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Genetic structure of *Lycopersicon pimpinellifolium* (Solanaceae) populations collected after the ENSO event of 1997–1998

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Abstract

The greatest extent of genetic variation and outcrossing for *Lycopersicon pimpinellifolium* occurs in northern Peru. This is also the area most affected by EI Niño Southern Oscillation (ENSO). Using morphological and the molecular markers SSRs and AFLPs, we studied the genetic structure of *L. pimpinellifolium* populations collected after the ENSO event of 1997–1998. This was the most intense in the last century and caused a vast increase in the size of *L. pimpinellifolium* populations. Populations in the area surveyed were not regionally differentiated. We did not find any cline or eco-geographic association for genetic diversity, and positive correlations between genetic and geographic distances were found only at very short distances. Flooding and water streams caused by ENSO might have facilitated a periodical seed migration from distant areas. Gene flow between populations could then occur, facilitated by the increase in the population sizes of plants and pollinators and by the high levels of stigmatic exsertion. Results revealed a significant lack of heterozygotes in comparison with those expected in a panmictic population without consanguinity. A high degree of endogamy was found in all populations. In this context, endogamy can be explained by the occurrence of crosses between relatives rather than by autogamy. In an area intensely disturbed by ENSO, we found a population that had not been reported by earlier collectors in this region. This yellow-fruited population remained morphologically and molecularly differentiated from all *L. pimpinellifolium* and *L. esculentum* populations analyzed.

Introduction

The Currant tomato, *Lycopersicon pimpinellifolium* (Jusl.) Mill., is a colored-fruited wild species, included in the *Lycopersicon* subgenus (cf. Müller 1940). The fruit and the plant resemble those of the *L. esculentum* Mill., but they are substantially smaller. All populations of *L. pimpinellifolium* described to date are self-compatible. This species can be reciprocally hybridized with *L. esculentum* and has served as a valuable source of germplasm for tomato breeders (Rick and Chetelat 1995; Galiana-Balaguer et al. 2001).

Lycopersicon pimpinellifolium is typically in coastal areas of Ecuador and Peru usually at elevations below 1000 m, becoming increasingly rare as altitude is gained following the river valleys to the east (Taylor 1986; Warnock 1991). This species was initially regarded as a relatively invariable species. The first populations characterized were

highly uniform in gross morphology and tended to reproduce autogamously. However, the detailed studies conducted by Rick et al. (1977, 1978) on 43 populations, using morphological variants and allozymes, revealed varying degrees of genetic variation and rates of outcrossing (0–40%) within its range of distribution. Central populations displayed a typically allogamous floral morphology, whereas peripheral populations were less variable and tended to be autogamous (Georgiady et al. 2002).

The knowledge of the tomato genome has increased significantly in the two last decades and molecular tools that facilitate genetic studies have been developed. Different DNA-marker systems are being routinely employed for the construction of genetic maps of tomato (Arens et al. 1995; Broun and Tanksley 1996; Haanstra et al. 1999; Saliba-Colombani et al. 2000; Areshchenkova and Ganal 2002; Bonnema et al. 2002), for phylogenetic studies with *Lycopersicon* species Miller and Tanksley 1990; Alvarez et al. 2001; Marshall et al. 2001; Spooner et al. 2005), for identification of cultivars and for studies of genetic diversity within *L. esculentum* (Williams and St Clair 1993; Villand et al. 1998; Baudry et al. 2001; Bredemeijer et al. 2002; He et al. 2003). Some of these studies include a few accessions of *L. pimpinellifolium* as references together with other wild *Lycopersicon* species. To our knowledge, DNA-markers have not been used in population genetic studies of *L. pimpinellifolium* beyond to those by Rick et al. (1977, 1978).

Rick et al. (1977) found the highest levels of variability in central populations of *L. pimpinellifolium* from the northwest coast of Peru. Rainfall is non-existent in much of this area and as a consequence natural populations are frequently composed of a few scattered individuals that grow in the more humid areas. However, an exceptional increase in the size of *L. pimpinellifolium* populations occurred in the summer of 1998 due to the effect of El Niño Southern Oscillation (ENSO). The ENSO is a weak southerly flow of warm water to approximately 6°S that develops annually along the coast, usually in winter. This phenomenon becomes more extensive and intense at highly irregular intervals. The ENSO of 1997–1998 was the most intense in the last century. It was associated with heavy rainfall and flooding on the northwest coast of Peru (WMO 1999), where it caused a significant increase in the spontaneous vegetation and vast areas were found densely covered with plants of *L. pimpinellifolium*. The Institute for Conservation and Breeding of Agricultural Diversity (COMAV) of the Universidad Politécnica de Valencia (UPV), in co-operation with the Universidad Nacional de Loja (Ecuador) and the Universidad Nacional de Piura (Peru), conducted a collecting expedition (ENSO98) to the area most affected by the climatic change after the 1997–1998 event. This expedition provided the largest *L. pimpinellifolium* germplasm collection reported to date from this area.

In this paper, we present a detailed study of the genetic structure of *L. pimpinellifolium* from the central area of its natural range with morphological traits and two molecular marker systems (Amplified Fragment Length Polymorphisms, AFLPs, and Simple Sequence Repeats, SSRs). The possible effect of ENSO on population structure by favoring plant migration and the subsequent gene flow among populations is also discussed.

Materials and methods

Areas surveyed

The expedition ENSO98 followed the FAO guidelines that encourage rational collection activities (Jaramillo and Baena 2000). The collaboration of local institutions facilitated the collection. The University of Loja and Piura hold seed samples of all accessions, so all the scientific and technical information obtained from the germplasm could be transferred, sharing the benefits derived from these resources.

The collection was performed in northwestern Peru in the areas most affected by the ENSO event (latitude 4–6 °S; longitude 79–81 °W) (Figure 1). The area surveyed corresponds with the central area of the *L. pimpinellifolium* range of distribution, where the greatest extent of genetic variation and outcrossing has been reported (Rick et al. 1977, 1978). The intense rain and floods enhanced the growth of *L. pimpinellifolium* in previously arid or semi-arid areas (Figure 2). This situation allowed us to collect a large number of samples or accessions (the collections were designated PL collections, from the Universities of Piura and Loja). Each sample or accession consisted of a

mixture of seeds coming from fruits collected from different plants randomly selected among those growing in a specific site. Each specific site was identified with passport data, including the geographic coordinates determined using a global positioning system (GPS) (Table 1). For the different analyses, the accessions were grouped into subpopulations (A, B, C, D, E, and F) according to both geographic and agro-ecologic criteria (Figure 1 and Table 1). The northernmost collection site was the desert coastal plain near Tambo Grande and Sullana, on the roadside from Piura to Talara (A). Southwards, we surveyed the lower Piura watershed, from Piura to Sechura (B, C). These areas provided a semi-xerophytic habitat typical of this Peruvian coast. *Lycopersicon pimpinellifolium* populations were also collected following the upper Piura watershed, on roadsides from Piura to Huancabamba (D). Here the environment changed, becoming more humid and foggy as altitude increased. *Lycopersicon pimpinellifolium* also abundant on the roadside from Piura to Olmos, in the department of Lambayeque (E). The ENSO had a great impact in this area and even a small lagoon had appeared as a consequence of the flooding. Here the collection was very intense as the land was completely covered with *L. pimpinellifolium* associated to other native species adapted to xerophytic climates such as *Prosopis pallida* L. ('mesquite' or 'algarrobo'), *Luffa operculata* Mill. ('esponja vegetal') or *Cucumis dipsaceus* Ehrenb. ex Spach. ('jaboncillo') (Figure 2). This amount of *L. pimpinellifolium* in the Sechura dessert and in the 'Pampa de Olmos' was already reported by Holle et al. (1978). These authors commented on the urgent need of collecting these populations, since it can be collected only in one of those rare years when the desert region gets sufficient moisture. Finally, morphologically diverse specimens of *L. pimpinellifolium* and *L. esculentum* occurred sympatrically in a small canyon in the surroundings of Olmos towards Jaen (F).

Plant material

Sixty-four accessions of *L. pimpinellifolium* were analyzed (Table 1). Fifty of them belonged to the PL collection. Other groups on previous expeditions conducted in the same area collected the remainder. Those fourteen accessions were provided by the Tomato Genetics Resource Center (TGRC, University of California, Davis, CA) that hosts one of the world's largest germplasm collections of *L. pimpinellifolium*. Nine accessions of *L. esculentum*, also collected at the ENSO98 expedition, were included in the assays. These were mostly landraces and naturalized cultivars found growing sympatrically with *L. pimpinellifolium* in areas intensely disturbed by ENSO. We also included in the molecular assays, ten Spanish tomato landraces, seven accessions of the wild form of tomato, *L. esculentum* var. *cerasiforme* (Dun.) A. Gray, from different origins, and representatives of other wild species i.e. *L. cheesmaniae* Riley, *L. parviflorum* Rick, Kesicki, Fobes and Holle, *L. peruvianum* (L.) Mill., *L. chilense* Dun. and *L. pennellii* (Corr.) D'Arcy (Table 1).

Culture management

Five plants were grown from seeds of each accession. These were cultivated under uniform conditions in the greenhouses of the COMAV, so comparisons could be made that were not feasible in the wild. Plants were randomly arranged in the greenhouse (spaced at 30×50 cm). The assay was conducted during the spring–summer growing season.

Morphological characterization

Morphological traits included those in two tomato descriptors (UPOV 1976; IPGRI 1996), and additional traits selected from previous experiences with wild species management, were scored per plant. The scored qualitative and quantitative traits are listed in Table 2.

Isolation of DNA

Young leaf tissue was sampled from the five plants per accession used in the morphological characterization. Genomic DNA was isolated from this tissue using the modified CTAB (hexadecyl trimethylammonium bromide) method described in Ferriol et al. (2003). For AFLP analysis, genomic DNA from the five plants per accession was

Table 1. List of *L. pimpinellifolium* and *L. esculentum* accessions collected in northwestern Peru during the ENSO98 expedition.

Accession number	Area code	Department or province (Country)[c]	Collection site[d]	Latitude (S)	Longitude (W)	Altitude (m)	Year of collection
L. pimpinellifolium							
PL603[a]	A	Piura (P)	Road Piura–Talara	4°51′37″	80°47′07″	75	1998
PL604			Road Piura–Talara	4°51′01″	80°50′57″	25	1998
PL660			San Isidro	4°48′37″	80°17′46″	200	1998
PL561			Tambo Grande	4°53′32″	80°22′31″	100	1998
PL650			Tambo Grande	4°53′32″	80°22′31″	100	1998
PL560			Tambo Grande	4°55′00″	80°20′35″	100	1998
PL562			El Pedregal	4°56′02″	80°32′22″	100	1998
LA2652[b]			Road Sullana	4°53′	80°41′	100	1984
LA2653			S. Fco de Chocán	4°45′	80°35′	50	1984
LA1688			El Pedregal	4°55′	80°22′	~112	1976
PL567	B	Piura (P)	Piura	5°11′13″	80°37′33″	40	1998
PL568			Piura	5°11′13″	80°37′33″	40	1998
PL575			Catacaos	5°16′54″	80°41′03″	25	1998
PL576			Catacaos	5°16′54″	80°41′03″	25	1998
PL647			Montesión	5°17′00″	80°41′14″	50	1998
PL577			Montesión	5°19′20″	80°42′49″	35	1998
LA1689			Piura, Castilla	5°12′	80°36′	~152	1976
LA1690[e]			Piura, Castilla	5°13′	80°38′	~152	1976
PL578	C	Piura (P)	Sechura	5°26′03″	80°45′35″	75	1998
PL579			Sechura	5°31′52″	80°49′13″	30	1998
PL580			Sechura	5°31′52″	80°49′13″	30	1998
LA1683			Miramar	5°31′	80°50′	~7	1976
PL563	D	Piura (P)	Morropón	5°09′15″	80°10′05″	110	1998
PL564			Morropón	5°14′39″	80°06′03″	75	1998
PL565			Buenos Aires	5°17′14″	79°57′29″	175	1998
PL566			Buenos Aires	5°17′14″	79°57′15″	175	1998
PL569			Buenos Aires	5°17′56″	79°56′35″	160	1998
PL570			Buenos Aires	5°19′46″	79°55′06″	150	1998
PL571			Victor Raul	5°20′46″	79°50′57″	190	1998
PL574			Chanchape	5°25′59″	79°44′32″	225	1998
PL573			Chanchape	5°26′07″	79°44′37″	225	1998
PL572			Chanchape	5°26′11″	79°44′41″	275	1998
LA0400			Hacienda Buenos Aires	5°15′	79°58′	~146	1976
LA1684			Chulucanas	5°07″	80°00′	~140	1976
PL581	E	Piura (P)	Road Piura–Olmos Km. 68	5°16′42″	80°06′21″	200	1998
PL582			Road Piura–Olmos Km. 84	5°23′26″	80°03′24″	190	1998
PL583			Road Piura–Olmos Km. 90	5°26′24″	80°01′54″	150	1998
PL658			Road Piura-Olmos Km. 98	5°29′13″	80°00′36″	160	1998
PL585			Road Piura–Olmos Km. 104	5°33′14″	79°58′41″	240	1998
PL586			Road Piura–Olmos Km. 104	5°33′14″	79°58′41″	240	1998
LA1380			Chanchape	5°16′	80°03′	~199	1971
PL588		Lambayeque (P)	Road Piura-Olmos Km. 111	5°35′54″	79°57′06″	210	1998
PL589			Road Piura–Olmos Km. 111	5°35′54″	79°57′06″	210	1998
PL587			Road Piura-Olmos Km. 08	5°35′21″	79°58′13″	325	1998
PL591			Road Piura-Olmos Km. 140	5°49′05″	79°49′59″	150	1998
PL592			Road Piura-Olmos Km. 140	5°49′05″	79°49′59″	150	1998
PL593			Road Piura-Olmos Km.140	5°49′05″	79°49′59″	150	1998
PL594			Road Piura-Olmos Km. 156	5°55′28″	79°46′29″	200	1998
PL595			Road Piura–Olmos Km. 156	5°55′28″	79°46′29″	200	1998
LA1381			Ñaupe	5°36′	79°54′	~168	1971
LA1469			El Pilar, Olmos	5°52′	79°47′	~229	1971

Table 1. Continued.

Accession number	Area code	Department or province (Country)[c]	Collection site[d]	Latitude (S)	Longitude (W)	Altitude (m)	Year of collection
PL516	F	Lambayeque (P)	Road Olmos-Jaén	5°59′42″	79°43′03″	230	1998
PL517			Road Olmos-Jaén	5°59′42″	79°43′03″	230	1998
PL596			Road Olmos-Jaén	5°59′42″	79°43′03″	230	1998
PL597			Road Olmos-Jaén	5°59′42″	79°43′03″	230	1998
PL598			Road Olmos-Jaén	5°59′42″	79°43′03″	230	1998
PL599			Road Olmos-Jaén	5°59′42″	79°43′03″	230	1998
PL600			Road Olmos-Jaén	5°59′42″	79°43′03″	230	1998
PL601			Road Olmos-Jaén	5°59′42″	79°43′03″	230	1998
PL602			Road Olmos-Jaén	5°59′42″	79°43′03″	230	1998
PL655			Road Olmos-Jaén	5°59′41″	79°43′03″	230	1998
LA 1470			Motupe direction Olmos-Bagua	6°01′	79°41′	∼241	1971
LA1471			Motupe to Jayanca	6°19′	79°45′	∼76	1971
LA2915			Olmos	5°59′	79°44′	∼241	1987
L. esculentum							
PL508		Loja (E)	Zapotebamba	4°02′23″	79°47′19″	950	1998
PL509			Zapotebamba	4°02′23″	79°47′19″	950	1998
PL510			Zapotebamba	4°02′23″	79°47′19″	950	1998
PL511			Zapotebamba	4°02′23″	79°47′19″	950	1998
PL515		Piura (P)	Huancabamba	5°16′48″	79°28′16″	2280	1998
PL648			Dos Pueblos	5°27′34″	80°45′57″	25	1998
PL518		Lambayeque (P)	Road Olmos-Jaén	5°59′42″	79°43′03″	230	1998
PL519			Road Olmos-Jaén	5°59′42″	79°43′03″	230	1998
PL520			Road Olmos-Jaén	5°59′42″	79°43′03″	230	1998
AN-L-42	Tomate morado	Andalucía (S)	Cádiz	–	–	–	–
CA-L-96	Tomate canario	Canarias (S)	Santa Cruz de Tenerife	–	–	–	–
C-L-120	Tomate de penjar	Cataluña (S)	Barcelona	–	–	–	–
CM-L-133	Tomate gordo	C. La mancha (S)	Cuenca	–	–	–	–
E-L-22	Tomate rosa	Extremadura (S)	Badajoz	–	–	–	–
MU-L-20	Cuarenteno	Murcia (S)	Murcia	–	–	–	–
MU-L-31	Muchamiel	Murcia (S)	Murcia	–	–	–	–
VL-115	Valenciano	C. Valenciana (S)	Castellón	–	–	–	–
VL-264	Valenciano	C. Valenciana (S)	Valencia	–	–	–	–
VL-268	Amarillo Gordo	C. Valenciana (S)	Valencia	–	–	–	–
L. esculentum var. *cerasiforme*							
Mex 0012		Yucatán (M)	Cansahcab to Dizdzautum	21°	89°	< 100	–
Mex 0070		Puebla (M)	Huenchinango	20°	98°	1.500	–
Mex 0120		Nayarit (M)	Santispac	22°	105°	< 100	–
ECU78		Zam-Chinchip(E)	Timbara	4°01′	78°33′	975	
LA1307		Ayacucho(P)	San Francisco	12°35′	73°49′	600	–
LA1312		Cuzco(P)	Paisanato	–	–	600	–
LA1388		Junín(P)	San Ramón	11°07′N	75°19′	700	–
GLP-65	*L cheesmanii*	Baltra(E)	Canal Norte	0°24′52″	90°17′04″	10	–
LA1960	*L. chilense*	Moquegua(P)	Road Moquegua to Torata	17°05′00″	70°52′00″	1850	–
LA0247	*L. parviflorum*	Huánco(P)	Chavinillo	9°46′00″	76°32′00″	3802	–
PE-45	*L. pennellii*	Ancash(P)	Road Santa-Huaraz	9°04′00″	77°59′00″	350	–
PE-16	*L. peruvianum*	Cajamarca(P)	San José	7°03′00″	78°16′00″	2370	–

Lycopersicon pimpinellifolium accessions provided by the Tomato Genetics Resource Center, Spanish tomato landraces held at the COMAV genebank and accessions of other wild *Lycopersicon* spp., used as reference in the analysis, are also included.

[a]PL: The code PL refers to the institutions (University of Piura and Loja) that participate in the ENSO98 expedition.

[b]LA: Accessions provided by the Tomato Genetics Resource Center.

[c]P: Peru; E: Ecuador; M: México; S: Spain.

[d]Locality closest to the collection site.

[e]The underlined accessions were also assayed in Rick et al. (1977).

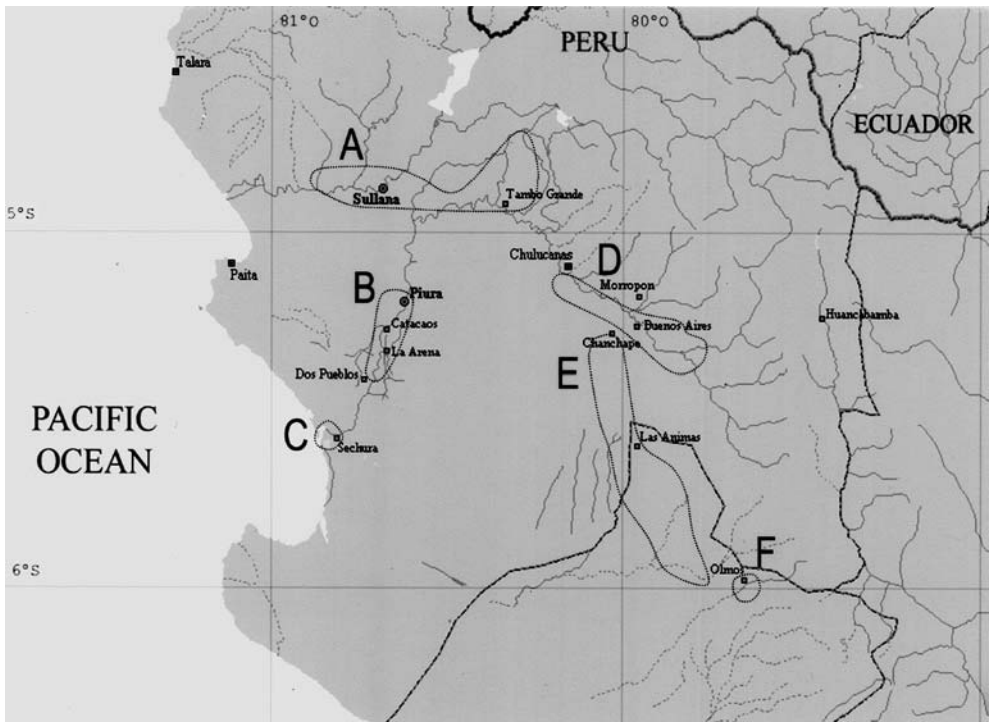

Figure 1. Map of the north-western region of Peru. The areas surveyed during the ENSO 98 expedition are indicated and named with a letter code.

pooled, whereas plants were individually analyzed with SSRs.

Amplified fragment length polymorphism (AFLP) analysis

The protocol described previously for AFLPs was followed (Ferriol et al. 2003; Nuez et al. 2004). The re-selective amplification of the AFLP technique was conducted using the primers *Eco*RI + A (5′ – GAC TGC GTA CCA ATT CA – 3′) and *Mse*I + C (5′ – GAT GAG TCC TGA GTA AC – 3′). Six different primer combinations were used in the selective amplification (*Eco*RI-AAC/*Mse*I-CAC, *Eco*RI-ACA/*Mse*I-CAC, *Eco*RI-AGG/*Mse*I-CAC, *Eco*RI-ACG/*Mse*I-CTA, *Eco*RI-ACT/*Mse*I-CAA, *Eco*RI-AGC/*Mse*I-CAA). Electrophoresis was

Figure 2. Habitat of *L. pimpinellifolium* in north-western Peru after the ENSO event of 1997–1998. (a) The natural vegetation was mainly composed of *Luffa operculata* and *L. pimpinellifolium* covering the trees (*Prosopis pallida*). (b) Detail of a plant of *L. pimpinellifolium* with red fruits.

Table 2. Qualitative and quantitative traits used in the morphological characterization of the accessions collected in northwestern Peru during the ENSO98 expedition.

Plant	Flower and inflorescence	Fruit
Quantitative traits		
Stem width (cm) SW	Number of petals NP	Fruit length (cm) FL
Height of the 3rd inflorescence (cm) H3I	Number of sepals NSp	Fruit width (cm) FW
Number of leaves between inflorescences NLI	Number of stamens NSt	Pedicel length (mm) PeL
Number of leaflets per leaf NLe	Petal length PL	Pedicel length from the abscission layer (mm) PeLA
Number of small leaflets per leaf NSLe	Petal width (mm) PW	Number of locules NL
Leaf length (cm) LL	Sepal length (mm) SpL	Width of pedicel scar (mm) WPS
Leaf width (cm) LW	Sepal width (mm) SpW	Fruit pubescence (0–4) FP
Leaflet length (cm) LeL	Style length (mm) SL	Intensity of external colour (0–4) IEC
Leaflet width (cm) LeW	Stigam exsertion beyond the anther tube (mm) SE	Intensity of flesh colour (0–4) IFC
Stem pubescence density (scored from 0 to 4) SPD	Stamen length (mm) StL	Lightness L
Stem pubescence length (0–4) SPL	Anther cone width (mm) ACW	Hue angle (arctg (b/a)) TAN
Leaflet pubescence density (0–4) LePD	Style hairiness (0–4) SH	
	Number of flowers per truss NFT	
	Inflorescence peduncle length (cm) IPL	
	Inflorescence length (cm) IL	
	Number of branches per inflorescence NBI	
Qualitative traits		
Plant growth type (determinate, semi-determinate, indeterminate)	Style position (inserted, same level as stamens, slightly exserted, highly exserted)	Fruit color (green, yellow, orange, red)
Stipules on the leaves (presence, absence)	Style shape (simple, fasciated, divided)	Epidermis color (colorless, yellow)
Anthocyanin coloration of stems (presence, absence)	Ovary shape (simple, slightly fasciated, fasciated)	Jointless pedicel (presence, absence)
Anthocyanin coloration of leaf veins (presence, absence)	Inflorescence type (uniparous, multiparous)	

conducted using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). Raw data were analyzed with GeneScan 3.1.2 analysis software (Applied Biosystems) and the resulting GeneScan trace files were imported into Genographer 1.6.0. (http://hordeum.oscs.montana.edu/genographer/). The AFLP fragments from 60 to 380 bp were scored in Genographer as present (1) or absent (0).

Simple sequence repeats (SSR) analysis

In the present study, we use 15 primer pairs (LE2A11, LE20592, LE21085, LEATPACAa, LEATPACAb, LECAB9, LECHI3, LEGAST1, LEGTOM5, LEILV1B, LELEUZIP, LEMDDNb, LESSF, LEWIPIG, LPHFS24), selected from those reported by Smulders et al. (1997) as polymorphic within the wild *Lycopersicon* species and with a high number of alleles. These SSRs represent different types of repetitions. PCR reactions were performed according to the procedure reported by these authors. The forward primers were labeled with different fluorescent dyes and 6 loci were simultaneously detected using an ABI PRISM 310 Genetic Analyzer.

Data analysis

Pearson correlation coefficients (r) and partial correlations (rp) were calculated among quantitative traits. Principal Components Analysis (PCA)

was performed with the standardized morphological quantitative data $((Z_{ij} = X_{ij} - \mathrm{CMIN}_i)/(\mathrm{CMAX}_i - \mathrm{CMIN}_i)$, where X_{ij} is the value of the i trait for the j accession, CMIN_i is the minimum value and CMAX_i the maximum value of the j accessions for the i trait) to obtain a graphic representation of the relationship structure of the characterized accessions (NTSYSpc v.2.02, Rohlf 1998). A unifactorial analysis of the variance (ANOVA) and a Duncan test for means comparison was conducted using the more outstanding traits (Statgraphics plus v 4.0).

In AFLP molecular analysis, the Dice similarity coefficient among accessions was calculated (Dice 1945). The similarity matrix was subjected to a Principal Coordinate Analysis (PCoA) (NTSYSpc v.2.02). The percentage of polymorphic loci (Pp), the gene diversity (Nei 1973) in each subpopulation, D_s, and in the global collection, D_t), and the differentiation coefficient (Nei 1977), $G_{ST} = ((D_t - D_s)/D_t)$, were also calculated (Popgene v.1.32, Yeh et al. 1997).

Genetic distances of Cavalli-Sforza and Edwards (1967) between accessions were calculated with SSRs data and used to construct a distance matrix. Previous studies showed that this distance is adequate for the analysis of intra-specific variability with SSR markers (Takezaki and Nei 1996). To compare the genetic distance calculated with the different marker systems we used a Mantel test (Mantel 1967) (NTSYSpc v.2.02). The data from the SSR analysis were also subjected to a Factorial Correspondence Analysis (FCA) (Escofier and Pagés 1990) to determine the relationships among the different plants. The mean number of alleles (Nm) and the polymorphic information content (Pic) per *locus*, the expected heterozygosity (H_e), assuming Hardy–Weinberg equilibrium, the observed heterozygosity (H_0), and the percentage of polymorphic loci (Pp) (per accession, in different subpopulations and per species) were also calculated. The genetic structure of populations was analyzed by using the Wright F parameters, F_{IS}, F_{IT} y F_{ST}, according to Weir and Cockerham (1984) (Genetix v.4.04, Belkhir et al. 1996–2002). A spatial autocorrelation analysis (Smouse and Peakall 1999), using 99 permutations, was performed with SSR data to study the correlation between genetic and geographic distance. This analysis allows the study of genetic structures at much shorter distances than other estimates of genetic differentiation, such as F_{ST}.

Results

Variability of qualitative traits

All plants displayed an indeterminate growth habit, leaves without stipules, anthocyaninless stems and leaves, simple styles and ovaries, uniparous inflorescences, and jointless pedicels as conforms to the standard *L. pimpinellifolium* phenotype (Taylor 1986). Variability was found for the color of the fruit flesh and epidermis. *L. pimpinellifolium* is typically a red-fruited species, but some PL accessions from the Lambayeque department (PL592 and PL593 from the area E, and PL597, PL598, PL599, PL601 and PL602 from the area F) exhibited orange or yellow fruits, a trait determined by the common *r* mutant, *yellow fruit flesh*. Furthermore, in most of the areas surveyed, plants carrying the recessive *y* mutation, *colorless fruit epidermis* that results in absence of pigment in the fruit epidermis, were found growing with plants with fruits of yellow epidermis. The percentage of plants with *colorless* fruits in the different areas ranged from 3 to 60%. The accession PL602 was mutant for traits *y* and *r*. The PL collection was also highly variable for the style position. Most accessions had flowers with slightly to highly exserted stigmas. However, a high proportion of plants with stigmas at the same level as the stamens were found among the accessions collected at the area F.

Correlations between traits

Significant positive correlations were found between traits measuring leaf size, such as length and width of leaves and leaflets ($r/rp_{\mathrm{LW-LL}} = 0.70/0.70$, $r/rp_{\mathrm{LW-LeL}} = 0.88/0.23$, $r/rp_{\mathrm{LW-LeW}} = 0.81/0.73$, $r/rp_{\mathrm{LL-LeL}} = 0.68/0.48$, $r/rp_{\mathrm{LL-LeW}} = 0.58/0.58$, $r/rp_{\mathrm{LeL-LeW}} = 0.72/0.64$), between traits related to flower size and display, such as length and width of petals and sepals, stamens and style length, and stigma exsertion ($r/rp_{\mathrm{PL-PW}} = 0.84/0.76$, $r/rp_{\mathrm{PL-SpW}} = 0.75/0.08$, $r/rp_{\mathrm{PL-SpL}} = 0.64/0.34$, $r/rp_{\mathrm{PL-StL}} = 0.76/0.16$, $r/rp_{\mathrm{PL-SE}} = 0.61/0.14$, $r/rp_{\mathrm{PW-StL}} = 0.60/0.30$, $r/rp_{\mathrm{PW-SpW}} = 0.86/0.40$,

$r/rp_{PW-SpL} = 0.71/0.51$, $r/rp_{SE-StL} = 0.44/0.69$), between traits related to inflorescence size and display, such as number of flowers per truss and inflorescence length ($r/rp_{NFT-IL} = 0.83/0.75$, $r/rp_{IL-IPL} = 0.62/0.71$), and between the traits measuring fruit size, such as fruit length and width ($r/rp_{FW-FL} = 0.95/0.96$).

Morphological multivariate analysis

A Principal Component Analysis was conducted using the quantitative data of all PL accessions, *L. pimpinellifolium* and *L. esculentum* (Figure 3a, b). The first and the second components accounted for 32.3% and 15.3% of the total variation. The first component separated the accessions of the two species Figure 3b). *L. esculentum* accessions displayed large and multiloculated fruits (FW, FL, WPS, NL, PeL and PeLA), flowers with a variable number of petals, sepals and stamens (from 5 to 6) (NSp, NP and NSt), and with sepals larger than petals, a wide anther cone and a short style with inserted stigma (SpW, ACW, SpL). Furthermore, the plants of the cultivated species were shorter, although more robust, than those of *L. pimpinellifolium*, and developed larger and more densely pubescent leaves (LW, LL, LeL, Lew).

Lycopersicon pimpinellifolium accessions were grouped on the basis of the first component, displaying typical characteristics of wild species, such as slender plants (H3I), and long inflorescences with a larger number of flowers (IL, NFT, IPL). The accessions of this species were distributed along the axis of the second component. This distribution was concordant with a gradual variation in the size of floral structures. The majority of the red-fruited accessions were distributed in the upper half of the graph, displaying attractive flowers with large petals (PL, PW), long styles and stamens (StL, SL), and stigmas exserted beyond the anther cone (SE). The degree of stigmatic exsertion of these red-fruited accessions was variable in all the areas surveyed (A = 1.4–3.4 mm, B = 1.1–2.7 mm, C = 2.3–3.8 mm, D = 0.8–2.5 mm, E = 2.1–4.3 mm, F = 1.8–2.3 mm). The accessions PL577, PL579, PL587, collected in different areas (B, C, and E), grouped separately from the others due to their large flowers with highly exserted.

The only yellow-fruited accessions from the area E (PL592 and PL593) appeared interspersed with other red-fruited accessions. However, most of the yellow-fruited accessions from the area F (PL598, PL599, PL601 and PL602) were grouped separately from the other in the lower part of the graph. In addition to fruit color differences, the yellow-fruited accessions were more pubescent, had smaller flowers with a lower degree of stigmatic exsertion, inflorescences with a lower number of flowers, and larger fruits (Table 3).

In general, the accessions of *L. pimpinellifolium* did not group according to their geographical origins. Similar morphological types were collected from the different areas. The accessions from the area F were the more variable. The different morphological types collected at this area appeared scattered in the PCA (Figure 3b). There also were no clinal patterns. We did not find any association between the morphological distribution observed in the PCA and different parameters such as altitude, latitude, longitude and distance to the coast.

Molecular polymorphism

Between 73 and 157 reproducible fragments were amplified per AFLPs primer combination, with an average of 111 bands per combination. For all the species together, a total of 665 bands were amplified, of which 622 (93%) were polymorphic. Among them, 447 fragments were amplified in *L. pimpinellifolium*, of which 337 (75%) were polymorphic. Simple sequence repeats were more polymorphic than AFLPs, as expected due to the higher mutation rates of the microsatellite regions. All the assayed SSR loci were polymorphic among *Lycopersicon* spp. An average of 11.5 SSRs alleles per locus were found in all the species, ranging from 3 to 24, whereas an average of 6.7 alleles, ranging from 2 to 20, were identified in *L. pimpinellifolium* (Table 4). LE2A11, LEATPACAa, LECAB9, LELEUZIP, LEMDDNb, LEWIPIG, LEGAST1 and LPHFS24 were polymorphic within *L. pimpinellifolium*, but had one allele much more frequent than the others. The remaining loci were highly polymorphic within this species.

Certain alleles were present in most *L. esculentum* cultivars and in accessions of the var. *cerasiforme*, but absent in *L. pimpinellifolium*. Also, some

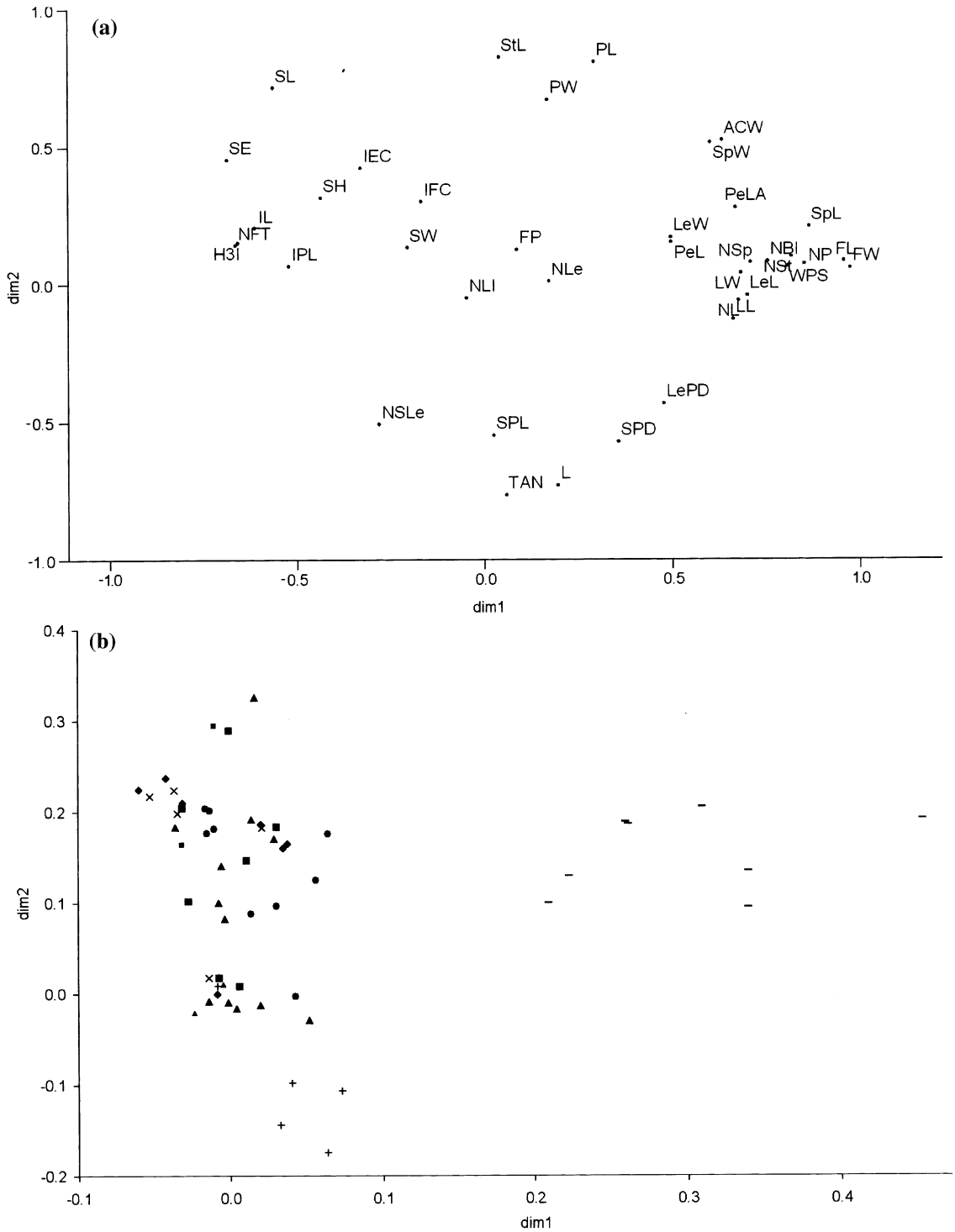

Figure 3. Principal Components Analysis (PCA) performed with the standardized quantitative morphological data of the *L. pimpinellifolium* and *L. esculentum* accessions collected at the ENSO98 expedition. (a) Diagram showing the relationships among the quantitative characters. The codes of the quantitative traits are included in Table 2. (b) Phenetic morphological structure among the nine accessions of *L. esculentum* (–) and the 50 accessions of *L. pimpinellifolium*. (Figure symbols: A (◆), B (■), C (■), D (•), red-fruited E (▲), yellow-fruited E (▲), red-fruited F (×), and yellow-fruited F (+)) .

Table 3. Morphological differences between the red-fruited accessions of *L. pimpinellifolium* collected at the ENSO98 expedition and those yellow-fruited collected in the same expedition at the F area (see Figure 1).

Accessions of *L. pimpinellifolium*	FL (mm)	FW (mm)	SPL (0–4)	SPD(0–4)	SE (mm)	PL (mm)	PW (mm)	ACW (mm)	StL (mm)	PeLA (mm)	NFT (mm)
Yellow-fruited (F)	12.09*	12.87*	3.83*	1.75*	0.74*	10.65*	3.01*	1.62*	6.71*	4.68*	17.00*
Red-fruited	9.66	10.51	2.48	1.05	2.56	15.37	4.86	2.03	8.79	6.59	19.72

FL = Fruit length, FW = Fruit width, SPI = Stem pubescence length, SPD = Stem pubescence density, SE = Stigma exsertion beyond the anther cone, PL = Petal length, PW = Petal width, ACW = Anther cone width, StL = Stamen length, PeLA = Pedicel length from the abscission layer, and NFT = Number of flowers per truss.

* The numbers in the same column are significantly different according to the Duncan means comparison test ($p < 0.05$).

alleles abundant in *L. pimpinellifolium* and were absent or rare in *L. esculentum*.

Molecular multivariate analysis

Principal Coordinates Analysis (PCoA) of AFLP data differentiated the green-fruited species (*L. pennellii*, *L. parviflorum*, *L. peruvianum* and *L. chilense*) from those red–orange and yellow-fruited (*L. esculentum*, *L. esculentum* var. *cerasiforme*, *L. pimpinellifolium* and *L. cheesmanii*) (data not shown).

Multivariate analyses, PCoA with AFLPs and FCA with SSRs, were also performed using only data from *L. pimpinellifolium* accessions, both PL and TGRC (Figure 4a, b). The two principal factors accounted for 10.2% and 8%, and 4.4% and 3.7% of the total variation, for AFLPs and SSRs, respectively. The correlation between the genetic distance matrices obtained with AFLPs and SSRs was 0.48 ($p < 0.05$) (Mantel 1967). Despite the differences found with the two marker systems, there were several common aspects to both analysis: (a) TGRC accessions were interspersed with PL accessions from different collection areas, but they did not represent all the different types included in the PL collection; (b) As with the results obtained previously with the morphological traits, PL accessions grouped independently of their origin. Similar molecular types were found even in different river basins, in areas separated by large geographic distances; (c) According to the two principal axes of variation of PCoA and FCA, the yellow-fruited accessions collected in the area F (PL597, PL598, PL599,

Table 4. Polymorphic information content (PIC) of the SSRs loci used in the characterization of the PL collection of the *L. pimpinellifolium*.

Locus (repetition)	Number of alleles	PIC	Approximate size (bp)
LE2A11/$(ATCT)_{5-1}$	2	0.17855	153,157
LE20592/$(TAT)_{15-1}(TGT)_4$	13	0.54986	150, 153, 156, 159, 162, 165, 169, 172, 175, 178, 181, 184, 187
LE21085/$(TA)_2(TAT)_{9-1}$	20	0.78623	99, 102, 108, 110, 111, 112, 113, 114, 115, 117, 118, 120, 122, 125, 128, 130, 133, 136, 140, 142
LEATPACAa/$(TA)_7$	13	0.50223	183, 187, 189, 197, 201, 205, 209, 211, 213, 215, 217, 219, 232
LEATPACAb/$(GA)_4$	4	0.37581	181, 183, 185, 187
LECAB9/$(TA)_6(CA)_3$	5	0.73055	116, 118, 124, 126, 128
LECHI3/$(TA)_{6-1}(GA)_4$	3	0.08487	119, 123, 125
LEGAST1/$(TA)_{12}(TG)_4$	6	0.70438	134, 138, 142, 144, 146, 148
LEGTOM5/$(TA)_{10}$	6	0.58119	175, 177, 179, 181, 185, 201
LEILV1B/$(T)_8(TA)_{10}(T)_5$	8	0.69476	136, 137, 138, 139, 140, 142, 146, 152
LELEUZIP/$(AAG)_{6-1}TT(GAT)_7$	3	0.01268	93, 99, 102
LEMDDNb/$(TG)_4(TA)_4$	3	0.62284	277, 278, 279
LESSF/$(CCCCA)_4$	10	0.25514	296, 298, 300, 302, 304, 306, 308, 310, 313, 315
LEWIPIG/$(CT)_{8-1}(AT)_4$	3	0.94621	248, 250, 256
LPHFS24/$(TA)_6$	2	0.26793	155, 159

The number of alleles amplified per locus and their size, is also included.

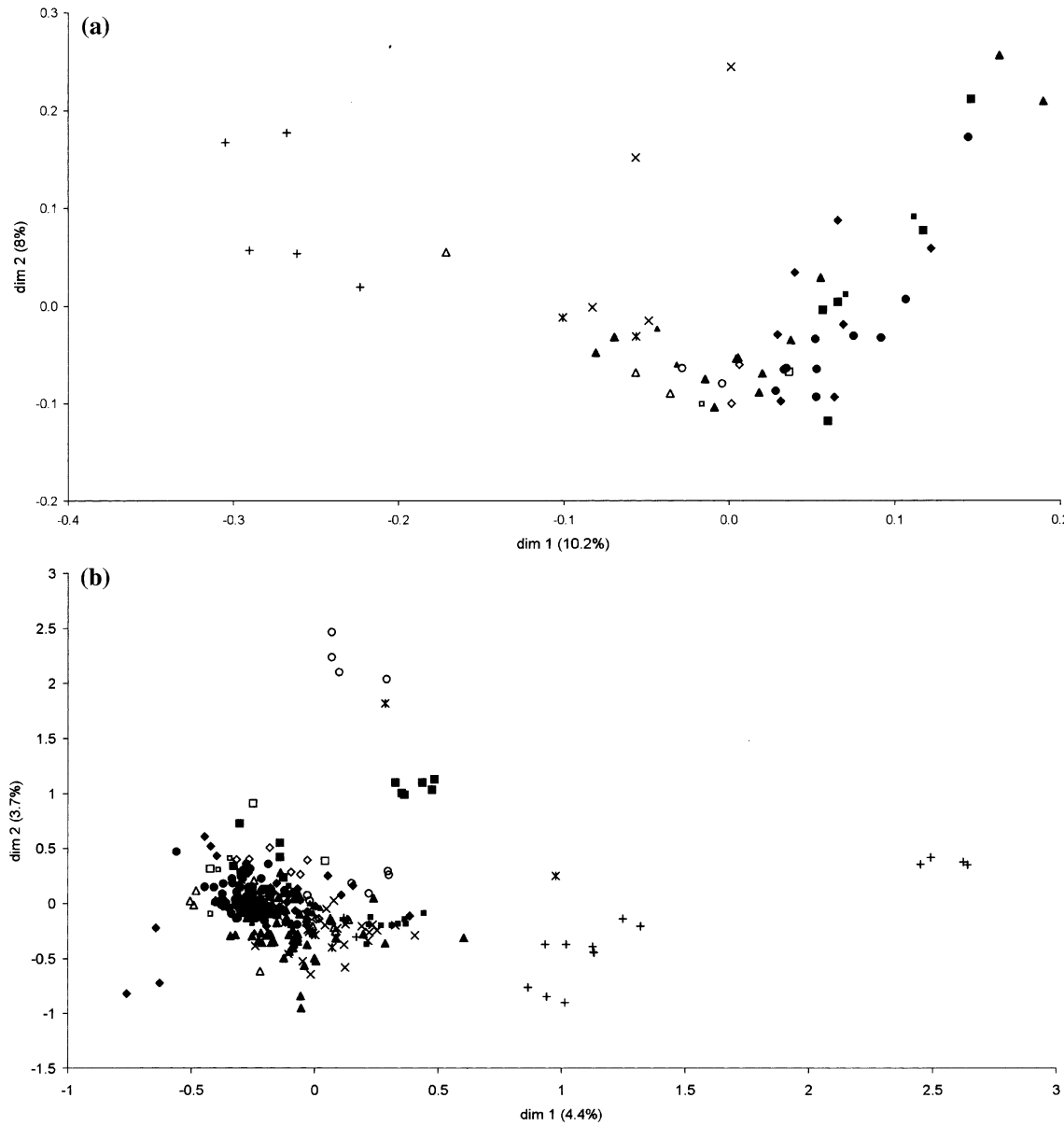

Figure 4. Diagrams showing the relationships among the accessions of *L. pimpinellifolium* collected by the ENSO98 expedition and those provided by the TGRC. (a) PCoA with AFLPs. Each symbol represents the analysis of a DNA bulk of 5 plants per accession. (b) Factorial Correspondence Analysis (FCA) with SSRs. Each symbol represents an individual plant. (see legend of Figure 3 for area symbols, black symbols have been used for PL accessions and white symbols for TGRC accessions, TGRC accessions of the F area (*).

PL601, PL602) grouped separately from the others. The accessions collected in this area were also the more variable molecularly, and appeared distributed in several differentiated groups. Further analyses were performed to clarify these three points.

Genetic diversity of the PL collection

The genetic diversity of the PL collection was compared with that of the previous collections in the area (TGRC sample) (Table 5). The different estimates of total genetic diversity calculated with

Table 5. Estimates of gene diversity in the PL accessions of *L. pimpinellifolium*, and in the accessions collected in the same area provided by the Tomato Genetics Resource Center.

Population	SSRs				AFLPs	
	N	He	Ho	Nm	*N*	D ± SD
PL collection	234	0.42	0.11	6.13	47	0.17 ± 0.19
TGRC	63	0.44	0.14	4.87	11	0.11 ± 0.18
Total	297	0.44	0.12	6.67	58	0.17 ± 0.18

N = Number of plants for SSRs, and number of accessions (DNA bulks) for AFLPs; He = Expected heterozygosity; Ho = Observed heterozygosity; Nm = Mean number of alleles; D = Gene diversity (Nei 1973).

the two marker systems (He, Ho, Nm, and D) provided values similar or only slightly higher for the PL collection. To avoid the effect that the higher number of plant/accessions of the PL collection in comparison with the TGRC sample could have on these estimates, we also calculated the mean value of Nm (using SSR data) in 100 groups of 63 plants randomly selected from the 234 of the PL collection, and the mean value of D (using AFLPs data) in 100 groups of 11 accessions, randomly selected among the 47 of the PL collection. The estimates obtained (Nm_{SSRs} = 5.4 ± 0.32 and D_{AFLPs} = 0.14 ± 0.01) were also similar to those calculated for the TGRC sample (Table 5). This result suggests that the ENSO event of 1997–1998 did not cause a significant increase of the total genetic diversity of *L. pimpinellifolium* populations in this area.

Structure of variation in the different areas surveyed

Estimates of gene diversity for each PL accession calculated with the two marker systems were highly correlated ($r_{DAFLPs-NmSSRs} = 0.73$). Therefore, the two systems gave similar information about the diversity of the PL collection. We did not find any cline or geographic association for variability, as both highly variable and nearly monomorphic accessions were found in all the areas surveyed Figure 5). The genetic diversity of subpopulations was also estimated and compared with total genetic diversity (Table 6). The total genetic diversity was only slightly higher than that of the different subpopulations. This could be a consequence of the lack of differentiation among subpopulations, as already suggested by the results of the morphological and molecular multivariate analysis. The lack of differentiation is confirmed by the low value of the F_{ST} (0.17).

The observed heterozygosity was in all cases lower than that expected under the Hardy–Weinberg equilibrium (Table 6). The low F_{ST} value along with the high value of F_{IS} = 0.69, suggests that this significant lack of heterozygotes is mostly explained by the endogamy of the subpopulations.

The spatial autocorrelation analysis allowed a more detailed study of the genetic structure of the

Table 6. Estimates of gene diversity in the subpopulations of *L. pimpinellifolium* collected in the different areas surveyed during the ENSO98 expedition.

Area code	SSRs						AFLPs		
	N	He ± SD	Ho ± SD	Nm	F_{IS}	F_{ST}	*N*	D ± SD	Pp
A	35	0.42 ± 0.29	0.15 ± 0.13	3.67	0.66 (0.58–0.69)	–	7	0.10 ± 0.18	29.3
B	24	0.36 ± 0.29	0.10 ± 0.12	2.60	0.73(0.59–0.78)	–	5	0.10 ± 0.18	24.8
C	10	0.24 ± 0.26	0.19 ± 0.24	1.73	0.26(0.25–0.56)	–	2	0.07 ± 0.15	16.7
D	50	0.34 ± 0.26	0.12 ± 0.09	3.40	0.65(0.59–070)	–	10	0.09 ± 0.17	26.6
E	70	0.35 ± 0.27	0.11 ± 0.09	4.33	0.68(0.59–0.72)	–	14	0.13 ± 0.19	38.7
F	45	0.37 ± 0.31	0.07 ± 0.06	3.60	0.82(0.80–0.86)	–	9	0.15 ± 0.20	40.5
Total	234	0.42 ± 0.28	0.11 ± 0.08	6.13	0.69(0.70–0.78)	0.17(0.13–0.22)	47	0.15 ± 0.18	67.3

N = Number of plants for SSRs, and number of accessions (DNA bulks) for AFLPs; He = Expected heterozygosity; Ho = Observed heterozygosity; Nm = Mean number of alleles; F_{IS}, F_{ST}: Statistics of Wright (Weir and Cockerham 1984), the values in brackets have been calculated for a 95% confidence interval, conducting bootstrap analysis with the *loci*; D = Gene diversity (Nei 1973); Pp = Percentage of polymorphic loci.

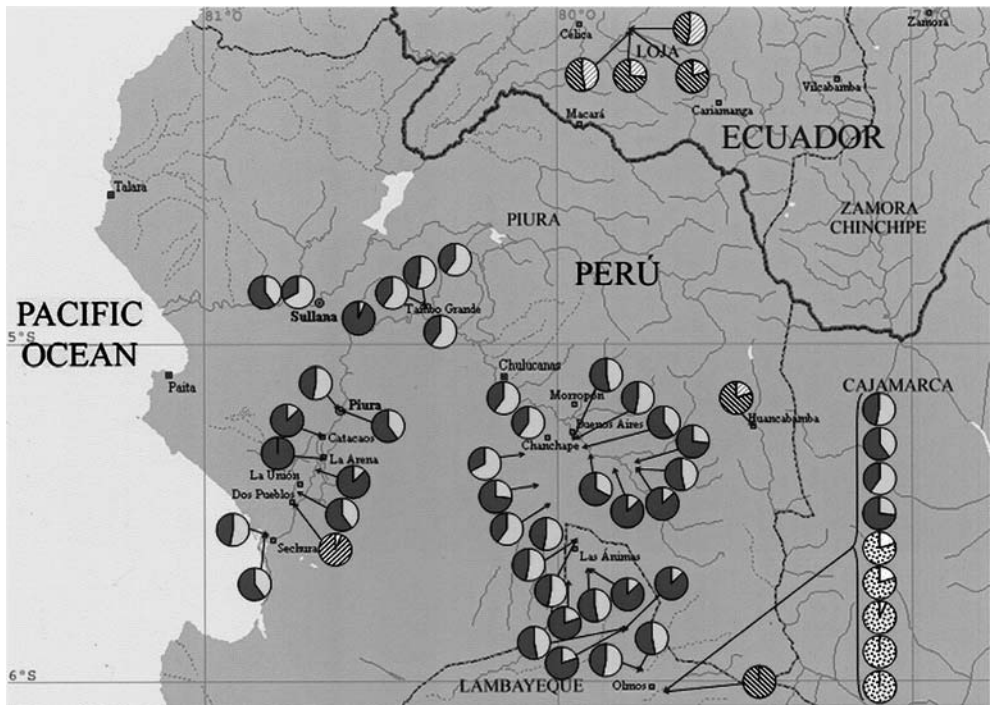

Figure 5. Percentage of polymorphic SSRs *loci* in the different accessions of *L. pimpinellifolium* and *L. esculentum* collected during the ENSO98 expedition. Striped circles represent *L. esculentum* accessions, solid circles represent accessions of *L. pimpinellifolium*, and dotted circles the yellow-fruited accessions of *L. pimpinellifolium* collected at the area F. The light color indicates the percentage of polymorphic *loci*.

PL population (Figure 6). Positive correlations between genetic and geographic distances were found at short distances, with a maximum value for the correlation coefficient of 0.17. For distances under 30 km, the accessions collected at nearer sites were similar and genetic distance increased with geographic distance. For large distances, the correlations were not significant.

Differentiation within L. pimpinellifolium

The yellow-fruited accessions collected at the area F displayed a degree of polymorphism much lower that most of the red-fruited accessions of *L. pimpinellifolium*, even lower than some *L. esculentum* cultivars (Figure 5). Two of them were monomorphic (PL599 and PL601). This group of accessions also showed a low degree of heterozygosity ($Ho_{PL597} = 0.033 \pm 0.129$, $Ho_{PL598} = 0.053 \pm 0.207$, $Ho_{PL599} = 0$, $Ho_{PL601} = 0$, $Ho_{PL602} = 0.081 \pm 0.177$), in comparison with the mean value of the red-fruited accessions of *L. pimpinellifolium* ($Ho = 0.120 \pm 0.194$) and *L. esculentum* ($Ho = 0.072 \pm 0.113$). Also notable is the high degree of fixation ($F_{IS} = 0.899$) of this subpopulation in comparison with the TGRC sample ($F_{IS} = 0.682$) and the PL red-fruited subpopulations ($F_{IS} = 0.707$). These accessions appear to be nearly pure lines.

To clarify the nature of this group of accessions, a FCA was conducted using the SSRs data from all PL accessions, *L. pimpinellifolium* and *L. esculentum*. Since the PL accessions of *L. esculentum* were found growing sympatrically with the wild *L. pimpinellifolium* accessions, they could share some of its alleles due to introgression. For this reason, we included in this analysis several Spanish landraces of tomato and a group of accessions of *L. esculentum* var. *cerasiforme* from different origins (Figure 7). Results are coherent with the morphological analysis as FCA separated the yellow-fruited accessions from the other PL *L. pimpinellifolium* accessions and also from those of the *L. e.* var. *cerasiforme*, that were interspersed with the Spanish tomato landraces and with the PL tomato accessions. The degree of differentia-

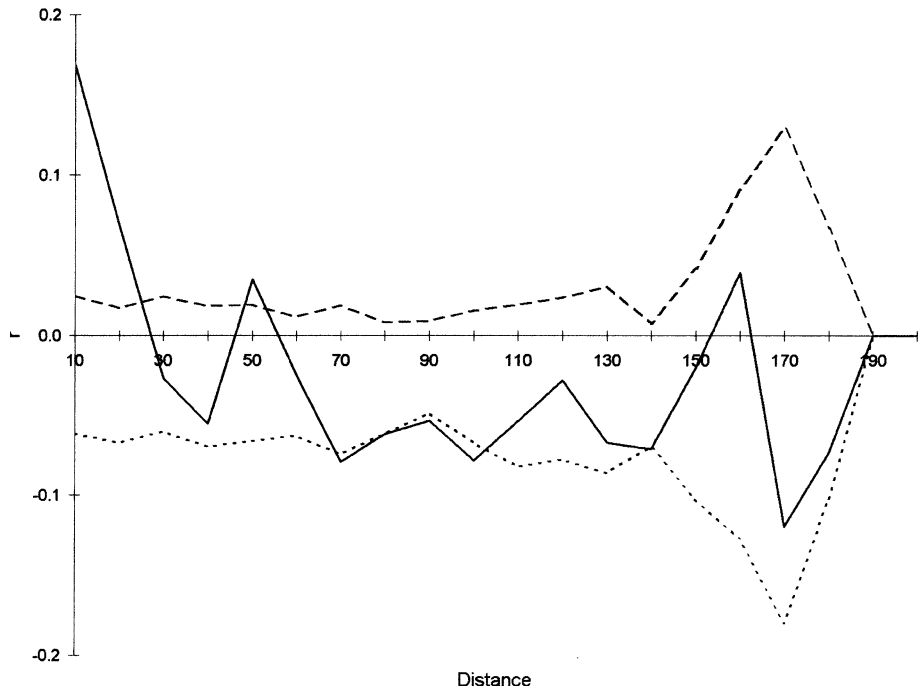

Figure 6. Diagram showing the variation of the coefficient of genetic correlation (r) among the accessions of *L. pimpinellifolium* collected by the ENSO 98 expedition with the geographic distance (km). The dotted lines indicate the limits of the confidence interval (95%) of the null hypothesis of absence of correlation.

tion of the yellow-fruited subpopulation from *L. esculentum* and *L. e.* var. *cerasiforme* was much higher than that of the red-fruited PL accessions of *L. pimpinellifolium* from the cultivated species (Table 7).

Discussion

Our morphological and molecular characterization of this extensive assemblage of germplasm has provided the most complete study published to date on the genetic structure of the populations of *L. pimpinellifolium* from the central area of its distribution range, where the highest levels of variation occur. The effect of a periodical climatic phenomenon such as the ENSO on these populations has also been studied for the first time.

The ENSO event of 1997–1998 increased exceptionally the size of the *L. pimpinellifolium* populations. However, this increase was not associated with a significant increase in the total genetic diversity of this species in the area. This suggests that, despite the small number of plants

Table 7. Differentiation of the PL populations collected during the ENSO98 expedition (*L. esculentum*, red-fruited *L. pimpinellifolium* (from different areas) and yellow-fruited *L. pimpinellifolium* (collected at the F area)) from the population of *L. esculentum* var. *cerasiforme* (including accessions from different origins) and the group of accessions of *L. pimpinellifolium* provided by the Tomato Genetics Resource Center.

	TGRC *L. pimp.*	PL *L. pimp.* red-fruited	PL *L. pimp.* yellow-fruited I	*L. e. cerasiforme*
PL *L. esculentum*	0.34/0.39[a]	0.35/0.32	0.59/0.47	0.29/–
TGRC *L. pimpinellifolium*	–	0.07/0.09	0.27/0.27	0.38/–
PL *L. pimpinellifolium* red-fruited	–	–	0.25/0.23	0.46/–
PL *L. pimp.* yellow-fruited F	–	–	–	0.67/–

[a]The first number refers to the F_{ST} Wright statistics (Weir and Cockerham 1984), calculated using the SSRs data, and the second refers to the Nei differentiation coefficient (Nei 1977), $G_{ST} = (D_t - D_s)/D_t$, calculated using AFLPs data.

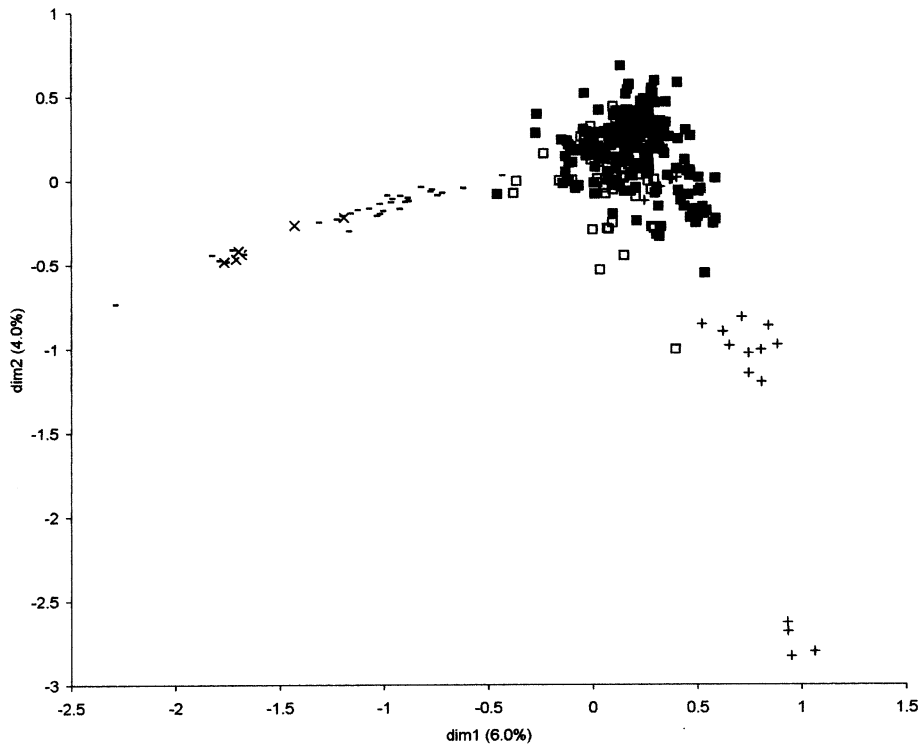

Figure 7. Factorial Correspondence Analysis (FCA) performed with SSRs showing the relationships among the accessions collected during the ENSO98 expedition. (Figure symbols: *L. esculentum* (−), red-fruited *L. pimpinellifolium* from different areas (■), and yellow-fruited *L. pimpinellifolium* from the F area (+)), the TGRC accessions of *L. pimpinellifolium* (□), the population of *L. esculentum* var. *cerasiforme* with accessions from different origins (×) and the group of tomato Spanish landraces (−).

that survive during the drought periods, most of the genetic variability remains in latent seeds coming from those large populations that can grow in the area during ENSO years.

According to our studies, *L. pimpinellifolium* is not regionally differentiated in the area surveyed. Rick et al. (1977) reported defined regional patterns and clines in the distribution of allozymes, within the entire range of distribution. The SSRs used in this study were more informative than the allozymes used by those authors who detected from 2 to 17 alleles per locus (with a mean of 4.8). However, we did not find SSR alleles exclusive or abundant in any sub-area of this zone influenced by the ENSO, except from those of the yellow-fruited population from the F area. The study of spatial auto-correlation reveals a significant population structure only at very short distances, probably due to an effect of pollen or seed dispersal (Ennos 1994; Smouse and Peakall 1999). For larger distances, the genetic distance does not increase with the geographic distance, and similar molecular types are found in different regions. This lack of correlation between geographic and genetic distances has also been reported in other species (Del Rio et al. 2002; McGregor et al. 2002).

The low degree of differentiation found in such a wide area can be a consequence of pollen and seed dispersal (human or animal) combined with high outcrossing rates. The flooding and water streams associated to ENSO could have facilitated seed transport, bringing near different types initially separated. This would have allowed the occurrence of gene flow between them. The floral morphology found in most PL accessions can also facilitate this gene flow. It was here where Rick et al. (1977, 1978) reported that the highest level of stigmatic exsertion from the entire *L. pimpinellifolium* range of distribution. Accordingly, most of the red-fruited accessions collected at the ENSO98 expedition had large and exposed flowers, which increase floral display and pollinator attraction. The values of stigmatic exsertion found in the PL accessions were even higher than those reported by Rick et al.

in their populations from Piura and Lambayeque (1.5–2.2 mm). Furthermore, the increase in the population size and the probable increase in the number and kinds of pollinator insects caused by the ENSO could have increased the rate of outcrossing.

The high degree of endogamy found in the populations does not stand in contradiction to the existence of outcrossing. The endogamy can be a consequence of the existence of autogamy, but it could also be due to the occurrence of crosses between sibs or between close relatives. In fact, despite the high endogamy (high F_{IS} values), these populations are not totally fixed.

The PL collection included accessions morphologically and molecularly different to those collected in previous expeditions. As an example, Rick et al. (1977) did not report the occurrence of the *r* mutation in this area, and the *y* mutation only appeared in their populations from Lambayeque (the wild allele + was fixed in the populations from Piura). We collected seven accessions with yellow or orange fruits. The two accessions collected at the E area (PL592 and PL 593) only differ from some red-fruited accessions in the fruit color. A similar situation was found for other yellow-fruited accessions of *L. pimpinellifolium* collected further north, in the area of Santa Isabel (Ecuador), by the COMAV (F. Nuez, unpublished results). However, the yellow-fruited accessions from the area F displayed differential morphological characteristics and were molecularly different from the remaining PL accessions and the TGRC collections. Their values of stigmatic exsertion and heterozygosity are only comparable to those reported previously in the accessions of *L. pimpinellifolium* from the departments of Ancash or Lima, far south of Lambayeque, and in the accessions of more northerly areas, such as Tumbes (Ecuador) (Rick et al. 1977).

This yellow flesh color, characteristic of the first tomatoes cultivated in Europe (Mathiolus 1544), has been reported in two different genetic backgrounds of the tomato ancestor *L. esculentum* var. *cerasiforme*, in the majority of the Bolivian and in certain Mexican accessions (Rick and Holle 1990). An independent mutational origin of *r* in Central and South America has been suggested. Certain Mexican accessions of *L. esculentum* var. *cerasiforme*, locally named 'coyote', display a combination of *r* and *y* that results in a pale tawny yellow fruit color, as occurs in the accession PL602. Some of the morphological characteristics exhibited by the F accessions make them similar to var. *cerasiforme*. For example, the low degree of stigmatic exsertion, the reduced flower size, and the bigger fruits (although fruit diameter was always less than 15 mm, the limit used to differentiate *L. pimpinellifolium* from var. *cerasiforme* (Rick et al. 1990). Moreover, molecular analyses indicated that these accessions are near to being pure lines, similarly to the var. *cerasiforme* that tends to be highly autogamous. It is difficult to set any morphological limits that clearly distinguish *L. e.* var. *cerasiforme* from *L. pimpinellifolium*. Intergrades of many sort exists, not only for fruit size, but also for fruit shape, leaf shape, pubescence, and number of flowers per truss. These forms are frequent in the north and northeast margins of the *L. pimpinellifolium* distribution, where classification is sometimes arbitrary (Rick et al. 1977; Widrlechner 1987). The F_{ST} and G_{ST} values indicated that this is a subpopulation of *L. pimpinellifolium* and does not belong to the cultivated species, *L. esculentum*. The degree of differentiation of these yellow-fruited accessions from the red *L. pimpinellifolium* ($F_{ST} = 0.27 - 0.25$) is similar to the differentiation degree of the *L. e.* var. cerasiforme from *L. esculentum* ($F_{ST} = 0.29$).

This subpopulation was not present in prior collections. It is possible that it was simply overlooked by earlier collectors, but it is also possible that this population had been transported from another area by ENSO or other causes. In fact, if this population had been growing here for a long time, genetic flow between this subpopulation and the other yellow- and red-fruited accessions had likely occurred. Although, the identity of the population could have been maintained due to its high autogamy, insects would have dispersed its pollen. However, our results indicate the absence of recent and intense genetic flow between this population and the remainder accessions form the area. In any case, further studies including *L. esculentum* accessions from the entire distribution range are necessary in order to determine the origin of this population and its unique alleles. The accession PL598 of this subspecies has provided results of particular interest to us because of its remarkably high content of vitamin C (Galiana-Balaguer et al. 2001).

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