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AFLP fingerprinting in *Capparis* subgenus *Capparis* related to the commercial sources of capers

Cristina Inocencio[1,*], Robyn S. Cowan[2], Francisco Alcaraz[1], Diego Rivera[1] and Mike F. Fay[2]

[1]*Department of Plant Biology, University of Murcia, Murcia E-30100, Spain;* [2]*Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey TW9 3DS, UK; *Author for correspondence (e-mail: cpretel@um.es)*

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Abstract

A genetic fingerprinting technique (AFLP) was used to determine the relationships among *Capparis* spp. Genetic distances, based on AFLP data were estimated for 45 accessions of *Capparis* species, from Spain, Morocco and Syria. The results of this analysis support the differentiation of four of the five taxa involved. The group of plants recognised as *C. spinosa* on the basis of morphological characters, includes several cultivars and appears in an intermediate position between *C. orientalis* and *C. sicula* and overlaps with *C. orientalis*. The other two species *C. aegyptia* and *C. ovata* are separate from the rest. *Capparis spinosa* had a low number of unique bands in comparison with the other species. Although these results cannot confirm the hybrid origin of *C. spinosa*, the distribution of the bands supports this hypothesis, the most likely parental species being *C. orientalis* and *C. sicula*.

Introduction

The subtropical genus *Capparis* L. is represented in the Mediterranean and Western Asia by several species included in subgenus *Capparis* Commercially speaking, capers are immature flower buds which have been pickled in vinegar or preserved in granular salt. Semi-mature fruits and young shoots with small leaves may also be pickled for use as a condiment. All commercial products are thought to come from the same species, *C. spinosa* L., which is also the type species of the section. The use of capers can be traced to ancient times. Capers have long been used as a condiment in Greece, being highly appreciated for their pungent and bitter flavour (Alvarruiz et al. 1990; Inocencio et al. 2000). Most of the material used for commercial purposes is gathered from the wild, but there are also several areas where caper plants are cultivated.

The systematics of the genus *Capparis* are based on morphological characters such as leaf shape, flowers, spines, etc. (Zohary 1960; Greuter and Burdet 1984; Higton and Akeroyd 1991). The type of the section, *C. spinosa*, is morphologically closely related to *C. orientalis* Duhamel and *C. sicula* Duhamel, and these two taxa have been included in *C. spinosa* by some authors (Table 1). Moreover, there is no clear idea about how many or which species are commercialised. (See Hammer 2001).

Caper cultivars are commonly referred to as *C. spinosa*, although several belong to other taxa (Tables 2 and 3) and have also been investigated in this study.

Traditional classification based on the observation of both quantitative and qualitative macro-morphological characters could have a wide margin of error due to a lack of objectivity by the researcher and the effect of environmental

Table 1. Taxonomic interpretation of the five taxa studied by different authors.

Species	Zohary (1960)	Greuter and Burdet (1984)	Higton and Akeroyd (1991)	Fici (2001)
C. orientalis Veill.	*C. spinosa* var. *rupestris* (Sibth.) Hook. F. and Th.	*C. orientalis* Veill.	*C. spinosa* subsp. *rupestris* (Veill. in Duhamel) Jafri	*C. spinosa* subsp. *rupestris* (Veill. in Duhamel) Jafri
C. spinosa L.	*C. spinosa* L. subsp. *spinosa*	*C. spinosa* L.	*C. spinosa* L. subsp. *spinosa*	*C. spinosa* L. (rocky habitats)
C. sicula Veill.	*C. ovata* var. *sicula* (Duhamel) Zohary	*C. sicula* Veill.	*C. spinosa* var. *canescens* (Coss.) A. & O. Bolòs	*C. spinosa* L. (clay)
C. aegyptia Lam.	*C. spinosa* var. *aegyptia* (Lam.) Boiss.	*C. aegyptia* Lam.	–	–
C. ovata Desf.	*C. ovata* var. Desf. *ovata*	*C. ovata* Desf.	–	–

Table 2. Cultivars known within *C. spinosa*.

Cultivar	Localities	References
Ciavulara	Pantelleria (Italy)	Fici and Gianguzzi (1997)
Nuccida, Nocella	Salina (Italy)	Barbera and Lorenzo (1984); Fici and Gianguzzi (1997)
Spinoso di Pantelleria	Pantelleria (Italy)	Fici and Gianguzzi (1997)
Testa di Lucertola	Pantelleria (Italy)	Barbera and Lorenzo (1984)
Tondino	Salina and Pantelleria (Italy)	Caccette (1985)
Colorá	Mallorca (Spain)	Rivera et al. (1999)
De las Muradas	Mallorca (Spain)	Rivera et al. (1999)
Figues Seques (*)	Mallorca (Spain)	Rivera et al. (1999)
Mallorquina (*)	Mallorca (Spain)	Rivera et al. (1999)
Peluda (*)	Mallorca (Spain)	Rivera et al. (1999)
Redona (*)	Mallorca (Spain)	Rivera et al. (1999)
Roses (*)	Mallorca (Spain)	Rivera et al. (1999)
Rubia (*)	Mallorca (Spain)	Rivera et al. (1999)

Those marked with asterisk (*) have been studied in this paper.

Table 3. Cultivars known within *C. orientalis*, *C. sicula* and *C. aegyptia*.

Taxa	Cultivar	Localities	References
C. orientalis Veill.	Fulla Redona (*)	Mallorca (Spain)	Inocencio (2001)
	Nucciddara, Nocellara	Pantelleria (Italy)	Barbera and Lorenzo (1984); Fici and Gianguzzi (1997)
C. sicula Veill. subsp. *sicula*	Spinoso, Spinoso di Salina	Salina (Italy)	Barbera and Lorenzo (1984); Fici and Gianguzzi (1997); Caccette (1985)
C. aegyptia Lam.	De Safi (*)	Safi (Morocco)	Inocencio (2001)

Those marked with asterisk (*) have been studied in this paper.

conditions on the phenotypic characters. Over recent decades a large quantity of data has been used, which in an indirect manner reflects the genetic variability of these taxa. DNA studies related to *Capparis* are very scarce, there is one molecular study involving *Capparis* prior to this paper, where RAPD techniques have been used for estimating the genetic variation in capers from Tunisia and Italy (Khouildi et al. 2000).

Following the development of PCR (polymerase chain reaction) in the mid-1980s, the rapid and reliable amplification of DNA was made possible (Mullis 1986; Innis 1990). Thus, direct genome analysis is today possible and increasingly powerful

Table 4. Plant materials: sic: *C. sicula*; spin: *C. spinosa*, localities, status and voucher.

Samples	Part used	Locality	Voucher	Status
sic sk	Leaves	Sidi-Karcem (Morocco)	MUB 60017	Wild
sic fes 2	Leaves	Fes (Morocco)	MUB 60016	Wild
sic esc	Leaves	Escombreras (Murcia, Spain)	MUB 60055	Wild
sic esc1	Leaves	Escombreras (Murcia, Spain)	MUB 60148	Wild
sic esc2	Leaves	Escombreras (Murcia, Spain)	MUB 60056	Wild
sic esc3	Leaves	Escombreras (Murcia, Spain)	MUB 60057	Wild
sic syr7	Leaves	Damasco (Syria)	MUB 60058	Wild
sic esc5	Leaves	Escombreras (Murcia, Spain)	MUB 60149	Wild
spin m	Leaves	Llubi (Mallorca, Spain)	MUB 60036	cv. Mallorquina
spin pm	Flowers	Llubi (Mallorca, Spain)	MUB 60046	cv. Peluda
spin pmA	Flowers	Llubi (Mallorca, Spain)	MUB 60127	cv. Peluda
spin pbA	Leaves	Campos (Mallorca, Spain)	MUB 60128	cv. Peluda
spin pb	Leaves	Llubi (Mallorca, Spain)	MUB 60042	cv. Peluda
spin fsm	Leaves	Campos (Mallorca, Spain)	MUB 60039	cv. Figues seques
spin fsmA	Leaves	Campos (Mallorca, Spain)	MUB 60129	cv. Figues seques
spin fsb	Leaves	Llubi (Mallorca, Spain)	MUB 60130	cv. Figues seques
spin fb	Flowers	Llubi (Mallorca, Spain)	MUB 60041	cv. Figues seques
spin rmA	Flowers	Campos (Mallorca, Spain)	MUB 60131	cv. Redona
spin rm	Flowers	Campos (Mallorca, Spain)	MUB 60044	cv. Redona
spin rtA	Flowers	Llubi (Mallorca, Spain)	MUB 60040	cv. Roses
spin rt	Flowers	Llubi (Mallorca, Spain)	MUB 60037	cv. Roses
spin rbA1	Flowers	Llubi (Mallorca, Spain)	MUB 60022	cv. Rubia
spin cas4	Leaves	Alicante (Spain)	MUB 60049	Wild

molecular techniques are available for use in scientific fields such as systematics and taxonomy. The use of genomic characters has complemented morphological classifications substantially. The aim of this research was to investigate the relationship between *Capparis* spp. (wild populations, presumed hybrids and local cultivars) using Amplified Fragment Length Polymorph (AFLP, Vos et al. 1995). This technique has been widely used for determining phylogenetic relationships in plants (Rieck 2001).

Materials and methods

A total of 45 accessions of *Capparis* (from 28 populations) from Spain (28 accessions from 15 populations), Morocco (11 accessions from eight populations) and Syria (five accessions from five populations) (Table 6). A total of five randomly selected individuals per population were studied. A previous extraction of DNA from caper leaves collected on the Iberian Peninsula shows the difficulties in using such material due to the high concentration of waxes in the leaves. DNA was extracted either from leaves or flowers, the latter used preferably whenever possible. Approximately 2 g of fresh plant material from five different individuals per population were randomly collected and dried in silica gel (Chase and Hills 1991) (Tables 4–6). The DNA was extracted from all the individuals collected in each population but only those which gave enough bands to be compared are represented in this paper.

All commercial kits were used according to the manufacturer's protocols. The genomic DNA of each accession was extracted from approximately 0.3 g of sample, according to 2 × CTAB method (Doyle and Doyle 1987). The DNA was left to precipitate for 3 days. All samples were purified in caesium chloride/ethidium bromide gradients (1.55 g/mL). This was followed by a step of QIAquick column purification (Qiagen, Ltd). DNA was quantified with a UV-1201 UV-VIS spectrophotometer (Shimadzu Europe, Milton Keynes, UK).

Total DNA (500 mg) was restricted using *Mse*I and *Eco*RI, and adaptors were ligated on to the restriction sites. In the preselective amplification, primers with one additional base at the 3′ end were used to amplify a subset of the restriction fragments (Table 7).

Table 5. Plant materials: ori: *C. orientalis*; aeg: *C. aegyptia*; ova: *C. ovata*, localitites, status and voucher.

Samples	Part used	Locality	Voucher	Status
ori frA	Flowers	Llubi (Mallorca, Spain)	MUB 60133	cv. Fulla redona
ori mur2	Flowers	Palma de Mallorca (Mallorca, Spain)	MUB 60063	Wild
ori mur1	Flowers	Palma de Mallorca (Mallorca, Spain)	MUB 60132	Wild
ori cas2	Leaves	Alicante (Spain)	MUB 60047	Wild
ori cas3	Leaves	Alicante (Spain)	MUB 60048	Wild
ori mur5	Leaves	Palma de Mallorca (Mallorca, Spain)	MUB 60051	Wild
ori cas1	Leaves	Alicante (Spain)	MUB 60134	Wild
ori za	Leaves	Zaio (Morocco)	MUB 60001	Wild
ori dr	Leaves	Driouch (Morocco)	MUB 60029	Wild
ori mur	Leaves	Palma de Mallorca (Mallorca, Spain)	MUB 60045	Wild
aeg saf3	Flowers	Safi (Morocco)	MUB 60026	cv. Safi
aeg saf1	Leaves	Safi (Morocco)	MUB 60024	cv. Safi
aeg llan	Leaves	Llano del Beal (Murcia, Spain)	MUB 60135	Wild
aeg tiz2	Leaves	Tizi-n-test (Morocco)	MUB 60008	Wild
aeg asn2	Leaves	Asni (Morocco)	MUB 60005	Wild
aeg tiz1	Leaves	Tizi-n-test (Morocco)	MUB 60009	Wild
aeg syr	Flowers	Palmira (Syria)	MUB 60152	Wild
aeg syr4	Leaves	Ugarit (Syria)	MUB 60153	Wild
aeg syr5a	Flowers	Ain Dara (Syria)	MUB 60154	Wild
aeg syr6	Flowers	Maaraba (Syria)	MUB 60155	Wild
ova d2	Flowers	Driouch (Morocco)	MUB 60032	Wild
ova za 2	Flowers	Zaio (Morocco)	MUB 60001	Wild

The products were re-amplified using an AFLP^{TM} Plant Mapping kit (Applied Biosystems). The primer trial was performed with the standard three bases on *Mse*I and only two bases on the *Eco*RI site, due to the small genome size of *Capparis* (Table 7).

AFLP fragments were resolved using an ABI 377 DNA sequencer (PE Applied Biosystems Inc.). Fragments were sized by running dye-labelled size standards in each lane. AFLP profiles were edited using GeneScan 2.0.2 and Genotyper 1.1 (PE Applied Biosystems Inc.). Bands were edited manually because some bands were just below the threshold permitted by the software in some individuals and just above the threshold in others.

Data analysis

A matrix of raw data was constructed based on the presence (1) or absence (0) of each polymorphic DNA fragment for all accessions. The matrix was analysed using the NJ algorithm in the software package PAUP version 4.0d10 for Windows (Swofford 2002), and trees were printed with Treeview (Page 1996). It was also analysed by principle co-ordinates analysis (PCoA) in the R package for Multivariate Analysis version 4.0 (Casgrain and Legendre 1999) using Jaccard's coefficient (Jaccard 1908).

Results

The results of the PCoA analysis are shown in Figure 1. The first coordinate (horizontal axis) accounted for 23% of the variation and the second coordinate (vertical axis) accounted for 18% of the variation. Four groups can be recognised: *C. aegyptia* plus *C. ovata* on the left hand side of the plot, forming two independent groups, that are separated by the vertical axis but close to each other. *Capparis sicula* is isolated from the other groups, but there is no clear differentiation between *C. spinosa* and *C. orientalis*.

The NJ dendogram is shown in Figure 2. Three main groups were found: *C. aegyptia* plus *C. ovata* at the base of the dendogram form a group in which *C. ovata* forms a single cluster and *C. aegyptia* was divided in two subgroups. One sample of *C. sicula* fell into this group, and this sample also showed a more distant position from the rest of the *C. sicula* group in the PCoA analysis (Figure 1). The remaining samples of *C. sicula* form an

Table 6. Number of accessions per population: Spain (28 accessions from 15 populations), Morocco (11 accessions from eight populations) and Syria (five accessions from five populations).

Locality	Number of individuals
Llubi (Mallorca, Spain)	1 (ori frA)
Palma de Mallorca (Mallorca, Spain)	4 (ori mur, ori mur1, ori mur2, ori mur5)
Alicante (Spain)	3 (ori cas2, ori cas3, ori cas1)
Llano del Beal (Murcia, Spain)	1 (aeg llan)
Escombreras (Murcia, Spain)	5 (sic esc, sic esc1, sic esc2, sic esc3, sic esc5)
Llubi (Mallorca, Spain)	1 (spin m)
Llubi (Mallorca, Spain)	2 (spin pm, spin pmA)
Llubi (Mallorca, Spain)	1 (spin pb)
Campos (Mallorca, Spain)	1 (spin pbA)
Campos (Mallorca, Spain)	1 (spin fsm, spin fsmA)
Llubi (Mallorca, Spain)	2 (spin fsb, spin fb)
Campos (Mallorca, Spain)	2 (spin rmA, spin rm)
Llubi (Mallorca, Spain)	2 (spin rtA, spin rt)
Llubi (Mallorca, Spain)	1 (spin rbA1)
Alicante (Spain)	1 (spin cas4)
Zaio (Morocco)	2 (ori za, ova za 2)
Driouch (Morocco)	1 (ori dr)
Safi (Morocco)	2 (aeg saf1, aeg saf3)
Tizi-n-test (Morocco)	2 (aeg tiz1, aeg tiz2)
Asni (Morocco)	1 (aeg asn2)
Driouch (Morocco)	1 (ova d2)
Sidi-Karcem (Morocco)	1 (sic sk)
Fes (Morocco)	1 (sic fes 2)
Palmira (Syria)	1 (aeg syr)
Ugarit (Syria)	1 (aeg syr4)
Ain Dara (Syria)	1 (aeg syr5a)
Maaraba (Syria)	1 (aeg syr6)
Damasco (Syria)	1 (sic syr7)

Table 7. Selective bases at the 3′ end of the primers for AFLP selective reaction.

Fluorescent label	*Eco*RI site	*Mse*I site
NED	AT	CAT
JOE	AA	CAT
FAM	AC	CAT

independent group. Finally, *C. orientalis* and *C. spinosa* were intercalated.

The total number of bands scored, excluding the *C. aegyptia*/*C. ovata* group, was 310, of which 157 were polymorphic. *Capparis spinosa* only had six unique bands (2%), while the other taxa showed a higher number of unique polymorphisms. *C. orientalis* and *C. spinosa* shared more polymorphic bands than any other pair of taxa. The data is summarised in Table 8.

Discussion

Interspecific relationships of the taxa studied

In previous morphological studies (Inocencio 2001) qualitative characters which provided the basis for morphological differentiation among the taxa under discussion were investigated. The AFLP analysis indicates that a similar differentiation is also possible following DNA information and four groups are defined (Figures 1 and 2). However, the AFLP study indicates that the genetic distance among the *Capparis* spp. studied is low. The species included in subgenus *Capparis*, mainly those distributed in Western Asia and Mediterranean countries, have been used by man for many centuries as food, indicating that a human influence on the evolution of the species probably provoked an introgression.

The samples from *C. sicula* form a homogeneous group well differentiated from *C. spinosa* and *C. orientalis*. Although *C. spinosa* and *C. sicula* are quite similar morphologically and have often been confused taxonomically, there are characters that can separate them, including DNA analysis. The groups representing *C. spinosa* and *C. orientalis* are not fully separable, and typical members of each species appear to represent different extremes of a continuum (Figures 1 and 2).

The PCoA analysis shows that *C. aegyptia* and *C. ovata* are quite separate from the other taxa but very close to each other, even when these species are well separated morphologically. Rapid morphological changes in response to different ecological factors could mislead our perception of genetic differences among species. These morphologically differentiated species have similar genetic background. The data from NJ shows several groups in *C. aegyptia*, the Iberian–North African and the Asian samples are separated by the *C. ovata* clade. These two groups correspond to the populations from Syria as opposed to Morocco and Spain, showing that geographical differences exist. However both *C. aegyptia* groups are morphologically close, and adaptations to specialised habitats may have homogenised external morphology.

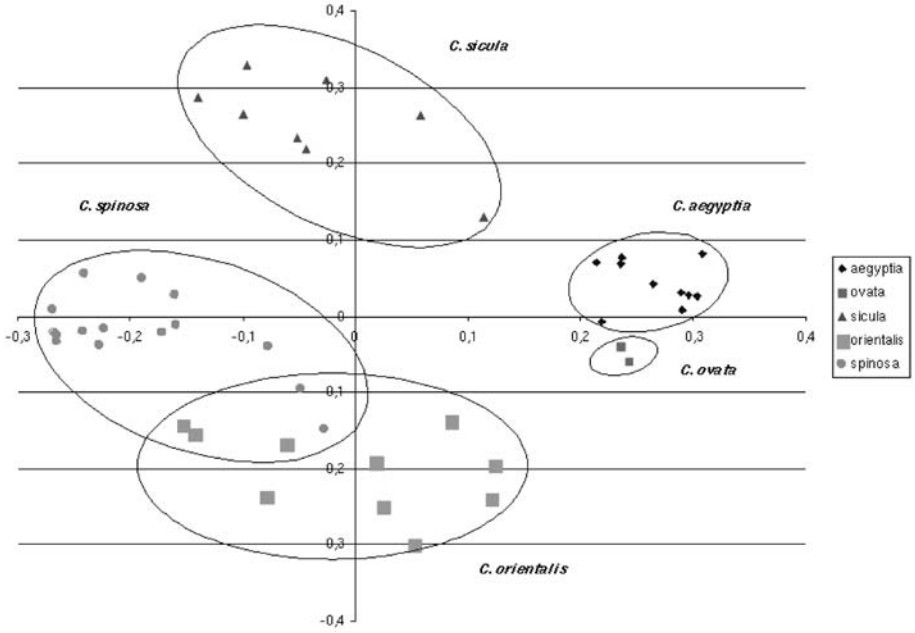

Figure 1. PCO analysis of *Capparis* taxa studied.

The sample 'sic. esc2' (Figure 2) illustrates the high overall similarity of some taxa, and lies at a point which is difficult to define as *C. sicula* or *C. aegyptia*/*C. ovata*. On morphological grounds it falls within our concept of *C. sicula* but since it belongs to a hybrid swarm between *C. sicula* and *C. aegyptia* it could be a hybrid product.

On the basis of this study three taxonomic groups are recognised corresponding to: *C. sicula*, *C. spinosa* plus *C. orientalis* and *C. aegyptia* plus *C. ovata*. The relationships between *C. spinosa* and *C. orientalis* and between *C. aegyptia* and *C. ovata* require further study.

Interspecific relationships between C. spinosa, C. orientalis *and* C. sicula

Capparis spinosa is rarely found in the wild, most known populations are cultivated and it possesses intermediate morphological characters between *C. orientalis* and *C. sicula*. All these factors suggest that this taxon may be of hybrid origin. In the Balearic Islands the cultivation of capers was extensive and there were many local cultivars recognised by farmers. All samples from *C. spinosa* studied are from these islands apart from a wild population in Alicante (Spain). The previous taxonomic study (Inocencio 2001) indicated that *C. spinosa* has been cultivated mainly in the Balearic islands and occasionally in other Mediterranean areas, where other species of *Capparis* are used as a source of capers. The occasional representation of *C. spinosa* outside of the Balearic islands and the economic difficulties for gathering samples of such scarce populations, has lead us to focus our study on the Balearic populations.

Based on the PCoA analysis this group of samples falls in an intermediate position between *C. sicula* and *C. orientalis*, close to the latter species. A low number of unique bands in *C. spinosa* gives this taxon less consistency as a species than the other two taxa which have a higher number of unique bands. The limits of the two groups representing *C. spinosa* and *C. orientalis* are not well defined. *Capparis spinosa* may have occurred in different locations where the populations of *C. sicula* and *C. orientalis* were growing together and then taken into cultivation and subjected to selection and clonal propagation. Thus *C. spinosa* may comprise cultivars that have descended from different ancestral lineages of *C. orientalis*. The bands shared between these two taxa are quite high in comparison to other combinations. This polymorphism is supported by the flavonoid

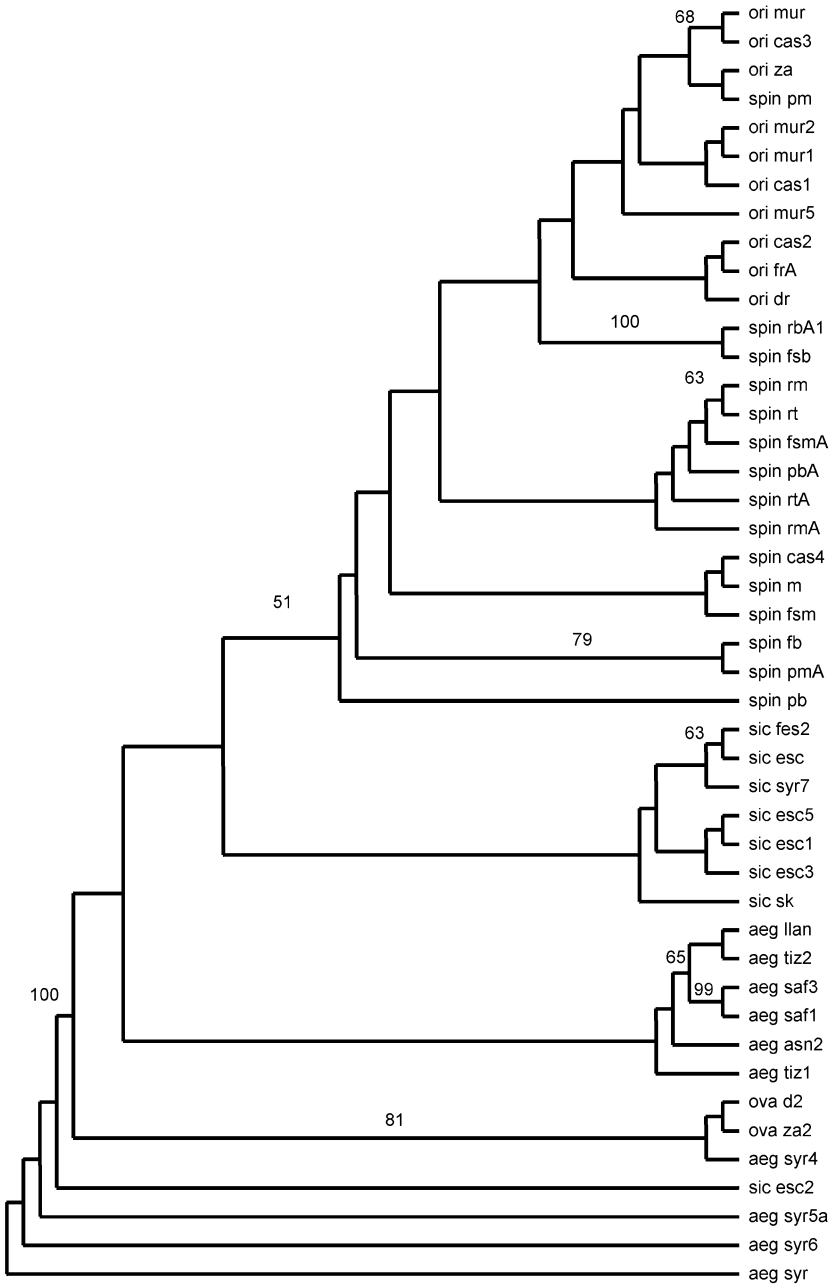

Figure 2. Neighbor-joining tree showing relationships of *Capparis* taxa studied. Numbers indicate the proportion (%) of 1000 bootstrap samples in which a particular clade was found (the branch length does not reflect the actual genetic distances).

composition of commercial capers both quantitatively and qualitatively (Inocencio et al. 2000).

In the Balearic Islands *C. orientalis* has occasionally been used as food because the morphological character of the plant, without thorns, makes it easier to collect its flowers and fruits. However the cultivation of this plant has been less extensive than that of *C. spinosa* due to its lower productivity.

The development of these two taxa under human selective pressure linked to the infrequent presence

Table 8. Number of shared bands and their relative frequencies. S = *C. sicula*; P = *C. spinosa*; O = *C. orientalis*.

Type of band	Number	Percentage (%)
S + O + P	157	50
S + P	20	6.5
O + P	52	17
S + O	12	4
S	33	11
P	6	2
O	30	9.5

of *C. sicula* in the Balearic Islands could explain why local *C. spinosa* has a greater genetic influence from *C. orientalis* than from *C. sicula*. This is similar in Southern France (the main area for the original materials of *C. spinosa* in Linnaeus' time).

Even if these kinds of studies cannot prove the hybrid character of one taxon, the results obtained make it difficult to accept the consistency of *C. spinosa* as a single species and support the hypothesis of a possible hybrid origin (Figure 1). The results suggest that *C. spinosa* rather than being a true species is a cultigen derived out of *C. orientalis* with some introgression from *C. sicula*.

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