



Long-distance colonization of the Western Mediterranean by *Cistus ladanifer* (Cistaceae) despite the absence of special dispersal mechanisms

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ABSTRACT

Aim There are few biogeographical and evolutionary studies that address plant colonization and lineage origins in the Mediterranean. *Cistus* serves as an excellent model with which to study diaspore dispersal and distribution patterns of plants exhibiting no special long-distance dispersal mechanisms. Here we analyse the pattern of genetic variation and divergence times to infer whether the African–European disjunction of *C. ladanifer* L. is the result of long-distance dispersal or of vicariance events.

Location Principally the Western Mediterranean region, with a focus on the Strait of Gibraltar.

Methods We used DNA sequence phylogenetic approaches, based on plastid (*rbcL/trnK-matK*) and nuclear (ITS) sequence data sets, and the penalized likelihood method, to date the diversification of the 21 species of *Cistus*. Phylogenetic relationships and phylogeographical patterns in 47 populations of *C. ladanifer* were also analysed using two plastid DNA regions (*trnS-trnG*, *trnK-matK*). These sequence data were analysed using maximum parsimony, Bayesian inference and statistical parsimony.

Results Dating estimates indicated divergence dates of the *C. ladanifer* lineage in the Pleistocene. Eight nucleotide-substitution haplotypes distributed on the European (four haplotypes) and African (five haplotypes) sides of the Strait of Gibraltar were revealed from *C. ladanifer* sequences. Both the haplotype network and the phylogenetic analyses depicted two main *Cistus* lineages distributed in both Europe and North Africa. An Iberian haplotype forms part of the North African lineage, and another haplotype distributed on both continents is related to the European lineage. Haplotype relationships with respect to outgroup sequences supported the hypothesis that the centre of genetic diversity is in northern Africa.

Main conclusions Based on lineage divergence-time estimates and disassociation between geographical and lineage haplotype distributions, we inferred at least two intercontinental colonization events of *C. ladanifer* post-dating the opening of the Strait of Gibraltar (c. 5 Ma). This result supports a hypothesis of long-distance dispersal rather than a hypothesis of vicariance. We argue that, despite limited dispersal abilities, preference for disturbed habitats was integral to historical colonization after the advent of the Mediterranean climate (c. 3.2 Ma), when *Cistus* species diverged and became established as a dominant element in the Mediterranean scrub.

Keywords

Cistaceae, ITS, molecular dating, phylogeography, *rbcL*, seed dispersal, Strait of Gibraltar, *trnK-matK*, *trnS-trnG*.

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INTRODUCTION

One of the most remarkable barriers to gene flow in the Western Mediterranean is the Strait of Gibraltar. The Iberian Peninsula and northern Africa are separated in this area by only 14.4 km of sea, yet 25% of the regional flora is exclusively African or European (Valdés, 1991). During the Messinian Salinity Crisis (5.96–5.33 Ma, Duggen *et al.*, 2003), when the Mediterranean Sea evaporated, land bridges facilitated plant range expansion (Vargas *et al.*, 1999; Caujapé-Castells & Jansen, 2003). Since the opening of the Strait of Gibraltar (*c.* 5 Ma), eliminating the land connection between Europe and Africa, plant range expansion across the Strait has depended on long-distance dispersal, although the emergence of islands in the Western Mediterranean region during Quaternary glaciations may have facilitated dispersal between the Iberian Peninsula and northern Morocco (Collina-Girard, 2001).

The genus *Cistus* L. (Cistaceae) comprises 21 species, distributed primarily in the Mediterranean region. Based on morphological features, *Cistus* (and most other genera of Cistaceae) is classified in the group of angiosperms that lack specialized structures for long-distance dispersal (van der Pijl, 1979; Herrera, 1992; Malo & Suárez, 1996; Bastida & Talavera, 2002). However, despite the absence of dispersal adaptations in

the genus, few *Cistus* species and subspecies are localized in Africa or Europe, and three species (*C. creticus*, *C. monspeliensis*, *C. salviifolius*) display circum-Mediterranean distributions, suggesting effective colonization ability. The highest number (14) of *Cistus* species is found in the Western Mediterranean and suggests active differentiation on both sides of the Strait of Gibraltar. *Cistus ladanifer* has an exclusively Western Mediterranean distribution, from the Iberian Peninsula and southern France to northern Morocco and Algeria (Demoly & Montserrat, 1993) (Fig. 1a). The disjunct distribution of *C. ladanifer* across the Strait of Gibraltar offers the opportunity to test whether this fragmentation is the result of vicariance or of long-distance dispersal (Kropf *et al.*, 2006).

The plastid genome (cpDNA) is considered structurally stable, haploid, non-recombinant and generally uniparentally inherited (primarily maternally in angiosperms) and exhibits geographically structured variation in a substantial number of plant species (Soltis *et al.*, 1997; Zhang *et al.*, 2005). We analysed haplotype polymorphisms of two plastid regions (*trnS-trnG*, *trnK-matK*) to assess the role and effects of geographical barriers on the phylogeography of *C. ladanifer*. Specifically, we examined plastid haplotype variation to: (1) reconstruct phylogenetic relationships between *C. ladanifer* and closely related species; (2) analyse phylogeographical patterns among populations and subspecies of *C. ladanifer*;

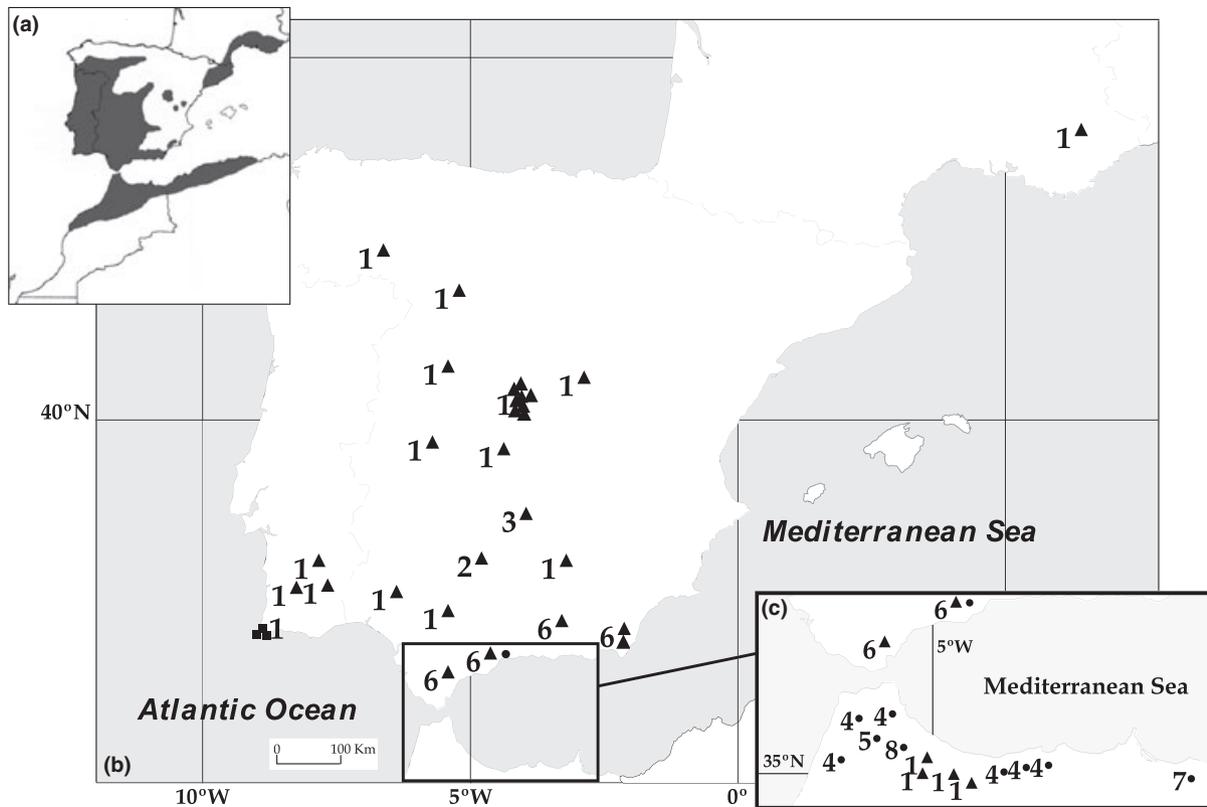


Figure 1 Distribution map (a) and geographical range of eight cpDNA haplotypes of *Cistus ladanifer* in the Iberian Peninsula and France (b) and northern Africa (c). Numbers indicate haplotypes. Each solid circle (●) represents one population of *C. ladanifer* subsp. *africanus*, each solid triangle (▲) represents one population of *C. ladanifer* subsp. *ladanifer* and each solid square (■) represents one population of *C. ladanifer* subsp. *sulcatus*.

and (3) infer patterns of genetic variation related to either vicariance events or long-distance dispersal of *C. ladanifer* across the Strait of Gibraltar. In addition, we estimated divergence times of *Cistus* lineages based on previously generated ITS and *trnK-matK* sequences (Guzmán & Vargas, 2005) and on a new set of *rbcL* sequences used successfully in angiosperm phylogenies (Wikström *et al.*, 2001; Bell & Donoghue, 2005; Lavin *et al.*, 2005; Linder *et al.*, 2005; Magallón & Sanderson, 2005). Finally, molecular analyses combined with palaeobotanical, ecological and distributional data were used to elucidate the colonization history of *C. ladanifer* in the Mediterranean.

MATERIALS AND METHODS

Study organisms

The Cistaceae is a medium-size family comprising eight genera and 180 species of typically heliophyte shrubs, subshrubs and herbs that occur in open areas with poor soils. The family is distributed in temperate and subtropical regions of the Northern Hemisphere, but the diversity of genera and species is highest in the Mediterranean floristic region (five of the eight genera are found there). The 21

species of *Cistus* form two well-defined lineages: the white-flowered group (plus *C. parviflorus*) (12 species) and the purple-flowered group (nine species) (Guzmán & Vargas, 2005). Species of both groups can occur in woodland understoreys, and some white-flowered species (*C. ladanifer*, *C. laurifolius*, *C. monspeliensis*) are dominant in evergreen scrub and are favoured by woodland disturbances such as fire. The Mediterranean white-flowered *C. ladanifer* is a self-incompatible and predominantly entomophilous species (Talavera *et al.*, 1993). The wide-open flower morphology and high quantity of pollen and nectar promote successful pollination by generalist insect pollinators, and over 100 species of beetles, bees and flies have been documented to visit the flowers (Bosch, 1992; Talavera *et al.*, 1993) (Fig. 2). The fruits of *C. ladanifer* are globular, lignified capsules with 6–12 valves (Demoly & Montserrat, 1993). Each fruit produces a large number of seeds (500–1000), with high heterogeneity in germination success related to fire regimes (Thanos & Georghiou, 1988; Valbuena *et al.*, 1992). Fruit valves open in summer, dehisce, and seeds fall close to the maternal plant (Bastida & Talavera, 2002). *Cistus ladanifer* is an obligate seeder, and therefore regenerates from plant and soil seed banks after disturbance (Arianoutsou & Margaritis, 1981; Valbuena *et al.*, 1992).

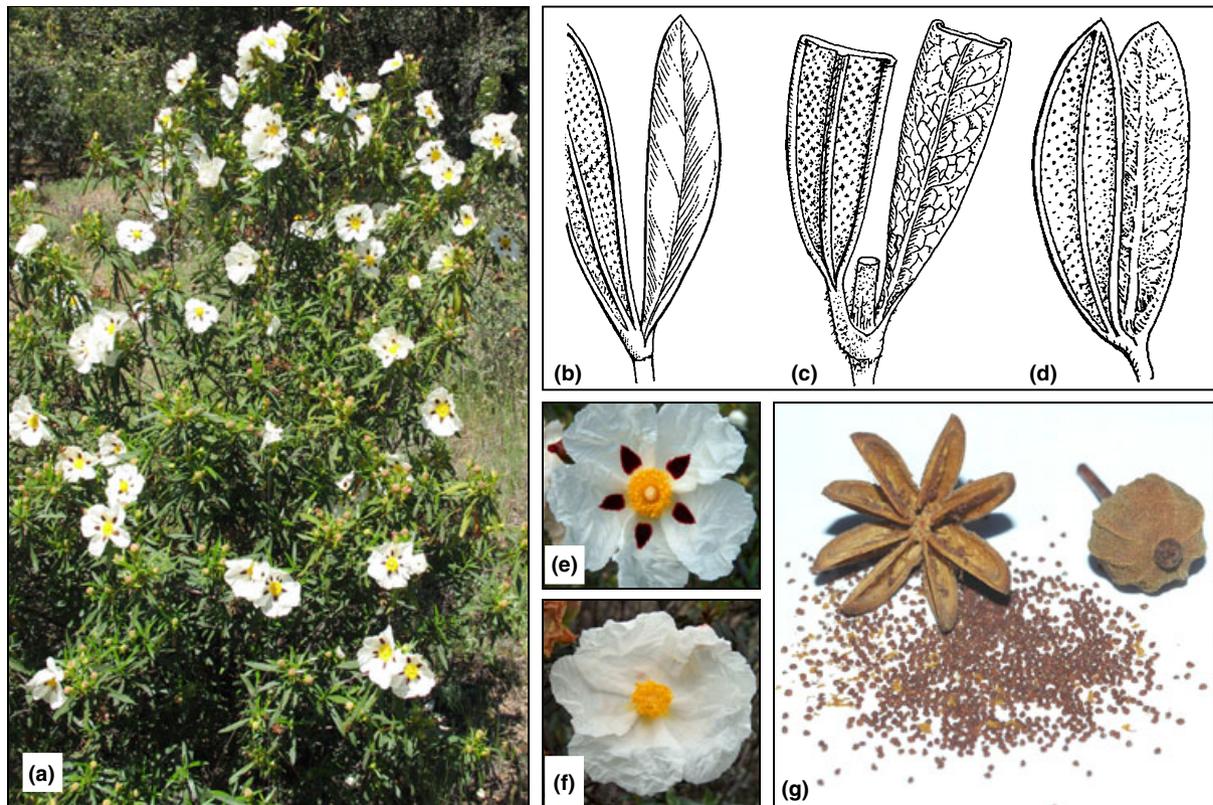


Figure 2 *Cistus ladanifer* leaf and flower morphology: (a) subsp. *ladanifer* var. *maculatus* (Spain, Madrid, El Escorial); (b) subsp. *ladanifer* leaves; (c) subsp. *africanus* leaves; (d) subsp. *sulcatus* leaves; (e) subsp. *ladanifer* var. *maculatus* flower; (f) subsp. *ladanifer* var. *ladanifer* flower; (g) mature and opened capsules (c. 1000 seeds). Photographs (a), (e), (f) by B. Guzmán, (g) by P. Vargas. Illustrations (b), (c), (d) by E. Sierra Ràfols (see Demoly & Montserrat, 1993).

Cistus ladanifer exhibits geographically structured morphological variation that has resulted in the recognition of three subspecies (Demoly & Montserrat, 1993). Leaf shape and nerve type between subspp. *ladanifer* and *sulcatus*, and leaf base and petiole types between subspp. *ladanifer* and *africanus* delimit subspecific taxa (Fig. 2). The distributions of the three subspecies occasionally overlap: subspp. *ladanifer* is primarily distributed in the Iberian Peninsula, France and northern Africa; subspp. *sulcatus* is endemic to south-western Portugal (Algarve region); and subspp. *africanus* is scattered in southern Spain (Cádiz, Málaga), but more commonly found in northern Africa. A hypothesis of phylogenetic relationships based on three nuclear and plastid markers strongly supported the monophyly of *C. ladanifer*, but did not resolve subspecies relationships (Guzmán & Vargas, 2005).

Sample strategy and DNA sequencing

Two data sets (*rbcl/trnK-matK* and ITS) were used to estimate divergence times of *Cistus* and related lineages. Thirty-four accessions of Cistaceae were sampled: 23 taxa of *Cistus*, four species of *Halimium*, two species each of *Tuberaria*, *Crocantimum* and *Helianthemum*, and one species of *Fumana* (Table 1, Fig. 3). In order to combine the data, the majority of the samples used in sequencing *trnK-matK* and ITS (Guzmán & Vargas, 2005) were employed to sequence *rbcl* (Table 1). Dipterocarpaceae sequences of *matK* and *rbcl* from GenBank were used as outgroup sequences for all analyses (Table 1; see Guzmán & Vargas, 2005). However, failure to align Cistaceae and Dipterocarpaceae ITS sequences led us to use *Helianthemum* and *Fumana* as the outgroup for the nuclear analysis (see Guzmán & Vargas, 2005).

A total of 55 individuals representing 47 distinct populations of *Cistus ladanifer* were sampled from the Western Mediterranean, with a focus on the Strait of Gibraltar (Fig. 1, Table 1). One individual per population was used, except for four Moroccan populations in which we sampled three individuals. A pilot study using only six *C. ladanifer* populations was initially performed to ascertain the most variable plastid sequences (*trnL-trnF*, *trnK-matK*, *trnS-trnG*, *rbcl*). Standard primers were used for amplification of the *trnK-matK* spacer (*trnK*-3914F, *matK*-1470R) (Johnson & Soltis, 1994), the *trnL* (UAA)-*trnF* (GAA) spacer (Taberlet *et al.*, 1991) and the *trnS* (GCU)-*trnG* (UCC) spacer (Hamilton, 1999). The *rbcl* exon was amplified from two overlapping segments using the following primer pairs: 1F-724R and 636F-1460R (Savolainen *et al.*, 2000). Procedures used for DNA sequencing of plastid and nuclear (ITS) regions followed Guzmán & Vargas (2005), with the exception of specific amplifications for *rbcl*, *trnK-matK* and *trnS-trnG*. Polymerase chain reaction (PCR) parameters were as follows: 1–3 min denaturation at 94°C; 24–28 cycles of 1 min at 94°C and 30 s–1 min at 48/50/55°C, respectively; and 1–2 min extension at 72°C.

The highest variation at the species level was observed in *trnK-matK* and *trnS-trnG* sequences, and thus we concatenated

them to build a combined matrix. The *trnL-trnF* and *rbcl* regions were discarded in the reconstruction of phylogeographical patterns of *C. ladanifer*. In addition, the monophyly and haplotype ancestry of *C. ladanifer* were evaluated by means of phylogenetic and phylogeographical analyses. For this, we analysed a data set comprising *C. ladanifer* accessions (one accession per haplotype) of *trnS-trnG* and *trnK-matK* sequences, plus those of the remaining white-flowered species, which form a well-supported clade (Guzmán & Vargas, 2005), and of two purple-flowered species (*C. albidus*, *C. symphytifolius*) as the outgroup (Table 1).

In order to determine pollen vs. seed gene flow, maternal inheritance of plastid DNA among *Cistus* species was assessed by analysis of *trnK-matK* and *trnS-trnG* sequences in two controlled hybrids: *C. parviflorus* × *C. laurifolius*, and *C. libanotis* × *C. ladanifer* (B. Guzmán, R. G. Page, P. Vargas, unpublished data). The hybrid resulting from each cross displayed a particular haplotype, which was used to establish maternal vs. paternal inheritance.

Phylogeny estimation

The plastid (*rbcl/trnK-matK*) and nuclear (ITS) data sets were analysed separately using PAUP* 4.0b10 (Swofford, 2002). Maximum parsimony (MP) analyses were conducted using heuristic searches with 1000 random addition replicates, and maximum likelihood (ML) with 100 replicates, tree bisection–reconnection (TBR), branch swapping, MulTrees (saving all optimal trees) and Steepest Descent in effect holding 10 trees per replicate. Internal node support for clades was assessed using 1,000,000 bootstrap replicates (fast stepwise addition, Mort *et al.*, 2000). The simplest model of sequence evolution that best fit the sequence data was determined using the hierarchical likelihood ratio test (hLRT) and the Akaike information criterion (AIC) in MrModeltest 1.1b (Posada & Crandall, 1998; Nylander, 2002) for each data set.

To infer divergence times, we used tree topologies and branch lengths obtained from ML results. The plastid and nuclear trees most congruent with the MP consensus tree of three DNA regions presented in Guzmán & Vargas (2005) were accepted for analysis. We chose the Langley and Fitch (LF) test (Magallón & Sanderson, 2005) to validate the constancy of substitution rates. The null hypothesis of constant rate was rejected, and therefore we applied the penalized likelihood (PL) method (Sanderson, 2002) as implemented in r8s. PL was run with the truncated Newton (TN) algorithm and the following parameters: collapse; num_time_guesses = 5; and num_restarts = 5. We pruned the extra outgroup (*Hopea* sp. in *rbcl/trnK-matK* and *Fumana thymifolia* in ITS data sets) as recommended in the r8s manual. The smoothing parameter for the PL method was calculated by a cross-validation procedure under the following criteria: cvstart = 0.5; cvinc = 0.2 and 0.5 (plastid and nuclear, respectively); cvnum = 10. Cross-validation suggested that the best smoothing parameter was 3.2 for *rbcl/trnK-matK* and 1000 for ITS data sets. Standard errors of divergence-time estimates were obtained

Table 1 Cistaceae taxa sequenced for the plastid *rcbL*, *trnK-matK* and *trnS-trnG* and the nuclear ITS regions. Taxonomy follows that of Guzmán & Vargas (2005), except for *C. albanicus*. Blotched (var. *maculatus*) and unblotched (var. *ladanifer*) petal individuals of *C. ladanifer* are indicated with solid (●) and open (○) circles following localities.

Taxon	Locality/source (number of individuals per population)*	Voucher	Haplotype number	<i>trnK-matK/matK</i> accession no.	<i>trnS-trnG</i> accession no.	ITS accession no.	<i>rcbL</i> accession no.
<i>Cistus</i> L.							
<i>Cistus albanicus</i> E.F. Warb. ex Heywood	Cultivated	R. G. Page 8BGA04 (MA)	–	DQ093010	FJ228736	DQ092964	FJ225880
<i>Cistus albidus</i> L.	Spain, Madrid, Aldea del Fresno	P. Vargas 25PV03 (MA)	–	DQ092974	FJ228730	DQ092932	–
<i>Cistus albidus</i> L.	Morocco, Tetuán	P. Vargas 41PV03 (MA)	–	–	–	–	FJ225860
<i>Cistus chinamadensis</i> Bañares et Romero	Canary Islands, La Gomera	R. G. Page 144BGA04 (MA)	–	DQ092987	–	DQ092943	–
<i>Cistus chinamadensis</i> Bañares et Romero	Canary Islands, La Gomera	Á. Fernández & J. Leralta 44BGA04 (MA)	–	–	–	–	FJ225866
<i>Cistus clusii</i> Dunal	Spain, Málaga, Mijas	R. G. Page 8BGA04 (MA)	–	DQ093009	FJ228739	DQ092963	FJ225879
<i>Cistus creticus</i> L.	Greece, Olympus	P. Vargas 209PV04 (MA)	–	DQ092978	–	DQ092936	FJ225862
<i>Cistus crispus</i> L.	Spain, Córdoba, Posadas	B. Guzmán 58BGA04 (MA)	–	DQ093013	–	DQ092967	FJ225882
<i>Cistus heterophyllus</i> Desf.	Morocco, Beni-Hadifa	B. Guzmán 99BGA04 (MA)	–	DQ092989	–	–	FJ225868
<i>Cistus heterophyllus</i> Desf.	Morocco	O. Filippi 7BGA04 (MA)	–	–	–	DQ092944	–
<i>Cistus horrens</i> Demoly	Canary Islands, Gran Canaria, San Bartolomé de Tirajana	B. Guzmán 5BGA05 (MA)	–	FJ225848	–	–	FJ225867
<i>Cistus ladanifer</i> L. ssp. <i>africanus</i> Dans.	Morocco, Asilah (3) ○	B. Guzmán 118BGA04 (MA)	4	FJ225805/6/7	FJ189405/6/7	–	–
<i>Cistus ladanifer</i> L. ssp. <i>africanus</i> Dans.	Morocco, Beni-Hadifa ○	B. Guzmán 102BGA04 (MA)	4	FJ225796	FJ189394	–	–
<i>Cistus ladanifer</i> L. ssp. <i>africanus</i> Dans.	Morocco, Grottes d'Hercules ○	P. Vargas 28PV03 (MA)	4	DQ093000	FJ189395	DQ092955	–
<i>Cistus ladanifer</i> L. ssp. <i>africanus</i> Dans.	Morocco, M'Diq	P. Vargas 45PV03 (MA)	4†	FJ225804	FJ189404	–	–
<i>Cistus ladanifer</i> L. ssp. <i>africanus</i> Dans.	Morocco, Taforalt ○	B. Guzmán 87BGA04 (MA)	7	FJ225800	FJ189399	–	–
<i>Cistus ladanifer</i> L. ssp. <i>africanus</i> Dans.	Morocco, Targuist ●	B. Guzmán 109BGA04 (MA)	4	DQ093001	FJ189400	–	FJ225874
<i>Cistus ladanifer</i> L. ssp. <i>africanus</i> Dans.	Morocco, Tetuan (3) ○	P. Vargas 60PV03 (MA)	8	FJ225801/2/3	FJ189401/2/3	–	–
<i>Cistus ladanifer</i> L. ssp. <i>africanus</i> Dans.	Morocco, Tleta-Ketama	V. Valcárcel 26VV03 (MA)	4	FJ225808	FJ189408	–	–
<i>Cistus ladanifer</i> L. ssp. <i>africanus</i> Dans.	Morocco, Hejar Lesfar (3) ○	J. Martínez 121JM03 (MA)	5	FJ225797/8/9	FJ189396/7/8	–	–
<i>Cistus ladanifer</i> L. ssp. <i>africanus</i> Dans.	Spain, Málaga, Sierra Bermeja ○	J. Martínez 253JM04 (MA)	6	FJ225809	FJ189409	–	–
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	France, Saint Chinian	O. Filippi 17BGA05 (MA)	1	FJ225826	FJ189428	–	–
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Morocco, Bab-Taza ●	B. Guzmán 115BGA04 (MA)	1	FJ225816	FJ189417	–	–
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Morocco Chaouen (3) ●	P. Vargas 46PV03 (MA)	1	FJ225822/3/4	FJ189424/5/6	–	–
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Morocco, Chaouen-Ketama	V. Valcárcel 20VV03 (MA)	1	FJ225814	FJ189415	–	–
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Morocco, Djebel Bouhalla	V. Valcárcel 32VV03 (MA)	1	FJ225815	FJ189416	–	–
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Portugal, Aljezur	B. Guzmán 24BGA04 (MA)	1	FJ225840	FJ189442	–	–
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Portugal, Aljustrel ○	B. Guzmán 18BGA04 (MA)	1	FJ225832	FJ189434	–	–
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Portugal, Ourique ○	B. Guzmán 22BGA04 (MA)	1	FJ225843	FJ189445	–	–
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain, Almería, Sierra de la Alhamilla ○	P. Vargas 179PV04 (MA)	6	DQ092997	FJ189411	DQ092952	–
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain, Almería, Isleta del Moro	P. Vargas 36PV05 (MA)	6	FJ225812	FJ189413	–	–
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain, Cádiz, Almoraima	P. Vargas 14PV03 (MA)	6	FJ225811	FJ189412	–	–
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain, Cáceres, Granadilla	B. Guzmán 3BGA04 (MA)	1	FJ225829	FJ189431	–	–

Table 1 Continued

Taxon	Locality/source (number of individuals per population)*	Voucher	Haplotype number	trnK-matK/matK accession no.	trnS-trnG accession no.	ITS accession no.	rbcl accession no.
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain, Ciudad Real, Sierra Madrona	B. Guzmán 6BGA06 (MA)	3	FJ225817	FJ189418	-	-
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain, Córdoba, Posadas •	B. Guzmán 56BGA04 (MA)	2	FJ225833	FJ189435	-	-
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain, Granada, Lanjarón	P. Vargas 124PV04 (MA)	6	FJ225813	FJ189414	-	-
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain, Guadalupe, Hiedelaencina	B. Guzmán 26BGA03 (MA)	1	FJ225834	FJ189436	-	-
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain, Huelva, Hinojos ◊	E. Narbona 18EN03 (MA)	1	FJ225818	FJ189419	-	-
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain, Jaén, Bailén	J. Martínez 255JM04 (MA)	1	FJ225827	FJ189421	-	-
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain, Madrid, Boadilla del Monte ◊	B. Guzmán 7BGA03 (MA)	1	DQ092996	FJ189429	-	FJ225872
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain, Madrid, Chapineria •	B. Guzmán 8BGA03 (MA)	1	FJ225821	FJ189423	-	-
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain, Madrid, El Atazar ◊	B. Guzmán 28BGA03 (MA)	1	FJ225825	FJ189427	-	-
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain, Madrid, El Escorial •	B. Guzmán 10BGA03 (MA)	1	FJ225819	FJ189420	-	-
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain, Madrid, El Pardo	B. Guzmán 18BGA05 (MA)	1	FJ225836	FJ189438	-	-
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain, Madrid, La Barranca	B. Guzmán 19BGA05 (MA)	1	FJ225835	FJ189437	-	-
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain, Madrid, La Cabrera •	B. Guzmán 29BGA03 (MA)	1	FJ225820	FJ189422	-	-
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain, Madrid, Manzanares El Real ◊	B. Guzmán 11BGA03 (MA)	1	FJ225828	FJ189430	-	-
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain, Málaga, Casabermeja	P. Vargas 17PV06 (MA)	6	FJ225810	FJ189410	-	-
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain, Orense, Laroca	J. Martínez 77BGA04 (MA)	1	FJ225831	FJ189433	-	-
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain, Salamanca	P. Vargas 146PV05 (MA)	1	FJ225837	FJ189439	-	-
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain Sevilla, Sierra Norte •	J. Martínez 258JM04 (MA)	1	FJ225838	FJ189440	-	-
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain, Toledo, Hinojosa de San Vicente	B. Guzmán 12BGA03 (MA)	1	FJ225830	FJ189432	-	-
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain, Zamora	J. Martínez 272JM04 (MA)	1	FJ225839	FJ189441	-	-
<i>Cistus ladanifer</i> L. ssp. <i>sulcatus</i> (Demoly) P. Monts.	Portugal, Cabo San Vicente	B. Guzmán 36BGA04 (MA)	1	DQ092998	FJ189447	DQ092953	-
<i>Cistus ladanifer</i> L. ssp. <i>sulcatus</i> (Demoly) P. Monts.	Portugal, Cabo Sardo	J. Arroyo 48BGA04 (MA)	1	FJ225844	FJ189446	-	-
<i>Cistus ladanifer</i> L. ssp. <i>sulcatus</i> (Demoly) P. Monts.	Portugal, Raposeira	B. Guzmán 38BGA04 (MA)	1	FJ225842	FJ189444	-	-
<i>Cistus ladanifer</i> L. ssp. <i>sulcatus</i> (Demoly) P. Monts.	Portugal, Sagres	B. Guzmán 29BGA04 (MA)	1	DQ092999	FJ189448	-	FJ225873
<i>Cistus ladanifer</i> L. ssp. <i>sulcatus</i> (Demoly) P. Monts.	Portugal, Vila do Bispo	B. Guzmán 39BGA04 (MA)	1	FJ225841	FJ189443	-	-
<i>Cistus laurifolius</i> L.	Spain, Madrid, Las Rozas	P. Vargas 12PV03 (MA)	-	-	-	DQ092958	-
<i>Cistus laurifolius</i> L.	Spain, Jaén, Sierra de Segura	B. Guzmán 13BGA03 (MA)	-	DQ093005	FJ228731	-	FJ225876
<i>Cistus libanotis</i> L.	Spain, Córdoba	R. G. Page 149BGA04 (MA)	-	DQ092993	FJ228732	DQ092948	FJ225870
<i>Cistus monspeliensis</i> L.	Morocco, Grottes d'Hercules	P. Vargas 30PV03 (MA)	-	-	-	DQ092965	-
<i>Cistus munbyi</i> Pomet	Portugal, Sagres	B. Guzmán 35BGA04 (MA)	-	DQ093012	FJ225849	-	FJ225881
<i>Cistus ochreatus</i> C. Sm. ex Buch	Morocco	O. Filippi 4BGA04 (MA)	-	DQ093006	FJ228738	DQ092960	FJ225877
<i>Cistus osbeckii</i> Webb ex Christ	Canary Islands, Gran Canaria	R. G. Page 150BGA04 (MA)	-	DQ092984	-	DQ092941	FJ225865
<i>Cistus parviflorus</i> Lam.	Canary Islands, Tenerife	O. Filippi 160BGA04 (MA)	-	DQ092980	-	DQ092938	FJ225863
<i>Cistus populifolius</i> L. ssp. <i>populifolius</i>	Greece, Crete	O. Filippi 6BGA04 (MA)	-	DQ092976	-	DQ092934	FJ225861
<i>Cistus populifolius</i> L. ssp. <i>major</i> (Dunal)	Spain, Ávila, Arenas de San Pedro	P. Vargas 5PV03 (MA)	-	DQ093003	FJ228733	-	-
<i>Cistus populifolius</i> L. ssp. <i>major</i> (Dunal)	Portugal, Ourique	B. Guzmán 20BGA04 (MA)	-	-	-	DQ092957	FJ225875

Table 1 Continued

Taxon	Locality/source (number of individuals per population)*	Voucher	Haplotype number	trnK-matK/matK accession no.	trnS-trnG accession no.	ITS accession no.	rbcl accession no.
<i>Cistus pouzolzii</i> Delile	Morocco, Ketama	S. L. Jury MA698247	-	-	-	DQ092962	FJ225878
<i>Cistus pouzolzii</i> Delile	France	R. G. Page 8BGA04 (MA)	-	DQ093007	FJ228734	-	-
<i>Cistus psilosepalus</i> Sweet	Spain, Ávila, Arenas de San Pedro	P. Vargas 7PV03 (MA)	-	DQ092994	FJ228737	DQ092949	FJ225871
<i>Cistus salvifolius</i> L.	Spain, Ávila, Arenas de San Pedro	P. Vargas 6PV03 (MA)	-	-	-	DQ092945	FJ225869
<i>Cistus salvifolius</i> L.	Spain, Granada, Sierra Nevada	P. Vargas 119PV04 (MA)	-	DQ092991	FJ228735	-	-
<i>Cistus symphytifolius</i> Lam.	Canary Islands, La Palma, La Cumbreita	B. Guzmán 143BGA04 (MA)	-	DQ092983	-	DQ092940	FJ225864
<i>Cistus symphytifolius</i> Lam.	Canary Islands, Tenerife, Villa Arico	P. Vargas 41PV05 (MA)	-	-	FJ228729	-	-
<i>Crocianthemum</i> Spach.							
<i>Crocianthemum chihuahuense</i> S. Watson	Mexico, Michoacán	G. Calderón MA527771	-	-	-	-	FJ225856
<i>Crocianthemum pringlei</i> S. Watson	Mexico, Guanajuato	G. Calderón MA527767	-	-	-	-	FJ225855
<i>Fumana</i> (Dunal) Spach							
<i>Fumana thymifolia</i> (L.) Spach ex Webb	Portugal, Ferrerías	B. Guzmán 53BGA04 (MA)	-	DQ092968	-	DQ092926	FJ225850
<i>Halimium</i> (Dunal) Spach							
<i>Halimium atriplicifolium</i> (Lam.) Spach	Spain, Granada, Sierra Nevada	P. Vargas 120PV04 (MA)	-	FJ225847	-	-	-
<i>Halimium atriplicifolium</i> (Lam.) Spach	Spain, Málaga, Coín	R. G. Page 155bBGA05 (MA)	-	-	-	DQ092931	FJ225859
<i>Halimium calycinum</i> (L.) K. Koch	Portugal, Cabo Sardao	B. Guzmán 49BGA04 (MA)	-	-	-	-	-
<i>Halimium oymoides</i> (Lam.) Willk.	Portugal, Coimbra	R. G. Page 158BGA04 (MA)	-	FJ225846	-	-	-
<i>Halimium oymoides</i> (Lam.) Willk.	Spain	R. G. Page 158bBGA04 (MA)	-	-	-	-	FJ225858
<i>Halimium umbellatum</i> (L.) Spach	Spain, Madrid, Tres Cantos	B. Guzmán 71BGA04 (MA)	-	DQ092972	-	DQ092930	FJ225857
<i>Helianthemum</i> Mill.							
<i>Helianthemum scopulicolum</i> L.	Cultivated	B. Guzmán 67BGA04 (MA)	-	DQ092970	-	DQ092928	FJ225852
<i>Helianthemum squamatum</i> (L.) Dum. Cours.	Cultivated	B. Guzmán 70BGA04 (MA)	-	DQ092969	-	DQ092927	FJ225851
<i>Tuberaria</i> Dunal							
<i>Tuberaria guttata</i> (L.) Fourr.	Portugal, Vila do Bispo	B. Guzmán 44BGA04 (MA)	-	DQ092971	-	DQ092929	FJ225853
<i>Tuberaria globularifolia</i> (Lam.) Gallego	Spain, Orense, Sierra de Xures	J. Martínez 269JM04 (MA)	-	FJ225845	-	-	FJ225854
Dipterocarpaceae							
<i>Hopea</i> Roxb.							
<i>Hopea hainanensis</i> Merr. & Chun	-	Cho <i>et al.</i> (unpublished data)	-	-	-	-	AJ247623.1
<i>Hopea wightiana</i> Wall.	Malaysia, Frim Arboretum	Gamage <i>et al.</i> (2003, 2006)	-	AB246461	-	-	-
<i>Shorea</i> Roxb. ex C.F. Gaertn							
<i>Shorea affinis</i> (Thwaites) P.S. Ashton	Sri Lanka, Kottawa Arboretum	Gamage <i>et al.</i> (2003, 2006)	-	AB246471	-	-	-
<i>Shorea talura</i> Roxb.	-	Yuan <i>et al.</i> (unpublished data)	-	-	-	-	AY328198.1

*One individual per population from northern Africa, except for four *Cistus ladaniifer* populations in which three individuals were sequenced.

†Haplotype 9 when coding indels.

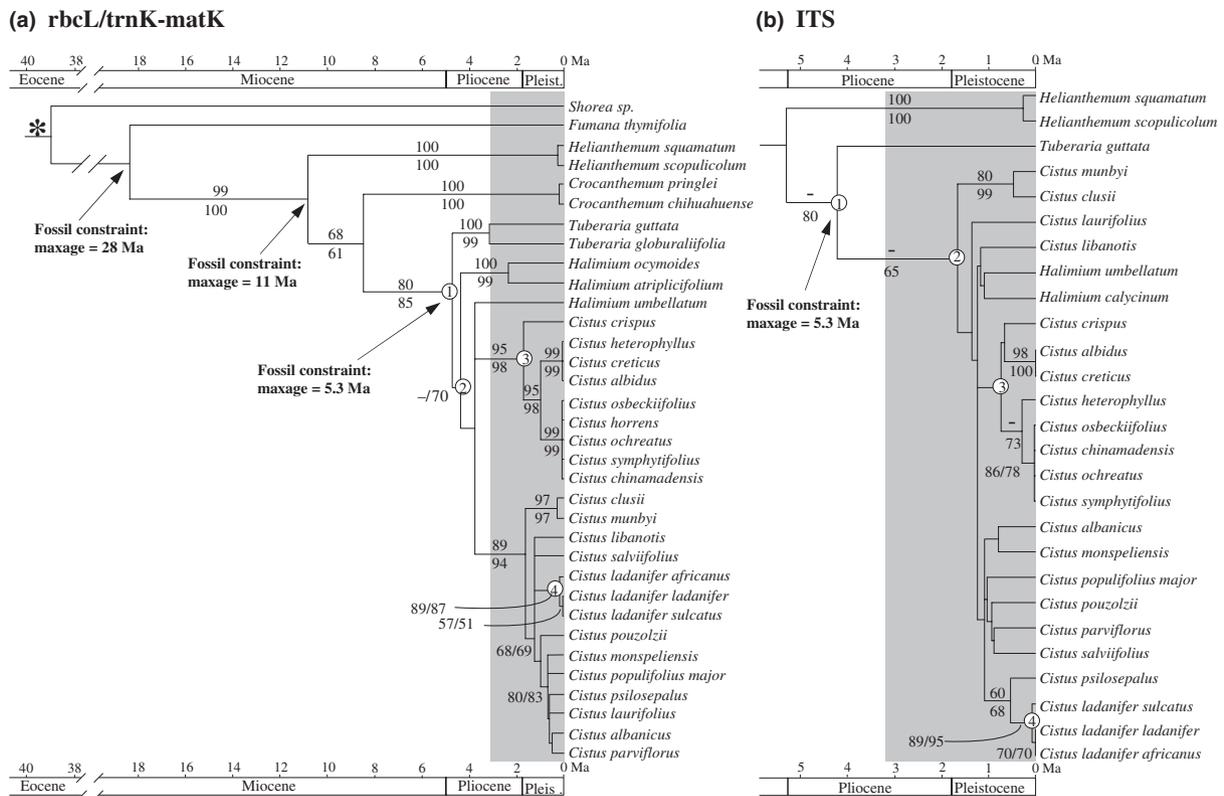


Figure 3 Chronograms of maximum likelihood (ML) trees of the Cistaceae genera based on the penalized likelihood (PL) analysis of (a) *rbcL/trnK-matK* and (b) ITS sequences. Branch lengths represent millions of years ago (Ma). Values above and below branches are bootstrap values for the ML and MP analyses, respectively. Three fossils and one estimated date (represented with an asterisk) of the Cistaceae divergence (Wikström *et al.*, 2001) were used to implement the analyses and are indicated by branch nodes. Shaded areas (grey) delineate the onset of the Mediterranean climate (*c.* 3.2 Ma). Nodes numbered as in Table 4.

using a non-parametric bootstrap procedure (Baldwin & Sanderson, 1998) and relative divergence times were converted into absolute time units using calibration points. Divergence times were calibrated using pollen records, as the Cistaceae are poor in macrofossil data. *Helianthemum* pollen has been identified from French Upper Miocene formations (11 Ma) (Naud & Suc, 1975) and *Tuberaria* pollen from Pliocene formations (5.3 Ma) in Germany (Menke, 1976). A macrofossil of a reproductive structure from Germany, described in Palibin (1909) as *Cistinocarpum roemeri* Conw., was used to constrain the Cistaceae crown group with a maximum age of 28 Ma (Middle Oligocene). We constrained the divergence age between the Dipterocarpaceae and Cistaceae with a minimum age of 23 Ma and a maximum age of 39 Ma (Wikström *et al.*, 2001). As we could not align the ITS sequences of Cistaceae and Dipterocarpaceae, we could not constrain this node in the ITS analysis.

Haplotype data analysis

The *trnK-matK* and *trnS-trnG* sequences of *C. ladanifer* were combined and aligned by eye. Relationships among haplotypes were inferred using *tcs* 1.21 software (Clement *et al.*, 2000), a method of statistical parsimony for constructing haplotype phylogenetic networks (Templeton *et al.*, 1992). The maxi-

mum number of differences resulting from single substitutions among haplotypes was calculated. In addition, we recoded indels as new characters and generated a matrix for analysis. Simmons & Ochoterena's (2000) 'simple indel coding' approach was followed to remove mononucleotide repeat stretches (poly-T and poly-A) because homology is highly uncertain for this type of polymorphism (Kelchner, 2000).

MP analyses of plastid haplotypes were performed using *PAUP** 4.0b10 (Swofford, 2002). Heuristic search and clade robustness parameters were as described above with the exception of holding 100 trees at each step. Bayesian inference (BI) was inferred using two identical searches with 10 million generations each (chain temperature = 0.2; sample frequency = 100). Probabilities converged on the same stable value after *c.* 40,000 generations in both runs. A 50% majority-rule consensus tree was calculated using the *sumt* command to yield the final Bayesian estimate of phylogeny.

RESULTS

Sequence variation

ITS and *trnK-matK* sequence attributes were reported in Guzmán & Vargas (2005). The length of *rbcL* sequences was 1403 bp, of which 167 were variable and 121

Table 2 Haplotypes found in 47 *Cistus ladanifer* populations. Variable sites (excluding mononucleotide repeat units) of the two plastid DNA fragment sequences (*trnK-matK*, *trnS-trnG*) are shown. Nucleotide position in each data set is numbered as starting from the 5' end of each sequence.

Nucleotide position	<i>trnK-matK</i>					<i>trnS-trnG</i>								
	155	162	964	1022	1046	91	118	321	331	384	461	470	498	501
Haplotype														
1	T	–	G	G	T	G	C	A	A	G	T	C	†	*
2	T	–	G	G	T	G	C	A	C	G	T	C	†	*
3	T	–	G	G	T	G	C	T	C	G	T	C	†	*
4	T	–	G	G	C	G	T	A	A	T	T	A	†	–
5	T	–	G	G	C	G	T	A	A	T	C	A	†	–
6	T	–	A	G	C	G	T	A	A	T	T	A	†	–
7	T	–	G	T	C	G	T	A	A	T	T	A	†	–
8	A	‡	G	G	C	T	T	A	A	T	T	A	–	–
9	T	–	G	G	C	G	T	A	A	T	T	A	–	–

*CAAACTAAA.

†AAA.

‡GAATT.

parsimony-informative in the whole data set (94/83 variable/parsimony-informative characters in the Cistaceae; 25/23 variable/parsimony-informative characters in *Cistus*). The aligned length of the combined *trnK-matK* and *trnS-trnG* sequences of all 11 white-flowered *Cistus* species was 2095 bp (1309–1314 bp for *trnK-matK*; 633–646 bp for *trnS-trnG*). Nucleotide substitutions and 3–10 bp indels of the *trnK-matK/trnS-trnG* matrix are shown in Table 2.

Cistaceae divergence times

The genera of Cistaceae differentiated during the Miocene–Pliocene (Fig. 3). All analyses were congruent with divergence of the *Cistus–Halimium* complex in the Pliocene–Pleistocene (crown ages: 5.02 ± 1.43 Ma, *rbcl/trnK-matK*; 1.81 ± 1.15 Ma, ITS). Both chronograms (Fig. 3, Tables 3 and 4) indicated the divergence of the *C. ladanifer* lineages in the Upper Pleistocene (0.19 ± 0.31 Ma, *rbcl/trnK-matK*; 0.07 ± 0.09 Ma, ITS).

Cistus ladanifer haplotype analysis

Artificial crossings generated two controlled hybrids for haplotype inheritance reconstruction. In both cases, F₁ individuals inherited plastid polymorphisms of *trnS-trnG* and *trnK-matK* sequences from maternal plants.

Analysis of the combined data of 112 *trnS-trnG* and *trnK-matK* sequences led to the recognition of eight nucleotide

haplotypes (Table 2). Four haplotypes (1, 2, 3, 6) were distributed in the Iberian Peninsula and France, whereas five haplotypes (1, 4, 5, 7, 8) were found in northern Africa (Table 1). Five of the eight haplotypes (2, 3, 5, 7, 8) were found exclusively in a single population. However, haplotypes 1, 4 and 6 were widespread. Haplotype 1 was found in two geographical areas (31 European and African populations, i.e. 65% of all populations). In northern Africa, haplotype 1 was detected only in subsp. *ladanifer* populations, whereas in the Iberian Peninsula this haplotype was shared by subspp. *ladanifer* and *sulcatus* (Fig. 1b). Haplotype 4 was distributed exclusively in six African populations (12.5% of all populations) of subsp. *africanus* (Fig. 1c). Haplotype 6 was exclusively found in six Iberian (Andalusian) populations of subsp. *africanus* (one population) and subsp. *ladanifer* (five populations). At the population level, identical plastid sequences were detected in the three individuals sampled within each of four Moroccan populations from an area of numerous haplotypes (Fig. 1, Table 1).

tcs constructed a single network of eight *C. ladanifer* haplotypes connected through haplotype 5 to the remaining white-flowered species (Fig. 4a). The network detected the African interior haplotype 4 as the one with the highest number of mutational connections (five connections). In the *C. ladanifer* clade, two haplotype subclades (subclade I: 1–2–3; subclade II: 4–5–6–7–8) were separated by three absent (extinct or not detected) haplotypes. These two haplotype groups were not entirely geographically structured, but did

Table 3 Settings and results of the maximum likelihood analyses for *rbcl/trnK-matK* and ITS data sets of the Cistaceae.

	Model	Base frequencies	Gamma distribution	Best score (–ln L)	No. trees
<i>rbcl/trnK-matK</i>	GTR + G	A = 0.279; C = 0.187; G = 0.244; T = 0.289	0.144	7653.80	29
ITS	GTR + I+ G	A = 0.180; C = 0.343; G = 0.301; T = 0.168	0.144	2730.673	20

Table 4 Penalized likelihood age estimates for the most important constrained and unconstrained nodes of the Cistaceae.

Node	<i>rbcL/trnK-matK</i>				ITS			
	Mean age (Ma)	SD (Ma)	Maximum age (Ma)	Minimum age (Ma)	Mean age (Ma)	SD (Ma)	Maximum age (Ma)	Minimum age (Ma)
1	5.29	0.03	5.30	4.59	5.13	0.89	5.3	0.12
2	5.02	1.43	3.97	0.02	1.81	1.15	5.3	0.07
3	1.77	0.91	12.38	0.68	0.58	0.18	1.42	0.16
4	0.19	0.31	3.97	0.02	0.07	0.09	0.83	0.0001

Nodes numbered as in Fig. 3.

Ma, million years ago; SD, standard deviation.

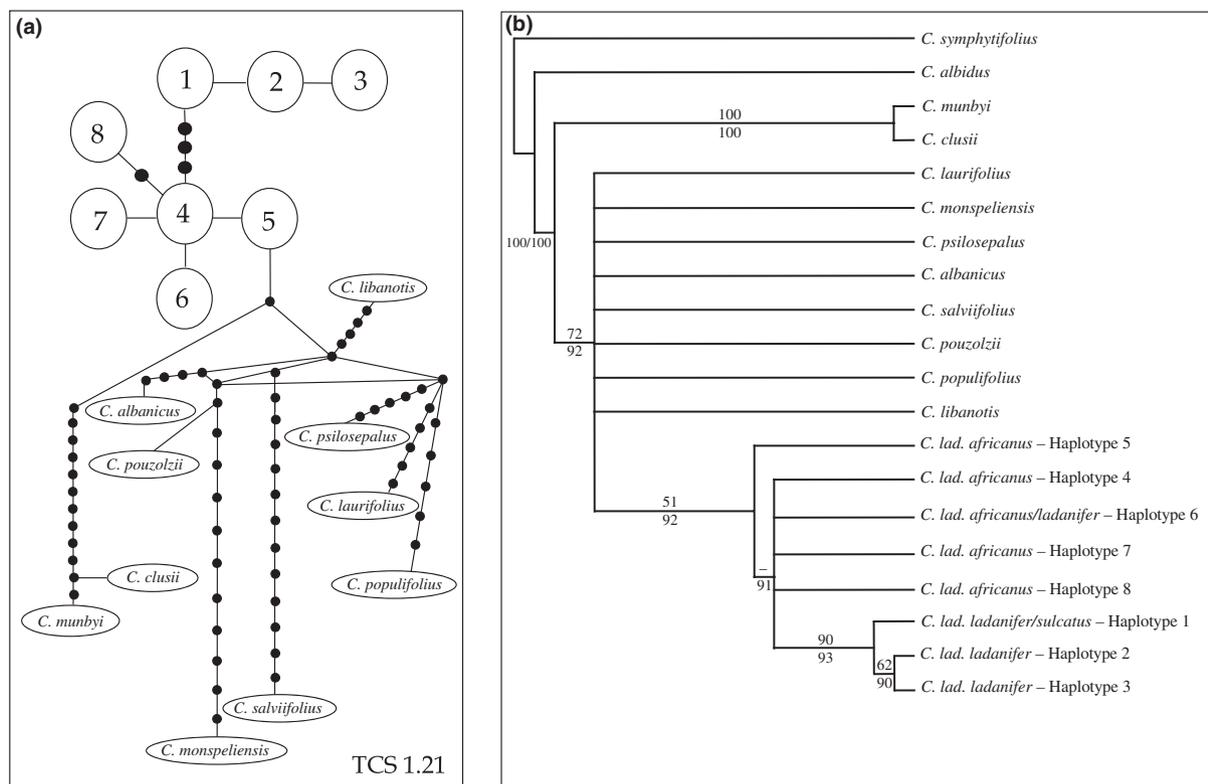


Figure 4 (a) Statistical parsimony network based on *trnS-trnG/trnK-matK* sequences of *Cistus ladanifer* and white-flowered related species; *C. ladanifer* haplotypes are indicated by numbers (1–8), lines indicate a single nucleotide substitution, and dots (•) represent 18 haplotypes (extinct or not detected). (b) Strict consensus tree of 10,920 shortest trees of 137 steps (CI = 0.92; RI = 0.87) from the combined analysis of *trnS-trnG* and *trnK-matK* sequences. Numbers above branches are bootstrap values. Numbers below branches show Bayesian posterior probabilities (PP). The dash (–) indicates no branch support over 50% bootstrap.

show significant alliance to south-western Europe (subclade I) and northern Africa (subclade II). Occurrence of the interior haplotype 1 on both continents bridged a high number of populations and distributional areas. A single mutation connects the Iberian tip haplotype 6 with the primarily African group of haplotypes, indicating remarkable disassociation between the population distributions and haplotype genealogy. rcs constructed a network with nine haplotypes from indel coding (one indel within the partial *trnK-matK* sequence and two indels within the *trnS-trnG* spacer sequence; Table 2). A new haplotype (9) was identified in eastern Morocco (in one

population from M'Diq), connected by one mutational step to haplotype 4 and by three mutational steps to haplotype 8 (results not shown). The two haplotype subclades distributed primarily in western Europe (subclade I) and northern Africa (subclade II) were also generated. We hereafter consider the network analysis with no indel coding for the sake of brevity.

Forty-seven of the 120 *trnK-matK/trnS-trnG* variable characters from the sequence matrix were phylogenetically informative. MP analysis generated 10,920 trees of 137 steps with a consistency index (CI) of 0.92 and a retention index (RI) of 0.87 (Fig. 4b). A Bayesian tree was reconstructed using

GTR + G, which was accepted as the simplest model of sequence evolution, and was largely consistent with the strict consensus MP tree (Fig. 4b). The MP tree recognized *C. ladanifer* accessions as a monophyletic group with a 51% bootstrap value (BS) and a posterior probability (PP) of 92. An extended sample of ITS, *ncpGS*, *trnS-trnG*, *rbcL*, *trnK-matK* and *trnL-trnF* DNA data strongly supported (100% BS; 100 PP) the monophyly of *C. ladanifer* (B. Guzmán & P. Vargas, unpublished data). Haplotypes 1, 2 and 3 form a well-resolved clade (90% BS, 93 PP). Bayesian analysis revealed haplotype 5 (Hejar Lesfar, Morocco) as sister (91 PP) to the other haplotypes, and the MP analysis retrieved the same relationship but with weak support (< 50% BS). Network analysis demonstrated congruent results with those of phylogenetic analyses (see above).

DISCUSSION

Colonization across the Strait of Gibraltar

The disjunct distribution of *C. ladanifer* (Fig. 1a) could be the result of one of two processes: (1) ancestral vicariance; or (2) ancestral or recent long-distance dispersal. The limited dispersability of pollen, fruit and seed suggests that the vicariance hypothesis might be biologically more acceptable than long-distance dispersal to account for the distribution patterns of *C. ladanifer* (Fig. 1). However, both the Betic Bridge (c. 16–14 Ma) and the flooding of the Strait of Gibraltar after the Messinian Salinity Crisis (c. 5.59–5.33 Ma) precede the Upper Pleistocene (0.19 ± 0.31 Ma, *rbcL/trnK-matK*; 0.07 ± 0.09 Ma, ITS) origin of *C. ladanifer* (Fig. 3). This strongly suggests that the disjunct distributions of *C. ladanifer* populations/lineages across the sea barrier are the result of recent long-distance dispersal events rather than of vicariance. Despite the presence of many species showing disjunct distributions in the Strait of Gibraltar area (2625 species, Valdés, 1991), there are few published studies addressing the role of this geographical barrier. Contrary to the conviction that plant dispersal is dramatically hindered by major sea barriers, an increasing number of population studies support the hypothesis that the Strait of Gibraltar is a permeable barrier, including studies of *Olea europaea* (Besnard *et al.*, 2002; Rubio *et al.*, 2006), *Pinus pinaster* (Gómez *et al.*, 2005), *Quercus suber* (Lumaret *et al.*, 2005), *Hypochaeris salzmanniana* (Ortiz *et al.*, 2007) and *Calicotome villosa* (Arroyo *et al.*, 2008). However, a substantial number of plant groups have failed to cross the Strait of Gibraltar, including *Saxifraga globulifera* (Vargas *et al.*, 1999), *Pistacia lentiscus* (Werner *et al.*, 2002), *Quercus ilex* (Lumaret *et al.*, 2002; Petit *et al.*, 2005), *Androcymbium gramineum* (Caujapé-Castells & Jansen, 2003), *Frangula alnus* (Hampe *et al.*, 2003) and certain lineages of five coastal plants (*Cakile maritima*, *Crithmum maritimum*, *Erygium maritimum*, *Halimione portulacoides*, *Salsola kali*; Kadereit *et al.*, 2005).

Two cpDNA lineages were identified across the geographical range of *C. ladanifer* (Fig. 4), one primarily European

(including haplotypes 1, 2 and 3) and the other African (including haplotypes 4, 5, 6, 7 and 8). The presence of haplotype 1 on both continents (Fig. 1b,c), the recent origin of *C. ladanifer* (Fig. 3) and haplotype relationships in the network analysis (Fig. 4) suggest the presence of an initial migrant possessing this interior haplotype, which differentiated on the Iberian Peninsula, resulting in the lineage of the haplotypes 2–3. Another lineage (haplotypes 4–5–6–7–8) evolved in northern Africa. The Iberian haplotype 6 is closely related to this African lineage (a single mutational step from the Moroccan haplotype 4) (Fig. 4a). The number of plastid mutations (4) is too high to place haplotype 6 in the primarily European lineage. Consequently, we inferred that haplotype 6 is the result of relatively recent long-distance dispersal from Africa to southern Iberia. Reconstructions of haplotype geographical ancestry point to a major centre of diversity in northern Africa. The *C. ladanifer* clade is connected to the haplotypes of the other 10 white-flowered species through the northern African haplotype 5 (Fig. 4a). Similarly, the phylogenetic reconstruction indicates haplotype 5 as sister to the other seven haplotypes of *C. ladanifer* (Fig. 4b). Considering the origin of *C. ladanifer* in northern Africa, population disjunctions may be the result of at least two colonization events from Africa to the Iberian Peninsula across the Strait of Gibraltar. Current geographical distributions and our estimates of divergence times lead us to hypothesize that the 12 *Cistus* species currently distributed in the western Mediterranean are the result of multiple colonizations after the opening of the Strait of Gibraltar between Africa and Europe.

Macaronesian plants provide further support for long-distance dispersal events and corroborate the colonization potential of *Cistus*. In addition to a single introduction of the ancestor of the five purple-flowered endemic species (Guzmán & Vargas, 2005), the occurrence of *C. monspeliensis* (a species of the white-flowered lineage) in the Canarian Islands indicates independent dispersal events from the mainland to the volcanic islands of Macaronesia (Hansen & Sunding, 1993). In addition to the findings for *Cistus*, a pattern of multiple colonization has similarly been inferred for small groups of genera that display both multiple introductions to the Canary Islands and the absence of special mechanisms for long-distance dispersal (Vargas, 2007).

Dispersal mechanisms vs. Mediterranean habitat requirements

Factors influencing the colonization success of plant groups include, among others, seed dispersal, seed germination rate, habitat preference, plant-growth conditions, breeding system and biotic interactions (Wang & Smith, 2002). The determination of paternal vs. maternal inheritance of cytoplasmic organelles is vital to distinguish between gene flow by pollen vs. seed dispersal. We showed with our controlled hybrids that the plastid sequences were maternally inherited, indicating that the haplotype network is consistent with plastid reconstruction of seed dispersal. *Cistus ladanifer* demonstrates a high

reproductive potential to colonize and form part of the Mediterranean scrub, particularly after fire (Pérez-García, 1997). The species exhibits high fruit and seed set (65–90%, Talavera *et al.*, 1993; B. Guzmán, E. Narbona, J. Arroyo & P. Vargas, unpublished data), compared with other hermaphroditic and self-incompatible plant species (Sutherland & Delph, 1984). Because of the high number of flowers per plant (> 500) and the high number of ovules per flower (often more than 1000), an individual has the potential to release thousands of seeds per year from its dehiscent capsules (Fig. 2). Massive seed dispersal by wind and gravity (anemobarochory), high germination rates and successful range expansion in Mediterranean environments may account for the rapid colonization of *C. ladanifer* after woodland disturbance (Luis-Calabuig *et al.*, 2000).

Bastida & Talavera (2002) found that 26% of *C. ladanifer* seeds dispersed no further than 20–60 cm from the canopy edges of the maternal plant, although herbivore endozoochory has been described as a mechanism for relatively long-distance dispersal (Malo & Suárez, 1996). Moreover, once in the soil, seeds of *C. ladanifer* can undergo a secondary dispersal by granivorous ants, which may increase seedling survival by moving the seed a few metres, and so beyond the influence of adult plants. The homogeneous distribution of haplotype 1 (haplotypes 2 and 3 are, however, found in one Iberian population each; haplotype 6 exclusive to southern Andalusia) in Europe may be the result of gradual expansion following a leading edge model during recolonization of the Mediterranean region after post-glacial climatic amelioration. This phylogeographical structure (high genetic variability in the refuge area and genetic uniformity in the recolonized areas) may be the result of a single colonizing lineage, similar to that observed in European *Hedera* (Grivet & Petit, 2002; Valcárcel *et al.*, 2003). Previous studies documented Mediterranean species surviving in refugia in southern Iberia, followed by range expansion in post-glacial periods (Hampe *et al.*, 2003; Valcárcel *et al.*, 2003; Vargas, 2003). The hypothesis that refugial populations of *C. ladanifer* survived past glaciations in northern Iberia and southern France, presumably bearing the ancestral haplotype 1, is congruent with our haplotype genealogy. However, palaeoclimatological evidence does not support this hypothesis, and range expansion of haplotype 1 from southern Iberian refugia is more plausible. During the harsh episodes of past glaciations, northern regions were probably climatically unsuitable for strict Mediterranean species such as *C. ladanifer* (Hewitt, 2000). Furthermore, pollen records attributed to *C. ladanifer* support a long-standing presence in southern Iberia, but a temporally interrupted occurrence at the northern end of the range (Pons & Reille, 1988).

Despite the absence of special dispersal mechanisms in *Cistus*, a wider window of opportunity for successful colonization of xerophyllous organisms appears to have occurred on both sides of the Strait of Gibraltar since the establishment of the Mediterranean climate (Rodríguez-Sánchez *et al.*, 2008). The absence of special mechanisms to long-distance dispersal

may have been overcome by the presence of large areas of suitable habitat that may have facilitated colonization of the Mediterranean basin. *Cistus ladanifer* is well represented during early successional stages after the disturbance of *Quercus suber* (Lumaret *et al.*, 2005) and *Quercus ilex* (Petit *et al.*, 2005) woodland communities by factors such as fire. Notably, *C. ladanifer* not only shares similar distributions to these Mediterranean trees, but also shows similar phylogeographical patterns of migration across the Strait of Gibraltar, indicating a common history of colonization (Fauquette *et al.*, 1999). The circum-Mediterranean distribution of three *Cistus* species (*C. creticus*, *C. monspeliensis*, *C. salviifolius*) further supports the hypothesis of active dispersal and colonization of angiosperms adapted to Mediterranean habitats.

In summary, phylogeographical results and divergence time estimates, coupled with congruent results in *C. monspeliensis*, *C. laurifolius* and *C. salviifolius* (M. Fernández-Mazuecos & P. Vargas, unpublished data), lead us to propose a pattern of active colonization and recolonization processes in the history of *Cistus*, clearly favoured by recurrent expansion into xeric habitats after the establishment of the Mediterranean climate.

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