4 Relationships between 47 *S. vespertilio* individuals corresponding to 5 populations (V1–V5; above), and 26 individuals of *S. lidii* corresponding to 3 populations (L1–L4; below) based on principal coordinates analysis using AFLP-based genetic similarities (see Table 1). First and second coordinates account for 12.3% and 9.5% of variation for *S. vespertilio* and 12.1% and 8.9% for *S. lidii*

two first coordinates account for 12.1% and 8.9% of the variation observed. The three populations we studied also plot in different areas of the graph with the most geographically distinct population (L4) clearly differentiated from the geographically more proximal L1 and L3 (Fig. 4). The latter two have different 'central tendencies', but are not easily differentially circumscribed (Fig. 4). These results seem to support a hypothesis of moderate genetic differentiation among populations within each species.

Discussion

Solanum vespertilio and *S. lidii* are unusual solanums closely related to the eggplant (Bohs and Olmstead 2001; Collonnier et al. 2001) with a unique combination of reproductive characteristics, including strong zygomorphy, heteranthery, self compatibility, and andromonoecy, and for *S. vespertilio*, tetramery (both corolla and stamens) and weak enantiosytly (Anderson et al. 2005). As in many other species that have evolved

on islands (Carlquist 1974; Bernardello et al. 2001; Tremetsberge et al. 2005), the isolation of the common ancestor of these solanum species from the African ancestral lineages and their evolution under the particular conditions of the Canary Islands may well have resulted in the acquisition of this unique combination of morphological characteristics. Each of the individual characteristics is not unknown in *Solanum*; it is their combination, and as rare endemics on islands, that makes them so notable. Our AFLP data from a large number of individuals confirm that *S. vespertilio* and *S. lidii*, like a number of Macaronesian species (Francisco-Ortega 2005), are related to continental African species. Molecular-based phylogenetic studies confirm that this sister species pair is clearly linked with the African *Solanum* lineages (Bohs and Olmstead 2001; Anderson et al. 2005). However, both *Solanum* species are genetically clearly differentiated from their continental African species, and we report here many exclusive AFLP fragments that reinforce the concept of their independence. It is interesting to note that *S. vespertilio*, occupying the younger of the two islands relevant here (Tenerife), and the one that is further away from the African source of these island species, is less similar to the African solanums studied than *S. lidii* (on the geographically more proximal, and older, Gran Canaria). The reproductive features of *S. vespertilio* are also more unusual than those of *S. lidii*.

As indicated, *S. vespertilio* and *S. lidii* share many morphological (and ecological) characteristics (Anderson et al. 2005), a sister species association that is supported herein by the relatively smaller genetic distance between them than between the Canarian *Solanum* species pair and their African congeners. On the other hand, both species are clearly genetically different: the GD between them is much greater than the GD among the populations of each. In addition, our data show unique AFLP fragments for each species, supporting the conclusion that *S. vespertilio* and *S. lidii* are reproductively isolated from one another. Although it is still unknown if viable sexual hybrids between them can be obtained, the fact that there are AFLP fragments exclusive and universal to all plants of *S. vespertilio* and absent in *S. lidii* and vice versa, also strongly support the contention of reproductive isolation. Of course, essentially the species do occur on different islands (though near, about 57 km apart) and are bee pollinated; thus, distance in the context of the pollination biology also makes it unlikely that there could or would be regular inter-species crosses. In addition, seed dispersal, to the extent it is known, is particularly interesting for these two species, but would also not provide support for long-distance dispersal. That is, two species of lizards, each endemic to the appropriate island, have been found to have seeds of these solanums present in their excrement (*Gallotia stehlini* for *S. lidii* and *Gallotia galloti* for *S. vespertilio*; A. Valido, pers. comm.). We recognize that *S. vespertilio*, now in the form of the newly published subspecies, *S. vespertilio* ssp. *doramae* (Marrero and Gonzalez Martin 1998) does occur on Gran Canaria. However the populations of *S. vespertilio* and *S. lidii* are isolated by some distance (ca. 30+ km), are in very different drainages and the *S. vespertilio* ssp. *doramae* now represented by only 2–3 individuals has always been very restricted in number and geographical extent (Bañares et al. 2004).

Solanum vespertilio and *S. lidii* possess a surprisingly high level of genetic diversity (>65% of loci are polymorphic within each species). And despite the comparatively restricted distribution and smaller population sizes of *S. lidii*, the diversity of both species is similar. The high genetic diversity in species with a small number of populations and few individuals within each population may be an indication that the number of individuals of these species was larger in the recent past (Ellstrand and Ellam 1993; Crawford et al. 2001; Leimu and Mutikainen 2005). Certainly in the millennia prior to the introduction of goats (i.e., ~2500 years ago), that may have been the case. And currently, though the plants mostly grow in zones where the vegetation is protected, the few remaining local farmers are allowed to cut plants. Not surprisingly, these spiny, rank opportunist native plants, that are locally considered poisonous, are often cut away along trails and roadways, and in grazing areas (even by the crews of the 'Servicios de Administración del Cabildo de Tenerife' in the

protected Anaga Mts regions). Another explanation of the relatively high genetic diversity may be the existence of outcrossing and thus some gene flow among populations (Husband and Barrett 1996). Even so, with the present number of individuals, well below the ''minimum viable population'' size (Pavlik 1996; Reed 2005), the genetic diversity of both species (and in particular that of *S. lidii* with few known populations, and those known of small size) is likely to decrease in future generations (Barrett and Kohn 1991).

The G_{ST} value for *S. vespertilio* is essentially identical to the mean value of 0.280 calculated from allozyme data for 23 species endemic to the Canary Islands (Francisco-Ortega et al. 2000), while the value for *S. lidii* is about 30% lower than that mean. The values presented by Francisco-Ortega et al. (2000) are for predominately outcrossing species, yet this self-compatible species pair exhibits values that are comparable to or lower. This is not as surprising as it might seem when the reproductive biology and natural history of these species are also considered. Although, self compatible (as is characteristic for most species of subgenus *Leptostemonum* that have been studied; Whalen and Anderson 1981), many of the panoply of unusual features cited for these solanums very likely also serve to promote outcrossing. In addition, observations of known pollinators in nature confirm a behaviour that would enhance outcrossing (i.e., frequently, next-flower visits are to other plants, rather than to other inflorescences on the same plant; Anderson, pers. obs.). Also, the G_{ST} values for the two *Solanum* species studied are much lower than the mean for endemic selfing species (near 0.600), but higher than the mean (0.174) for endemics with mixed-mating systems (Hamrick and Godt 1997). The G_{ST} values for the two Canarian species may result from both historical and biological factors, as is often the situation for island plants (Crawford et al. 2001). In some instances, extremely different G_{ST} values have been found for species sharing very similar life history attributes and occurring on the same island (Helenurm and Hall 2005). In the case of the solanums, a decrease in population size or even extinction of some populations that once served as bridges between extant populations, could also contribute to the relatively low G_{ST} value in these self-compatible species. Also, there may be gene flow between populations, particularly those in close spatial proximity (pop. V2–V5 of *S. vespertilio* in or near the Anaga Mts.). That conjecture is at least not contradicted for *S. vespertilio* where the geographically most distinct population (V1), is also genetically the most different. In fact, we have found no fragments exclusive and universal within any population. In addition, there are few fragments specific to any population. Both of these data points argue against isolation of most populations. At present, this genetic flow may have been interrupted by the shrinking area occupied by the populations and by the disappearance of populations that acted as a bridge between more distant populations. However, we have also been encouraged in extensive and focused field work (particularly by A. Santos) to find more *S. vespertilio* than is generally known in the Anaga region of Tenerife.

From the perspective of the species as a whole, it seems clear that with the present sizes, populations of *S. vespertilio* or *S. lidii* that are isolated will quickly lose a good deal of their diversity by genetic drift and inbreeding (Young et al. 1996). This effect, associated with the potential loss of vigour associated with inbreeding depression and consequent loss of fitness, might well lead to the rapid extinction of isolated populations, even if the environment is favourable for the growth and maintenance of populations (Frankham 1998; Reed 2005).

Given the small number of populations, and particularly, the small number of individuals of these species, the conservation of these eggplant relatives, both ex situ and in situ, is of great priority. Seeds of *S. vespertilio* and *S. lidii* are orthodox, and thus can be conserved ex situ for many years. The results that we have obtained indicate that a high proportion of alleles can be conserved by sampling seeds from different individuals even from a single population (because there are few alleles exclusive to a population) of each of the species. This strategy could be appropriate in the case that it becomes desirable to use *S. vespertilio* or *S. lidii* in the genetic improvement of the related cultivated eggplant (Collonnier et al.

2001). However, given that populations have different frequencies of alleles, conservation measures aimed at preserving the diversity not only of alleles, but also of the characteristic genetic composition of specific populations (a feature that may be associated with its fitness in particular environments; Francisco-Ortega et al. 2000), should include sampling different populations and saving the seeds separately. With this method, it will be possible to conserve not only a high proportion of alleles, but also the genetic composition of different populations. By using this system of *ex situ* conservation, if existing wild populations become extinct, they could be re-established with individuals from the same population conserved in seed banks. This strategy has already been proposed to re-establish extinct populations of the Galápagos endemic *Solanum cheesmaniae* from seeds that were collected several decades ago and that are conserved in germplasm banks (Nuez et al. 2004). Re-establishment via this method avoids introducing individuals from other populations, an unfortunate practice that could result in a change in the fitness and reduction of local variation (Fahselt 1988). To date, programs to transplant rare Canarian endemics are limited, and in order to preserve the genetic structure of populations some of the re-introduced plants have been removed (Francisco-Ortega et al. 2000).

The *in situ* conservation should be focused on preserving the natural areas where plant populations thrive. This method of preservation should include reducing, or eliminating, human disturbance of the environment where populations are growing. Most extant populations are included in rural parks and nature reserves which obviously contribute to the protection and survival of the populations. However, these reserves also include a few 'grandfathered' rights for grazing access. Thus, it would be of great help for the conservation of the natural populations if education programs for local people, for the Canary Islands Natural Parks services, and the Servicio de Proteccion de la Naturaleza de la Guardia Civil (SEPRONA) were established, so that the trail and roadside clearing that has resulted in the removal of large and vigorous plants of *S. vespertilio*, or cutting of *S. vespertilio* plants in pasture areas by traditional pastoralists is reduced. Monitoring populations to evaluate their size and the changes in their molecular diversity will provide relevant information future management strategies. Such a program is in place for the populations of *S. lidii* on Gran Canaria (A. Santos, pers. comm.). Transplanting to re-establish or increase the number of individuals in small populations should be done with seeds collected from the same population to avoid dramatic changes in the population structure that may affect the genetic integrity of the populations (Francisco-Ortega et al. 2000).

Because of their differential characteristics and genetic differentiation from other solanums related to eggplant, conservation of *S. vespertilio* and *S. lidii* is of great interest. Also, both species have many features relevant for the study of the evolution of unusual reproductive systems in *Solanum* (e.g., Anderson and Symon 1989). Therefore, measures for the conservation both *ex situ* and in situ of *S. vespertilio* and *S. lidii* should be implemented. The data presented herein provide the foundation for initiation of these programs.

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