



Research article

Unexpected synchronous differentiation in Mediterranean and Canarian *Cistus* (Cistaceae)

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ABSTRACT

Diversification rates of insular oceanic lineages have been historically considered to be higher than those of mainland counterparts. In order to test this hypothesis in a group of Canarian endemics (*Cistus*), we estimated phylogeny and conducted both network analyses of haplotypes and molecular clock estimates using plastid DNA sequences of the *trnS-trnG* and the *trnK-matK*. Net differentiation rates in the Canarian purple-flowered lineage parallel those of the mainland (Mediterranean) purple-flowered sister group, as revealed by a similar number of species (five vs. three), haplotypes (seven vs. eight) and haplotype clades (four vs. four). Splitting dates in the Canarian ($0.33 \pm 0.14/0.28 \pm 0.16$ Ma) and Mediterranean ($0.66 \pm 0.18/0.38 \pm 0.19$ Ma) lineages reveal concordance in tempo of diversification. All these results are interpreted as evidence of two synchronous evolutionary histories given that *Cistus* ancestors had to cope with factors promoting differentiation in two hotspots of plant diversity: the Macaronesian and Mediterranean floristic regions. Analysis of Canarian populations suggests that a geographical rather than ecological differentiation was primarily responsible for early stages of a non-adaptive radiation of the Canarian lineage. Differentiation patterns of mainland populations appear to be more complex.

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Introduction

The canonical hypothesis of differentiation in oceanic islands predicts speciation associated with radiation (Carlquist, 1967; Baldwin et al., 1998), which is a rapid proliferation of species from a single ancestor that implies a great morphological and physiological divergence among lineages in response to environmental (adaptive radiation) or non-environmental (non-adaptive radiation) causes (Schluter, 2000). Scholars traditionally accepted in the 20th century that island lineages often diversify in relatively short periods of time compared with their continental lineages (Carlquist, 1965, 1974; Sang et al., 1994; Kim et al., 1996; Crawford and Stuessy, 1997; Francisco-Ortega et al., 1997; Baldwin et al., 1998; Grant, 1998). This may be due to a considerable habitat heterogeneity, island age, island size, isolation from source areas, number of empty ecological niches and generally lower competition compared with mainland habitats. These factors have been suggested to have contributed to adaptive radiation of remarkable insular lineages (e.g., Hawaiian silversword alliance, Baldwin and Robichaux, 1995; *Echium*, García-Maroto et al., 2009; Hawaiian lobelioids, Givnish et al., 1996; *Sonchus*, Kim et al., 1996; *Argyranthemum*, Francisco-Ortega et al., 1997).

Alternative models of evolution in islands have been postulated in more recent years: (1) the niche pre-emption hypothesis argues that endemic groups with many species should be more effective at excluding repeat colonizations by relatives than groups represented by fewer species, because larger groups should pre-empt more of the habitat space available for colonization (Silvertown, 2004); (2) higher species diversity leads to a higher rate of diversification (Emerson and Kolm, 2005); (3) the colonization window hypothesis considers that island establishment rather than continental extinction is the prime determinant of spatio-temporal relationships of plant groups and predicts that opportunities for colonization have been temporally constrained to discrete waves of colonization (Carine, 2005); (4) anagenetic speciation, which implies divergence through time without further specific differentiation, appears to be significant in the origin of endemic species in most archipelagos (Stuessy et al., 2006); (5) early and recurrent colonization of islands are expected to be related to plant groups with diaspora displaying long-distance dispersal mechanisms (Vargas, 2007); and (6) the island immaturity-speciation pulse, in which the opportunities for speciation have a broadly predictable relationship to the life cycle of oceanic islands (Whittaker et al., 2007) (see also Stuessy, 2007; Whittaker et al., 2008).

The characteristics of *Cistus* offer an ideal opportunity to test previous models of evolution of the Canarian flora. The 21 species of *Cistus* are frutescent and suffrutescent shrubs primarily

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distributed in central-southern Europe, the Mediterranean and Macaronesia, although the centre of diversity is found in the western Mediterranean (Guzmán and Vargas, 2005). In addition, five endemic species are distributed across the Canary Islands (Table 1). Located along the northwestern Atlantic coast of Africa, the Canary archipelago consists of seven islands (Lanzarote, Fuerteventura, Gran Canaria, Tenerife, La Gomera, La Palma, El Hierro) that exhibit a broad range of geological ages (from 1.12 Ma of El Hierro to 20.6 Ma of Fuerteventura; Carracedo et al., 2002). The altitudinal gradients (from sea level to 3718 m) and the influence of the humid trade winds on the northern slopes of the islands have generated a high diversity of habitats responsible for the high degree of endemism (40%) found in the archipelago (Whittaker and Fernández-Palacios, 2007). These geographical and ecological conditions fostered ideal conditions to promote speciation in disparate plant groups of the Canarian flora (for review see Carine, 2005; Vargas, 2007). Causes related to moderate levels of speciation in some smaller genera are, however, intriguing. Despite the adaptation of the genus to Mediterranean environments (fire-dependent seed germination (Roy and Sonié, 1992; Trabaud and Renard, 1999), insect-dependent pollination (Talavera et al., 1993), flower-dependent reproduction (Herrera, 1987) and spring-dependent phenology (Herrera, 1986)), the Canarian *Cistus* occur in a variety of habitats and have diverged in leaf morphology and reproductive features (Table 2). Phylogenetic studies of the genus *Cistus* revealed a single origin for the Canarian lineage and a close relationship with western Mediterranean relatives (Guzmán and Vargas, 2005, 2009). Specifically, the Canarian lineage of five endemic species is sister to a mainland group of three purple-flowered species distributed in the Iberian Peninsula and northern Africa (*Cistus heterophyllus*) and the Mediterranean Basin (*Cistus albidus*, *Cistus creticus*).

Biogeographical analyses that incorporate a temporal framework to date diversification of island lineages and their mainland relatives provide testable hypotheses about the relationships between island and mainland floras. In particular, the tempo of diversification between sister groups (e.g., continental and insular lineages of *Cistus*) may serve to test whether the above-mentioned hypotheses hold true. In this study, we employed analyses of plastid haplotype variation of the five Canarian endemics of *Cistus* and their sister group to: (1) evaluate phylogeographical relationships between populations and related species; (2) infer patterns of historical differentiation within each group; and (3) test whether diversification of species and haplotype lineages was accelerated in the insular clade compared with the mainland clade.

Materials and methods

Sample strategy and DNA sequencing

Seventy samples representing 21 populations (43 individuals) of the five *Cistus* endemics to the Canary Islands and 18 populations (27 individuals) of three mainland purple-flowered species of *Cistus* were sequenced to investigate plastid (*trnS-trnG*, *trnK-matK*) haplotype variation (Table 1). Although a higher number of samples (43) were analysed from the Canary Islands, the mainland group is represented by a sample (27 individuals) covering a larger area (i.e., the Mediterranean floristic region). We increased the number of sequenced individuals (two–five) in 12 Canarian and five mainland populations to test haplotype constancy within populations. To investigate phylogenetic relationships among haplotypes and to estimate divergence times, we sequenced one sample of the two other purple-flowered (*Cistus*

crispus, *Cistus parviflorus*), the 10 white-flowered, two *Halimium* species and one *Tuberaria* species as outgroup sample.

Standard primers were used for amplification and sequencing of the *trnK-matK* (*trnK*-3914F, *matK*-1470R) (Johnson and Soltis, 1994) and the *trnS* (GCU)–*trnG* (UCC) (Hamilton, 1999) spacers. One internal primer (*trnSGpolyTf*; 5'-TTAGATTCTATTACATTCT-3') was designed to amplify and sequence the *trnS-trnG* spacer due to mononucleotide repeat stretches found in the purple-flowered species (poly-T at site 190, poly-A at site 580). PCR amplifications were performed as in Guzmán and Vargas (2005) for *trnK-matK* sequences, whereas those for *trnS-trnG* sequences were 1–3 min pretreatment at 94 °C, 28 cycles of 1 min at 94 °C, 1–2 min at 50–53 °C and 1–2 min at 72 °C. Procedures used for DNA sequencing are given in Guzmán and Vargas (2005).

Haplotype data analysis

The sequences of *trnK-matK* and *trnS-trnG* were combined and aligned by eye, given the low number of indels across sequences. Technical problems in the sequencing of the *trnS-trnG* spacer caused by poly-T stretches led us to eliminate 101 bp (190–291 bp sites). Haplotypes (h) were determined by sequence variation in the concatenated *trnK-matK/trnS-trnG* sequences. Relationships among Canarian haplotypes, on one hand, and among mainland purple-flowered species on the other, were inferred using the software TCS 1.21 (Clement et al., 2000). The program implements a statistical parsimony approach using the algorithm described in Templeton et al. (1992) to construct haplotype networks. The maximum number of differences among haplotypes as a result of single substitutions was calculated with 95% confidence limits and treating gaps as missing data.

Estimating lineages divergences

Tree topologies and branch lengths from the combined data set of *trnS-trnG* and *trnK-matK* sequences were obtained using Bayesian inference (BI) and maximum parsimony (MP). BI was analysed in Mr. Bayes 3.2.1 (Ronquist and Huelsenbeck, 2003) using the best model of molecular evolution (GTR+G) selected by Mr. Modeltest (Posada and Crandall, 1998; Nylander, 2002). Two identical searches with 3,000,000 generations each (chain temperature=0.2; sample frequency=100) were performed. In both runs, probabilities converged on the same stable value approximately after generation 30,000. A 50% majority-rule consensus tree was calculated using the *sumt* command to yield the final Bayesian estimate of phylogeny. We used posterior probability (PP) as an estimate of robustness. MP analyses were performed as in Guzmán and Vargas (2005), except for clade support. We performed a full heuristic bootstrap using 1000 replicates with random taxon addition, TBR branch swapping and the options Multrees and Steepest Descent in effect but limiting the number of rearrangements per random replicate to 10,000,000 to overcome computational limitations. In order to estimate divergence times, we employed the 50% majority-rule consensus tree obtained by BI.

To check the constancy of substitution rates, we used the Langley and Fitch (LF) test (Magallón and Sanderson, 2005). We rejected the null hypothesis of constant rate ($\chi^2=175.64$; d.f.=29). Then, divergence times were estimated using the r8S 1.71 program (Sanderson, 2002) with a penalized likelihood (PL) approach that was implemented with the truncated Newton (TN) algorithm. Initial results were obtained under the following parameters: cvstart=0.5; cvinc=0.5; cvnum=10 with cross-validation enforced. The rate smoothing with the lowest cross-validation scores was selected and the dating procedure was

Table 1
Cistaceae taxa sequenced for the *trnK*–*matK* and *trnS*–*trnG* DNA spacers.

Taxon	Locality/source (number of individuals per population ^a)	Voucher	Haplotype coding (h) by number or letter	<i>trnK</i> – <i>matK</i> accession no.	<i>trnS</i> – <i>trnG</i> accession no.
Cistus L.					
<i>Cistus albanicus</i> E.F. Warb. ex Heywood	Cultivated	R. G. Page 8cBGA04(MA)	–	DQ093010	FJ228736
<i>Cistus albidus</i> L.	Spain, Madrid, Aldea del Fresno	P. Vargas 25PV03 (MA)	A	DQ092974	FJ228730
<i>Cistus albidus</i> L.	Spain, Cádiz, Grazalema	P. Vargas 252PV06 (MA)	A	GQ926985	GQ927044
<i>Cistus albidus</i> L.	Portugal, Sagres	B. Guzmán 34BGA04 (MA)	A	GQ926988	GQ927047
<i>Cistus albidus</i> L.	Morocco, Tetuán	P. Vargas 41PV03 (MA)	A	DQ092975	GQ927042
<i>Cistus albidus</i> L.	Morocco, Ketama	B. Guzmán 111BGA04 (MA)	A	GQ926989	GQ927048
<i>Cistus albidus</i> L.	Morocco, Fardiwa (2)	J. Martínez 118JM03 (MA)	A	GQ926986/7	GQ927045/6
<i>Cistus albidus</i> L.	Morocco, Talamagait	B. Guzmán 94BGA04 (MA)	B	GQ926984	GQ927043
<i>Cistus albidus</i> L.	Morocco, Ouazanne (2)	J. Martínez 210JM04 (MA)	A, C	GQ926991/2	GQ927050/1
<i>Cistus albidus</i> L.	Morocco, Beni-Hadifa	B. Guzmán 105BGA04 (MA)	D	GQ926990	GQ927049
<i>Cistus chinamadensis</i> Bañares et Romero subsp. <i>gomeræ</i>	La Gomera, Roque Agando	R. G. Page 144BGA04 (MA)	2	DQ092987	GU080223
<i>Cistus chinamadensis</i> Bañares et Romero subsp. <i>gomeræ</i>	La Gomera, Roque Agando (3)	A. Fernández & J. Leralta 44BGA04 (MA)	2	DQ09286, GQ927006/7	GQ281688, GQ927065/6
<i>Cistus chinamadensis</i> Bañares et Romero subsp. <i>Chinamadensis</i>	Tenerife, Chinamada (3)	C. Rodríguez 4BGA06 (MA)	1	GQ927008/9/10	GQ927067/8/9
<i>Cistus clusii</i> Dunal	Spain, Málaga, Mijas	R. G. Page 8bBGA04 (MA)	–	DQ093009	FJ228739
<i>Cistus creticus</i> L.	Morocco, Azilal (5)	P. Vargas 68PV05 (MA)	A, C, F	GQ926997– GQ927001	GQ927056– GQ927060
<i>Cistus creticus</i> L.	Morocco, Afourer (3)	P. Vargas 7PV05 (MA)	C, E	GQ926993/4/5	GQ927052/3/4
<i>Cistus creticus</i> L.	Morocco, Asni	P. Vargas 86PV05 (MA)	E	GQ926996	GQ927055
<i>Cistus creticus</i> L.	Morocco, Essaouira (2)	P. Vargas 131PV03 (MA)	A, E	GQ927002/3	GQ927061/2
<i>Cistus creticus</i> L.	Greece, Olympus	P. Vargas 209PV04 (MA)	A	DQ092978	GQ281684
<i>Cistus creticus</i> L.	Greece, Crete, Veneratos	P. Vargas 94PV05 (MA)	A	GQ927004	GQ927063
<i>Cistus crispus</i> L.	Spain, Córdoba, Posadas	B. Guzmán 58BGA04 (MA)	G	DQ093013	GQ281694
<i>Cistus heterophyllus</i> L.	Morocco, Beni-Hadifa	B. Guzmán 99BGA04 (MA)	A	DQ092989	GQ281690
<i>Cistus heterophyllus</i> L.	Morocco, Gurugú Mountain	B. Guzmán 84BGA04 (MA)	A	DQ092989	GQ927101
<i>Cistus heterophyllus</i> L.	Morocco, Targuist	B. Guzmán 108BGA04 (MA)	A	GQ927005	GQ927064
<i>Cistus horrens</i> Demoly	Gran Canaria, Ayacata (3)	B. Guzmán 2BGA05 (MA)	3, 4	FJ225848, GQ927037/8	GQ281689, GQ927096/7
<i>Cistus horrens</i> Demoly	Gran Canaria, San Bartolomé de Tirajana (3)	B. Guzmán 5BGA05 (MA)	3	FJ225848, GQ927039/40	GQ927098– GQ927100
<i>Cistus ladanifer</i> L.	Spain, Madrid, El Atazar	B. Guzmán 28BGA03 (MA)	–	FJ225825	FJ189427
<i>Cistus laurifolius</i> L.	Spain, Madrid, Las Rozas	P. Vargas 12PV03 (MA)	–	DQ093004	GQ927103
<i>Cistus libanotis</i> L.	Spain, Córdoba	R. G. Page 149BGA04 (MA)	–	DQ092993	FJ228732
<i>Cistus monspeliensis</i> L.	Portugal, Sagres	B. Guzmán 35BGA04(MA)	–	DQ093012	FJ225849
<i>Cistus monspeliensis</i> L.	Tenerife, Villa de Arico	P. Vargas 42PV05 (MA)	–	GQ927041	GQ927102
<i>Cistus munbyi</i> Pomel	Morocco	O. Filippi 4BGA04 (MA)	–	DQ093006	FJ228738
<i>Cistus ochreatus</i> C. Sm. ex Buch	Gran Canaria	R. G. Page 8BGA04 (MA)	1	DQ092985	GQ281687
<i>Cistus ochreatus</i> C. Sm. ex Buch	Gran Canaria	R. G. Page 150BGA04 (MA)	1	DQ092984	GQ927104
<i>Cistus ochreatus</i> C. Sm. ex Buch	Gran Canaria, Artenara (3)	P. Vargas 60PV05 (MA)	1	GQ927031/2/3	GQ927090/1/2

Table 1 (continued)

Taxon	Locality/source (number of individuals per population ^a)	Voucher	Haplotype coding (h) by number or letter	<i>trnK-matK</i> accession no.	<i>trnS-trnG</i> accession no.
<i>Cistus ochreatus</i> C. Sm. ex Buch	Gran Canaria, Tamadaba (3)	B. Guzmán 4BGA05	1	GQ927034/5/6	GQ927093/4/5
<i>Cistus osbeckiifolius</i> Webb ex Christ	Tenerife, Cañadas del Teide	P. Escobar 48/05 (MA)	5	GQ281699	GQ281685
<i>Cistus osbeckiifolius</i> Webb ex Christ	Tenerife, Cañadas del Teide (3)	P. Vargas 177PV05 (MA)	5	GQ927028/9/30	GQ927087/8/9
<i>Cistus parviflorus</i> Lam.	Greece, Crete	O. Filippi 6BGA04 (MA)	–	DQ092976	GQ281683
<i>Cistus populifolius</i> L.	Spain, Ávila, Arenas de San Pedro	P. Vargas 5PV03 (MA)	–	DQ093003	FJ228733
<i>Cistus pouzolzii</i> Delile	Morocco, Ketama	S. L. Jury 698247MA	–	DQ093008	GQ927105
<i>Cistus psilosepalus</i> Sweet	Spain, Ávila, Arenas de San Pedro	P. Vargas 7PV03 (MA)	–	DQ092994	FJ228737
<i>Cistus salviifolius</i> L.	Spain, Ávila, Arenas de San Pedro	P. Vargas 6PV03 (MA)	–	DQ092990	GQ281691
<i>Cistus symphytifolius</i> Lam	Tenerife, Vilaflor (2)	P. Vargas 174PV05 (MA)	2, 5	GQ927024/5	GQ927083/4
<i>Cistus symphytifolius</i> Lam	Tenerife, Villa de Arico	P. Vargas 41PV05 (MA)	5	GQ927011	FJ228729
<i>Cistus symphytifolius</i> Lam	Tenerife, Aguamansa	C. García-Verdugo 7CG05 (MA)	5	GQ927026	GQ927085
<i>Cistus symphytifolius</i> Lam	Tenerife, Barranco de las Ánimas	P. Escobar 51/05 (MA)	5	GQ927027	GQ927086
<i>Cistus symphytifolius</i> Lam	Tenerife, Güimar (2)	P. Vargas 48PV05 (MA)	7	GQ927021/2	GQ927080/1
<i>Cistus symphytifolius</i> Lam	Tenerife, Teide	P. Escobar 45/05 (MA)	7	GQ927023	GQ927082
<i>Cistus symphytifolius</i> Lam	Tenerife, Teno (5)	C. García-Verdugo 7CG06 (MA)	6, 7	GQ927014/5/6/7/8	GQ927073/4/5/6/7
<i>Cistus symphytifolius</i> Lam	La Palma, Barranco de Garome (2)	C. García-Verdugo 35CG05 (MA)	2	GQ927019/20	GQ927078/9
<i>Cistus symphytifolius</i> Lam	La Palma, La Cumbrecita	B. Guzmán 143BGA05 (MA)	2	DQ092983	GQ281686
<i>Cistus symphytifolius</i> Lam	La Palma, Roque de los Muchachos (2)	V. Valcárcel 64VV05 (MA)	2	GQ927012/3	GQ927071/2
<i>Halimium</i> (Dunal) Spach					
<i>Halimium ocymoides</i> (Lam.) Willk.	Portugal, Coimbra	R. G. Page 158BGA04 (MA)	–	FJ225846	GQ281679
<i>Halimium umbellatum</i> (L.) Spach	Spain, Madrid, Tres Cantos	P. Vargas 71BGA04 (MA)	–	DQ092972	GQ281676
<i>Tuberaria</i> Dunal					
<i>Tuberaria guttata</i> (L.) Fourr.	Portugal, Vila do Bispo	B. Guzmán 44BGA04 (MA)	–	DQ092971	GQ281674

Material source, number of individuals per population^a, voucher reference, haplotypes (numbers for Canarian accessions and letters for mainland accessions) and GenBank accession numbers are indicated. Taxonomy follows different accounts (see Guzmán and Vargas, 2005).

^a One individual per population except when indicated in brackets.

repeated with the following parameters: collapse; num_time_guesses=5 and num_restarts=5. Cross-validation suggested 32 as the best smooth parameter. Standard deviations were obtained by bootstrapping data using the ML approach (PAUP*, Swofford, 2002). Confidence intervals of estimated ages were calculated by bootstrapping the Bayesian consensus tree (Baldwin and Sanderson, 1998; Sanderson and Doyle, 2001). To convert relative divergence times into absolute time units, we used the split age between the complex *Cistus-Halimium* and the rest of the Cistaceae, obtained in a previous study (Guzmán and Vargas, 2009), to constrain the node to a maximum age of 3.26 Ma. A likelihood ratio test was performed in r8s (rrlike command) to test whether the Canarian and the mainland lineages have evolved from the most recent ancestor at the same constant rate.

Alternatively, divergence time estimates and associated confidence intervals (Kishino et al., 2001) were obtained utilizing a relaxed Bayesian molecular clock method, which allows for variability in the evolutionary rate over time. We used the Multidistribute package (<http://statgen.ncsu.edu/thorne/multidiv>

[time.html](#)) developed by Thorne and Kishino (2002). We followed the procedure outlined in Rutschmann (2005) but adjusted some parameters to the present data set. The prior distribution of time from the ingroup root to present (rttm) and its standard deviation (rttmsd) were set to 0.32 based on the estimated age of the split between the *Tuberaria* genus and the *Cistus-Halimium* assemblage (Guzmán and Vargas, 2009). The median of branch lengths was 0.003, so rtrate=0.003/0.32=0.009; rtratesd was set equal to rtrate. Brownmean and brownsd were set to 0.4, bigtime was set to 100.0 and commonbrown was set to 0. The split age between the *Cistus-Halimium* complex and the *Tuberaria* genus was constrained as upper bound=0.326. After a burn-in stage of 100,000 cycles (not sampled), the Markov chain was sampled 500,000 times every 100 cycles.

Lineages-through-time plots (LTT) for the Canarian and the mainland groups were calculated using PL estimated dates and the APE package (Paradis et al., 2004) in R software v2.31 (2006) to visualize the number of lineages present at sequential time points.

Table 2

Ecological and morphological variation in purple-flowered *Cistus*; data from Martín Bolaños and Guinea (1949), Demoly and Montserrat (1993) and our own observations.

	Distribution	Climate conditions	Altitude (m)	Habitat	Stigma length	Fruit hairiness
<i>Cistus albidus</i>	Iberia, S France, N Italy, N Africa, Corsica, Sardinia	Dry, Mediterranean	0–1200	Degradated <i>Quercus ilex</i> and <i>Pinus halepensis</i> woodlands	Similar to stamens	Pubescent
<i>Cistus creticus</i>	Mediterranean Basin	Dry, Mediterranean	50–650	Dense maquis	Similar to stamens	Pubescent
<i>Cistus heterophyllus</i>	SE Spain, N Africa	Dry, Mediterranean	100–500	Sandy maquis	Similar to stamens	Glabre
<i>Cistus chinamadensis</i> ssp. <i>chinamadensis</i>	Tenerife (Canary Islands)	Subhumid, temperate-warm	535–650	Laurel forest	Above stamens	Pubescent
<i>Cistus chinamadensis</i> ssp. <i>gomeræ</i>	La Gomera (Canary Islands)	Subhumid temperate-warm	950–1300	Laurel forest	Above stamens	Pubescent
<i>Cistus horrens</i>	Gran Canaria (Canary Islands)	Semiarid, temperate to warm	300–1500	Pine forest	Above stamens	Glabre
<i>Cistus ochreatus</i>	Gran Canaria (Canary Islands)	Subhumid temperate-warm	860–1400	Pine forest	Above stamens	Glabre
<i>Cistus osbeckiifolius</i>	Tenerife (Canary Islands)	Dry, temperate-cold	1400–2400	High mountains	Above stamens	Pubescent
<i>Cistus symphytifolius</i>	El Hierro, Gran Canaria, La Gomera, La Palma, Tenerife (Canary Islands)	Temperate	350–2000	Pine and thermophilous forest	Above stamens	Glabre
	Leaf shape			Leaf and petioles trichomes	Leaf base	Leaf venation
<i>Cistus albidus</i>	Ovate–oblong, elliptic or oval–lanceolate			Dense tomentum of stellate hairs	Sessile	Reticulate
<i>Cistus creticus</i>	Oval and oblong–elliptic			Dense tomentum of single, glandulous and stellate hairs	Petiolate	Penninerve
<i>Cistus heterophyllus</i>	Ovate–rhomboid or lanceolate–elliptic			Dense tomentum of stellate hairs	Petiolate	Pinnate
<i>Cistus chinamadensis</i> ssp. <i>chinamadensis</i>	Elliptic–lanceolate			Tomentum of stellate hairs	Petiolate	Reticulate
<i>Cistus horrens</i>	Elliptic–lanceolate			Tomentum of stellate hairs	Petiolate	Reticulate
<i>Cistus chinamadensis</i> ssp. <i>gomeræ</i>	Oval			Glandulous and simple hairs	Petiolate	Reticulate
<i>Cistus ochreatus</i>	Oval			Glabre	Petiolate	Reticulate
<i>Cistus osbeckiifolius</i>	Lanceolate			No stellates hairs but densely covered by simple hairs	Petiolate	Reticulate
<i>Cistus symphytifolius</i>	Oblong–lanceolate			Only glandulous hairs on nerves	Petiolate	Reticulate

Results

Analysis of Canarian Cistus haplotypes

The sequence length of the Canarian purple-flowered *Cistus* was 1304bp for *trnK–matK* and 568–590bp for *trnS–trnG* (Table 3A). The combined data set of 86 *trnS–trnG* and *trnK–matK* sequences from 43 individuals distinguished seven haplotypes (h1–h7). Of these, five are found on Tenerife, three on Gran Canaria and one each on La Gomera and La Palma (Table 1; Fig. 1A). Two haplotypes were found on more than one island (h1 in Gran Canaria and Tenerife; h2 in Tenerife, La Palma and La Gomera). Three haplotypes (h1, h2 and h5) were shared by two taxa each: h1 by *C. ochreatus* and *C. chinamadensis* subsp. *chinamadensis*; h2 by *C. symphytifolius* and *C. chinamadensis* subsp. *gomeræ*; and h5 by *C. osbeckiifolius* and *C. symphytifolius*. Two haplotypes (h3 and h4) were found only in *C. horrens* from Gran Canaria, and two more haplotypes (h6 and h7) were found only in *C. symphytifolius* populations from Tenerife. Haplotype constancy was inferred because nine (75%) of the 12 populations, from which several individuals were analysed, had a single haplotype (Table 1). Only one population of *C. horrens* (Gran Canaria) and two populations of *C. symphytifolius* (Tenerife) showed two different haplotypes.

TCS constructed a single network with seven haplotypes connected by a single mutation (Fig. 1B), four haplotype clades

Table 3

List of haplotypes found in 21 populations of five species endemic to the Canary Islands (A) and in 18 populations from three mainland purple-flowered species of *Cistus* (B).

Nucleotide position	trnK–matK			trnS–trnG			
	352	597	973	153	327	352	
A. Canary Islands							
Haplotypes							
h1	A	G	A	T	T	A	
h2	A	G	A	T	A	A	
h3	A	G	A	G	T	A	
h4	A	T	A	G	T	A	
h5	G	G	A	T	T	A	
h6	G	G	A	T	T	C	
h7	G	G	G	T	T	A	
Nucleotide position	102	497	513	2	79	279	512
B. Mainland							
Haplotypes							
hA	C	G	A	T	C	A	G
hB	C	G	A	C	C	A	G
hC	A	G	A	T	C	A	G
hD	C	G	G	T	C	C	T
hE	C	G	A	T	A	A	T
hF	C	A	A	T	A	A	T

Variable sites (excluding indels) in the data sets of two plastid DNA fragments (*trnK–matK*, *trnS–trnG*) are shown, as numbered from the 5' to the 3' ends.

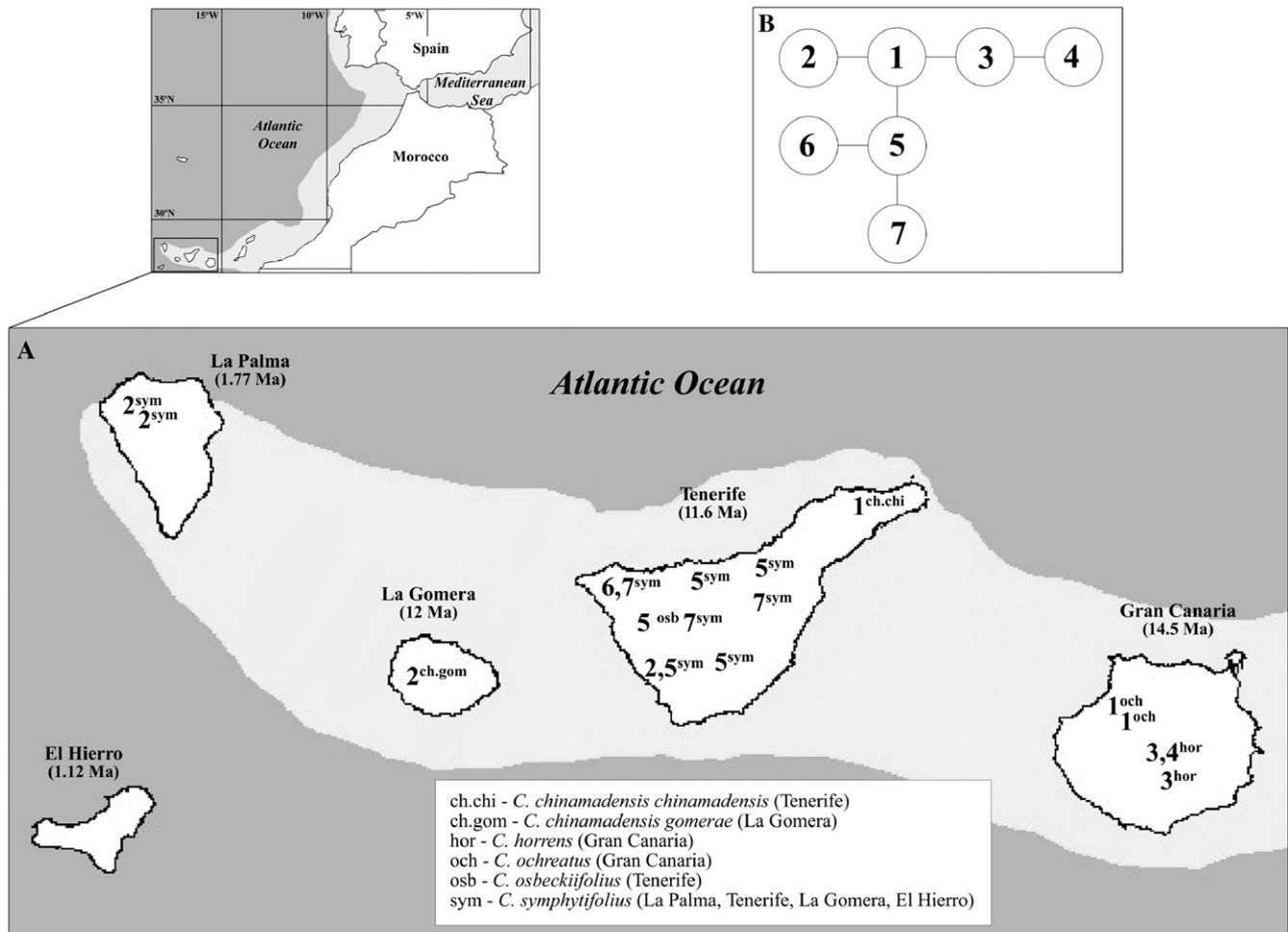


Fig. 1. Geographical distribution (A) of the seven cpDNA (*trnS-trnG*, *trnK-matK*) haplotypes of the five purple-flowered Canary species of *Cistus*. Numbers indicate the seven haplotypes. Superindex letters indicate *Cistus* taxa (abbreviated as in the inset). Statistical parsimony network (B) representing relationships of the seven plastid haplotypes. Lines between haplotypes in the network indicate a single nucleotide substitution.

and no loops. This analysis indicates that every haplotype needed to build up the network was sampled. Two interior haplotypes (h1 and h5) display the highest number of mutational connections (three connections each). Three clades of haplotypes are related to geography. Three haplotypes (h1, h3 and h4) show an eastern distribution (northeast Tenerife, Gran Canaria), three other haplotypes (h5, h6 and h7) are restricted to Tenerife and one haplotype (h2) has a western distribution (La Palma, La Gomera, southwest Tenerife).

Analysis of mainland *Cistus* haplotypes

The sequence length of the purple-flowered *Cistus* species from the mainland was 1304 bp for *trnK-matK* and 563–603 bp for *trnS-trnG* (Table 3B). The combined data set of 54 *trnS-trnG* and *trnK-matK* sequences from 27 individuals distinguished six haplotypes (hA–hF), which are distributed in Europe (one haplotype) and Africa (six haplotypes) (Table 1; Fig. 2A). The most common and widespread haplotype (hA) was found in 15 individuals (55.55% of all samples) from populations of the two continents. This haplotype accounts for 73% of *C. albidus* individuals, 31% of *C. creticus* and 100% of *C. heterophyllus*. One more haplotype (hC) was recorded across two taxa (*C. albidus* and *C. creticus*). Two haplotypes (hB and hD) were exclusively found in African populations of *C. albidus*, and two more haplotypes

(hE and hF) in African populations of *C. creticus*. We retrieved heterogeneity of haplotypes in four of the five (80%) populations where we analysed more than one individual (Table 1).

TCS constructed a single network with the six haplotypes, four haplotype clades and no loops (Fig. 2B). It was inferred that there were two missing haplotypes needed to build up the network. Two groups of haplotypes (hA–hB–hC/hE–hF) were separated by one missing haplotype, which is also connected with hD through another missing haplotype (Fig. 2B). The network depicted hA and a missing one (extinct or not found) as the two interior haplotypes with the most mutational connections (three connections).

Phylogenetic analyses

The aligned length of the concatenated *trnK-matK* and *trnS-trnG* sequences of the *Cistus*, *Halimium* and *Tuberaria* species was 2168 bp. Seventy-four of the 249 variable characters were parsimony informative. The MP analysis generated 852 trees of 306 steps with a consistency index (CI) of 0.90, a retention index (RI) of 0.93 and a rescaled consistency index (RC) of 0.84. The strict consensus tree (results not shown) had lower resolution than that of the BI tree. Relationships among the Mediterranean purple-flowered haplotypes in one hand, and between the Canary haplotypes 1 and 2 on the other, were not resolved in the MP analysis. The 50% majority consensus tree of the BI using

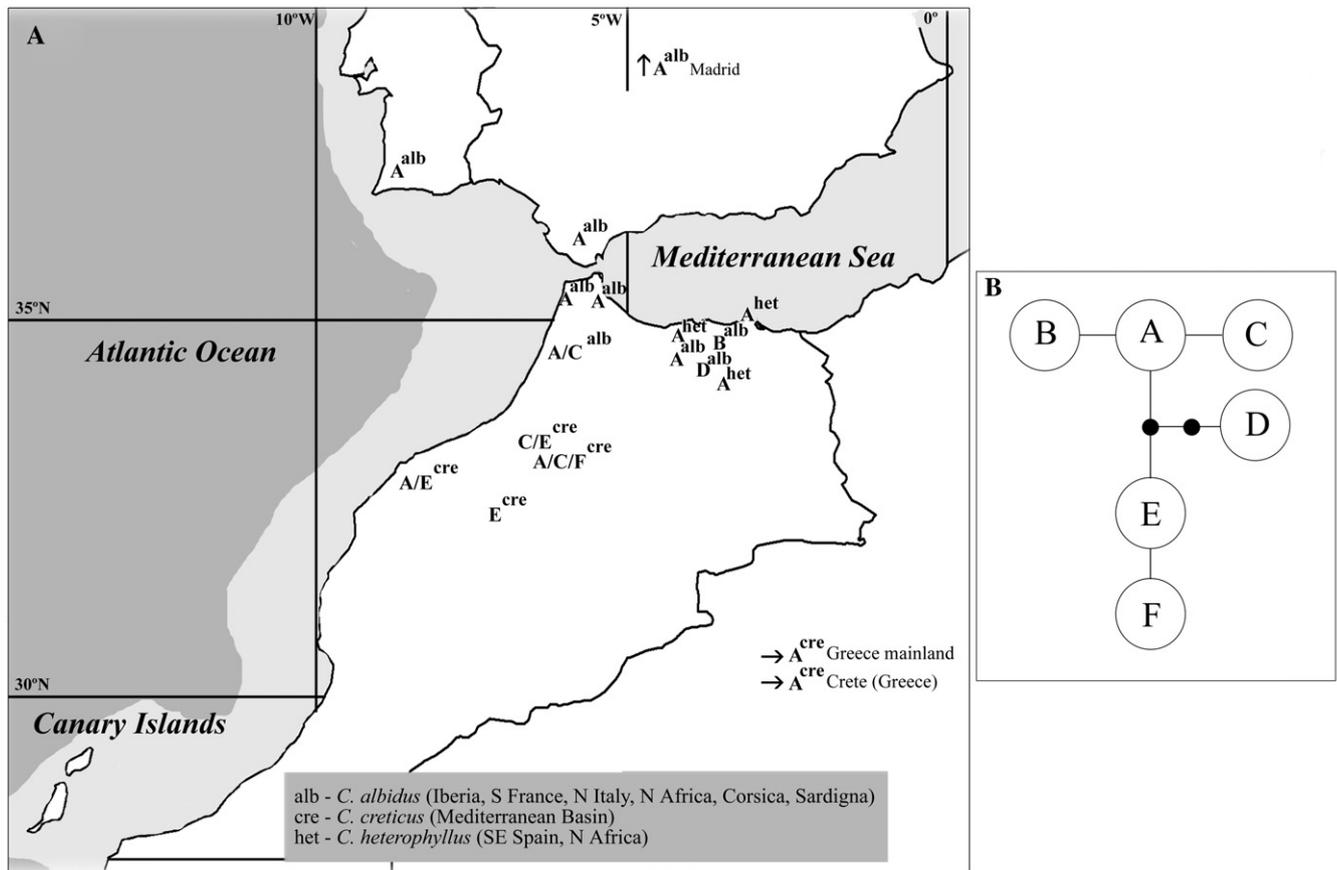


Fig. 2. Geographical distribution (A) of the six cpDNA (*trnS-trnG*, *trnK-matK*) haplotypes of the three purple-flowered species of *Cistus* distributed in mainland. Letters indicate the six haplotypes. Superindex letters indicate *Cistus* taxa (abbreviated as in the inset). Statistical parsimony network (B) representing relationships of the six plastid haplotypes. Lines between haplotypes in the network indicate a single nucleotide substitution and dots (●) represents missing haplotypes (extinct or not found).

the simplest model of evolution (GTR+G) is shown in Fig. 3. The BI and MP analyses retrieved a mainland purple-flowered species (*C. crispus*) sister to the group of the Canarian and the mainland purple-flowered clades (100 PP, 100% BS). Both analyses also recognized the Canarian species and three mainland purple-flowered species (*C. albidus*, *C. creticus*, *C. heterophyllus*) as two monophyletic groups (100 PP, 100% BS and 100 PP, 93% BS, respectively). Within the Canarian clade, both analyses retrieved the h2 as sister (94 PP, 63% BS) to the other Canarian haplotypes. Three more haplotype lineages are distributed in Tenerife and Gran Canaria (h1), Gran Canaria (lineage h3-h4) and Tenerife (lineage h5-h6-h7). Our data further illustrate that a minimum of two colonizations of the Canaries has occurred in *Cistus*; one for the five purple-flowered endemics and one for the white-flowered species *C. monspeliensis* (Fig. 3). Within the mainland purple-flowered clade, MP and BI analyses retrieved hF as sister to the other haplotypes (92 PP, 57% BS). Three more haplotype lineages (hE/hD/hA-hB-hC) are primarily distributed in northern Africa, with only hA also distributed in other areas.

Pairwise sequence divergence between samples of the purple-flowered lineage (including *C. crispus*) were similar (ranging from 0.05% to 1.1%) irrespective of the model of sequence substitution selected (Table 4). The same is true for values within the mainland lineage (ranging from 0.05% to 0.27%) and the Canarian lineage (ranging from 0.05% to 0.21%).

Divergence times

The results of both dating analyses (relaxed Bayesian molecular clock and PL) are shown in Table 5. We did not find major

incongruence between ages estimated with both methods. Hereafter, we will discuss results retrieved from the PL approach for the sake of brevity and comparison with other plant groups.

The molecular clock analyses estimated that the divergence of *Halimium ocymoides* and the two major lineages of the genus *Cistus* (one including the purple-flowered species plus *Halimium umbellatum* and the other of the white-flowered species plus the purple-flowered *C. parviflorus*) occurred in the Middle Pliocene (3.13 ± 0.08 Ma) (node 1 in Table 5). In the purple-flowered lineage, *C. crispus* is sister to the ancestor of two sublineages displaying similar divergence times. The ancestor of the mainland lineage (*C. albidus*, *C. creticus*, *C. heterophyllus*) split about 0.66 ± 0.18 Ma, whereas the Canarian divergence took place about 0.33 ± 0.14 Ma (nodes 13 and 17 in Table 5, respectively). PL showed that the diversification of the white-flowered *Cistus* clade (white-flowered species plus *C. parviflorus*) occurred at a rate of 3.67×10^{-9} nucleotide substitutions per site per year (s/s/yr). In contrast, higher local rates per branch were found within the Canarian (7.99×10^{-9} s/s/yr) and the mainland (6.67×10^{-9} s/s/yr) clades (Table 4). The relative rate test showed non-significant differences (LRStat=0.25; d.f.=5) between the two models tested (constant and different rates between the clades that are evolving from the same ancestor), so the null hypothesis of constant rates in both the Canarian and the mainland clades cannot be rejected.

The LTT plots for Canarian and mainland lineages are shown in the inset of Fig. 3. The shape of the plots hints at a comparable behaviour pattern in the two groups. Our analysis reflects that shortly after a long period of stasis (570,000 and 900,000 years in the mainland and Canarian lineages, respectively), the same

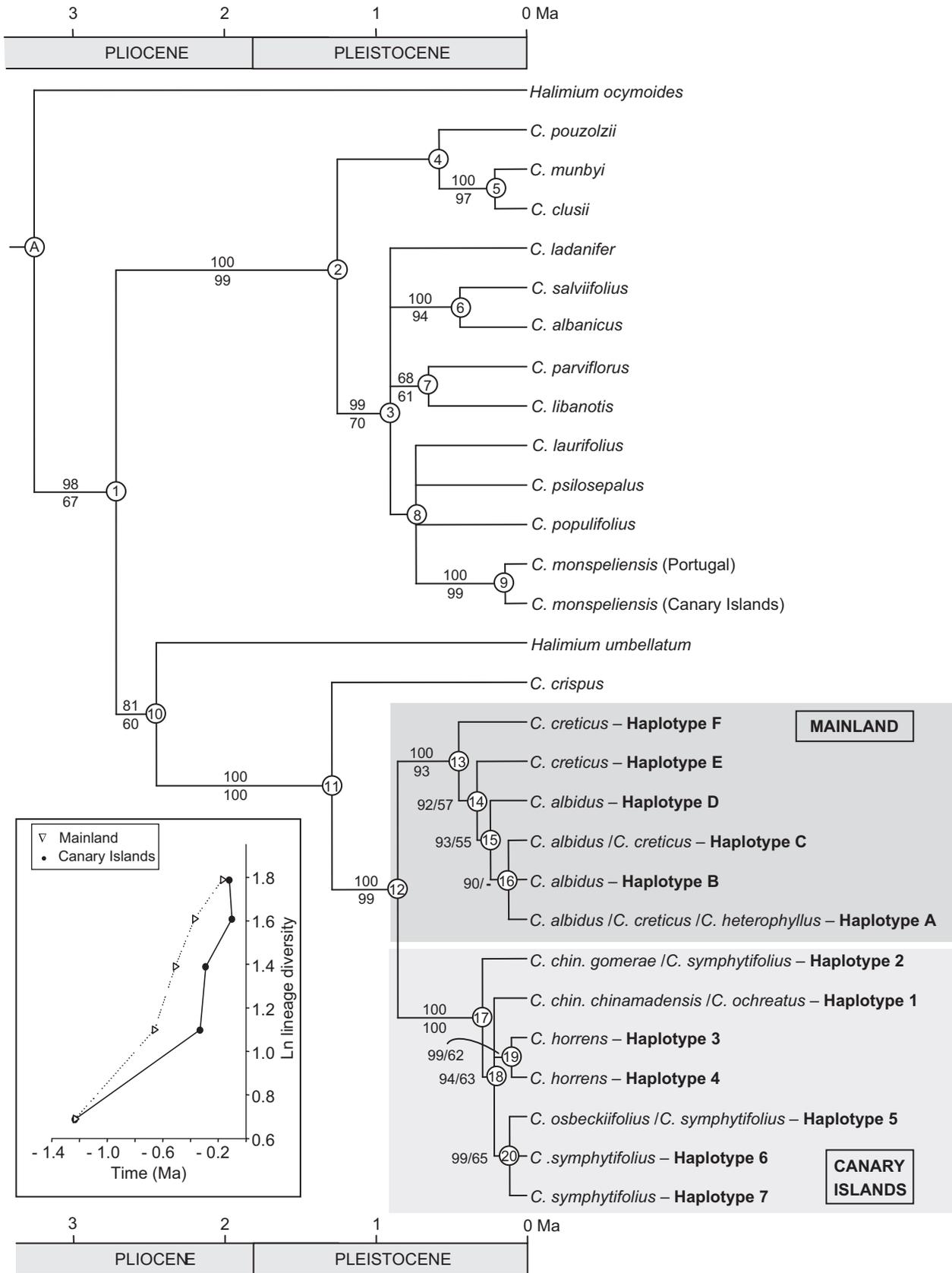


Fig. 3. Chronogram of the consensus Bayesian inference tree based on the combined data sets of *trnS-trnG* and *trnK-matK* sequences. Branch lengths represent million of years (Ma). Support values are given above (posterior probabilities) and below (bootstrap) branches from the BI and MP analyses, respectively. One estimated date (node A) of the *Cistus*-*Halimium* complex divergence (Guzmán and Vargas, 2009) was used to calibrate the analysis. Outgroup taxa (*Tuberaria guttata*) have been removed for legibility. Lineages-through-time plots of the Canary Islands (●) and the purple-flowered mainland (▽) lineages are also shown (inset).

Table 4

Penalized Likelihood estimates of divergence rates and pairwise divergences of *trnS-trnG* and *trnK-matK* sequences calculated with five different models of nucleotide evolution for the purple-flowered mainland and Canarian lineages of *Cistus*. s/s/yr=nucleotide substitutions per site per year.

	Divergence rates (s/s/yr)	Pairwise sequence divergence (%)				
		JC	F81	K-2-p	HKY85	GTR
White-flowered lineage	3.67×10^{-9}	0.1–1.19	0.1–1.19	0.1–1.19	0.1–1.19	0.1–1.19
Purple-flowered lineage	6.96×10^{-9}	0.05–1.1	0.05–1.1	0.05–1.1	0.05–1.1	0.05–1.1
Mainland lineage	6.67×10^{-9}	0.05–0.27	0.05–0.27	0.05–0.27	0.05–0.27	0.05–0.27
Canarian lineage	7.99×10^{-9}	0.05–0.21	0.05–0.21	0.05–0.21	0.05–0.21	0.05–0.21

Table 5

Penalized likelihood (bootstrapping of 1000 trees) and relaxed Bayesian molecular clock estimates of ages for constrained and unconstrained nodes.

Node	Penalized likelihood (PL)				Relaxed Bayesian clock			
	Mean age (Ma)	SD (Ma)	Maximum age (Ma)	Minimum age (Ma)	Mean age (Ma)	SD (Ma)	Maximum age (Ma)	Minimum age (Ma)
A (3.26)	3.26	0.00	3.26	3.26	2.4	0.52	3.17	1.23
1	3.13	0.08	3.23	2.78	2.21	0.50	3.03	1.15
2	2.15	0.29	3.02	1.03	1.33	0.41	2.23	0.63
3	1.90	0.28	2.52	1.00	1.08	0.34	1.85	0.50
4	1.19	0.48	2.59	0.14	0.91	0.36	1.70	0.31
5	0.26	0.22	2.27	0.004	0.26	0.19	0.72	0.01
6	0.90	0.29	2.01	0.14	0.47	0.27	1.12	0.06
7	1.51	0.41	2.74	0.0004	0.75	0.30	1.43	0.27
8	1.69	0.31	2.72	0.58	0.88	0.30	1.56	0.39
9	0.25	0.21	1.58	0.004	0.21	0.16	0.61	0.01
10	3.03	0.13	3.23	2.57	2.02	0.49	2.86	1.03
11	1.78	0.28	2.61	0.96	1.15	0.41	2.07	0.46
12	1.23	0.25	1.95	0.51	0.68	0.31	1.41	0.22
13	0.66	0.18	1.42	0.16	0.38	0.19	0.83	0.11
14	0.51	0.16	1.15	0.09	0.27	0.15	0.64	0.06
15	0.37	0.14	0.82	0.03	0.19	0.12	0.50	0.03
16	0.17	0.10	0.49	0.004	0.10	0.09	0.33	0.003
17	0.33	0.14	0.88	0.07	0.28	0.16	0.68	0.07
18	0.29	0.11	0.65	0.05	0.17	0.11	0.46	0.02
19	0.10	0.09	0.59	0.004	0.08	0.08	0.28	0.002
20	0.12	0.08	0.49	0.004	0.08	0.08	0.29	0.002

The first node (A) is assigned a maximum age (indicated in parentheses) as derived from a previous molecular clock study (Guzmán and Vargas, 2009). Numeric codes for each node of the phylogeny of Cistaceae correspond to those shown in Fig. 3. Ma=million years; SD=standard deviation.

number of haplotype lineages (four) was generated in both groups. This result is also compatible with the extinction of ancient haplotypes.

Discussion

Synchronous processes of divergence in Mediterranean and Canarian lineages

All phylogenetic analyses are congruent with a sister group relationship of three mainland (*C. albidus*, *C. creticus*, *C. heterophyllus*) and the five Canarian (*C. chinamadensis*, *C. horrens*, *C. ochreatus*, *C. osbeckiifolius*, *C. symphytifolius*) endemics of purple-flowered *Cistus* (Fig. 3). Levels of diversity in species number (three vs. five) in the mainland and the Canary Islands are comparable with those of haplotype diversity. Despite purple-flowered *Cistus* being across the Mediterranean Basin but more restricted in the Canary Islands, a similar number of inferred haplotypes (eight vs. seven) and the same number of haplotype clades (four) suggest a case of synchronous differentiation (Figs. 1–3).

This Canarian–Mediterranean group displays a moderate differentiation into eight purple-flowered species, which appear to have undergone rapid and recent differentiation as suggested

by the maximum sequence divergences of 1.1% (Table 4). Our divergence time estimates agree with this hypothesis. Once *C. crispus* branched off about the Pliocene–Pleistocene limit (1.78 ± 0.28 Ma) (Fig. 3), the split of the Canarian and mainland lineages took place (1.23 ± 0.25 Ma; Table 5). The question remains as to whether insular species are the result of a faster differentiation process with respect to mainland relatives.

A pattern of faster evolution on the Canary Islands compared with mainland groups has been described on the basis of molecular data assuming a clock-like average constancy of base-pair substitutions (Böhle et al., 1996; Kim et al., 1996; Krupkin et al., 1996; Francisco-Ortega et al., 1997; Baldwin et al., 1998; Francisco-Ortega et al., 1999; Thiv et al., 1999; Caujapé-Castells et al., 2001). The much higher maximum sequence divergence found between mainland species compared with that of their insular relatives has been explained on the basis of the faster speciation rates on islands (Francisco-Ortega et al., 1996). In contrast, our analyses suggest a synchronous process of evolution as illustrated by the similarity of maximum pairwise sequence divergences found in the Canarian and Mediterranean lineages (highest values of 0.21% and 0.27%, respectively) (Table 4). As demonstrated elsewhere, there is no universal clock, and rates of evolution can vary even between closely related lineages (Sanderson, 2002). Although we rejected the hypothesis of rate

constancy among lineages of *Cistus-Halimium* ($\chi^2=175.64$; d.f.=29), a constant rate of molecular evolution has occurred since the split of the mainland and Canary lineages of purple-flowered species (LRStat=0.25; d.f.=5). Given the relatively low number of purple-flowered species in existence (nine), an early stage of radiation is suggested by the estimated ages of 0.66 ± 0.18 Ma in the Mediterranean and 0.33 ± 0.14 Ma in the Canary Islands. This result supports one more aspect of the hypothesis of synchronous differentiation: similar differentiation times of *Cistus* lineages in the evolution of the Mediterranean and Canary floras (Fig. 3).

Palaeoclimatical events coincide with the two simultaneous evolutionary histories of *Cistus* and the two hotspots of plant diversity: the Macaronesian and Mediterranean floristic regions. The divergence of *H. umbellatum*, the purple- and the white-flowered *Cistus* lineages (3.13 ± 0.08 Ma) coincides with the onset (3.2 Ma) and establishment (2.8 Ma) of the Mediterranean climate (Suc et al., 1995). In addition, when the mainland and the Canarian purple-flowered lineages split (node 12 in Table 5), synchronous divergence occurred with the advent of the Pleistocene (< 1.8 Ma), as revealed by the *trnK-matK/trnS-trnG* chronogram (Fig. 3). Interestingly, radiation occurred in mainland lineages of *Cistus* not only in the purple-flowered group but also in the white-flowered lineage (2.15 ± 0.29 Ma; see node 2 of Table 5, Fig. 3). Establishment of the Mediterranean climate may have been crucial to triggering rapid speciation in the mainland. We hypothesize that no significant difference between mainland (Mediterranean) and island (Canarian) differentiations may have also occurred in other plant groups because the two areas conform to two hotspots of rapid differentiation (Myers et al., 2000).

Testing adaptive radiation in Canarian *Cistus*

Adaptive radiation is the evolution of ecological and phenotypic diversity within a rapidly multiplying lineage that may be detected by four evolutionary characteristics: (1) common ancestry, (2) rapid speciation, (3) trait utility and (4) phenotype–environment correlation (Schluter, 2000).

Common ancestry of Canarian *Cistus* is clearly documented from our results. The phylogenetic analysis (Fig. 3) provides strong support for the monophyly of the Canarian *Cistus*, in agreement with previous nuclear and plastid phylogenies (Guzmán and Vargas, 2005). This is consistent with the pattern found in many Macaronesian plant groups (*Echium*, Böhle et al., 1996; *Argyranthemum*, Francisco-Ortega et al., 1996; *Sonchus* alliance, Kim et al., 1996; *Pericallis*, Panero et al., 1999), but not in others (see Vargas, 2007). This implies a single mainland-to-island invasion event in the history of the Canarian purple-flowered *Cistus*.

Relatively rapid speciation in Canarian *Cistus* is interpreted by the number of species (five) and lineages (four) that originated in a short period of time ($330,000 \pm 140,000$ years considering the most conservative estimate). The most remarkable examples of plant radiation in oceanic islands appear to parallel this figure: 25 species of the silversword alliance in 5.5 ± 0.3 Ma (Baldwin and Sanderson, 1998); 28 species of *Echium* generated in 6.56 ± 1.4 Ma (García-Maroto et al., 2009); and 23 species of *Sideritis* in 3.3 ± 2.09 Ma (Kim et al., 2008).

Utility of particular reproductive traits is found in Canarian endemics. Island and mainland populations of plants often differ in their reproductive biology because altered pollination conditions have influenced the floral biology and mating systems of island plants (Sakai et al., 1997; Barrett, 1998b). Continental *Cistus* species are primarily self-sterile (Gard, 1910; Bosch, 1992; Talavera et al., 1993), but Canarian *Cistus* endemics are self-compatible (Warburg and Warburg, 1930). This condition pro-

motes colonization, as only one immigrant is sufficient to establish a new population (Baker, 1955). One additional mechanism promoting outcrossing by separating anthers and stigmas spatially within a flower is herkogamy (Barrett, 1998a). It is remarkable that the only species of *Cistus* with styles longer than stamens are found in the Canary Islands. The common ancestor of the Canarian and the mainland purple-flowered lineages appeared to have a style equal in length to the stamens (based on the hypothesis of character evolution for style length presented in Guzmán and Vargas (2005)). When the ancestor of *Cistus* colonized the Canary Islands, this reproductive feature may have been maintained in the mainland lineage, whereas it shifted in the Canarian lineage. Similarly, a strong trend to separation of flower sex parts has been documented in up to 20% of oceanic insular floras (Sakai et al., 1997).

Phenotype–environment correlations, however, are rarely observed in *Cistus*. Studies of large groups of Macaronesian plants have determined a correlation between phylogeny and ecology, which was used to describe a pattern of diversification via inter-island colonization between similar ecological zones (Francisco-Ortega et al., 1996; Kim et al., 1996; but see Percy and Cronk, 2002). Despite the Canarian *Cistus* occurring in particular habitats (*C. chinamadensis* in laurel forests of Anaga and central areas of La Gomera; *C. horrens* in pine forests of southern Gran Canaria; *C. ochreatus* in pine forests of Tamadaba (Gran Canaria); *C. osbeckiifolius* in high mountain habitats of Tenerife; *C. symphytifolius* in a wide range of habitats of Tenerife and La Palma; see Table 2), the phylogeographical (Fig. 1A) and phylogenetic (Fig. 3) analyses are not congruent with a relationship between lineages and ecology. Indeed, several species inhabiting different habitats share the same haplotype, whilst different haplotypes are found in the same species (Table 1; Fig. 1A). We consider that a limited period of time precluded diversification of Canarian *Cistus*, giving us the opportunity to witness a relatively early stage of radiation.

The facts that *C. symphytifolius* has a widespread distribution (Fig. 1A), shares the greatest haplotype divergence between populations (also within *C. horrens*), forms part of different lineages (Figs. 1A, 3) and displays the highest allozyme diversity (Batista et al., 2001) lead us to hypothesize an early divergence of *C. symphytifolius* (or an ancestor) to bring about new species. If *C. symphytifolius* retained the characteristics of the former species differentiating into the other four species (*C. horrens*, *C. ochreatus*, *C. osbeckiifolius*, *C. chinamadensis*), we would have a case of anacladogenesis given that the progenitor species (*C. symphytifolius*) persists in the same island (Stuessy and Crawford, 1990). In particular, Tenerife may have served as a centre of dispersal for the Canarian *Cistus* species as it is the island with the highest number of haplotypes (five) and species (*C. chinamadensis* subsp. *chinamadensis*, *C. osbeckiifolius*, *C. symphytifolius*) (Fig. 1A). Other studies stressed the historical role of Tenerife as a centre of dispersal in the Canary Islands (Francisco-Ortega et al., 2002; Allan et al., 2004).

In summary, our study group and results do not serve to support previous hypotheses that species diversity drives speciation (Emerson and Kolm, 2005), a window of opportunity for colonization (Carine, 2005), niche pre-emption (Silvertown, 2004) or anagenetic speciation (Stuessy et al., 2006). A single, recent colonization of the Canary Islands by *Cistus* agrees with a general prediction for plant groups with diaspora displaying no special mechanism for long-distance dispersal (Vargas, 2007). The five endemic species and four lineages indicate a rather cladogenetic process, albeit with a low speciation rate, of *Cistus* evolution in the Canarian archipelago (Stuessy and Crawford, 1990). We argue that the time of first colonization of a plant group greatly influences the testing of all these hypotheses (Stuessy 2007). Since as

recently as 500,000 years ago (Late Pleistocene), Canarian *Cistus* appears to have initiated a geographical rather than habitat-dependent radiation. Given that dispersal is a faster process than phenotype–environment differentiation, and that several haplotypes and species are related to particular habitats, the question remains as to whether adaptive radiation could take place in *Cistus* over time.

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