Adaptive Radiation in Mediterranean Cistus (Cistaceae)

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Abstract

Background: Adaptive radiation in Mediterranean plants is poorly understood. The white-flowered Cistus lineage consists of 12 species primarily distributed in Mediterranean habitats and is herein subject to analysis.

Methodology/Principal Findings: We conducted a “total evidence” analysis combining nuclear (ncpGS, ITS) and plastid (trnL-trnF, trnK-matK, trnS-trnG, rbcL) DNA sequences and using MP and BI to test the hypothesis of radiation as suggested by previous phylogenetic results. One of the five well-supported lineages of the Cistus-Halimium complex, the white-flowered Cistus lineage, comprises the highest number of species (12) and is monophyletic. Molecular dating estimates a Mid Pleistocene (1.04±0.25 Ma) diversification of the white-flowered lineage into two groups (C. clusii and C. salviifolius lineages), which display asymmetric characteristics: number of species (2 vs. 10), leaf morphologies (linear vs. linear to ovate), floral characteristics (small, three-sepalled vs. small to large, three- or five-sepalled flowers) and ecological attributes (low-land vs. low-land to mountain environments). A positive phenotype-environment correlation has been detected by historical reconstructions of morphological traits (leaf shape, leaf labdanum content and leaf pubescence). Ecological evidence indicates that modifications of leaf shape and size, coupled with differences in labdanum secretion and pubescence density, appear to be related to success of new species in different Mediterranean habitats.

Conclusions/Significance: The observation that radiation in the Cistus salviifolius lineage has been accompanied by the emergence of divergent leaf traits (such as shape, pubescence and labdanum secretion) in different environments suggests that radiation in the group has been adaptive. Here we argued that the diverse ecological conditions of Mediterranean habitats played a key role in directing the evolution of alternative leaf strategies in this plant group. Key innovation of morphological characteristics is supported by our dated phylogeny, in which a Mediterranean climate establishment (2.8 Ma) predated the adaptive radiation of the white-flowered Cistus.

List of haplotypes found in 16 species and subspecies of the white-flowered Cistus lineage. Variable sites of the sequences of four plastid DNA regions (trnL-trnF, rbcL, trnK-matK, trnS-trnG) are shown. Nucleotide position for each data set is numbered from the 5’ to the 3’ DNA ends.

Introduction

The concept of adaptive radiation implies a rapid ecological diversification, which should be reflected in a greater morphological and/or physiological divergence among species in brief periods of rapid diversification from a single ancestor [1,2]. Two mechanisms could generate adaptive radiations: (1) extrinsic causes due to new environmental circumstances [3,4]; (2) intrinsic characters of organisms [key innovation] that allow a taxon to utilize existing niche space in a novel manner [5]. Remoteness and the rich diversity of habitats of island systems help ensure little competition and different environments to test the potential of plant radiations [6,7]. In contrast to the wealth of studies documenting adaptive radiations in oceanic islands [see 3,8,9] and particular mainland habitats [see 10,11], we have found in literature no study fully focused on the Mediterranean region.

The Mediterranean climatic type, characterized by a strong seasonality (hot dry summers, cool wet winters), occurs in California, South Africa, central Chile, southern Australia, and typically in the Mediterranean Basin [12,13]. In all five of these areas the native vegetation is a dense scrub characterized by annuals, drought-tolerant deciduous and semi-deciduous mala-cophyllous species, and woody evergreen sclerophyllous species [14]. Sclerophyllous species are adapted to low water availability during summer by means of small, leathery and dark leaves covered with thick cuticles and small, thick-walled cells [15]. Small leaves and low specific leaf area have been viewed as adaptations to Mediterranean-type climates in many species of evergreen plants [16]. Indeed, sclerophylls are so successful that unrelated genera and families of woody plants converged into similar leaf traits. Two alternative origins have been proposed for the evolution of Mediterranean, woody plants: resprouters corresponding to older lineages (Tertiary with tropical to subtropical conditions) and seeders (such as Cistus) to younger lineages (Quaternary with Mediterranean conditions) [17]. Few studies have, however, addressed the origin of Mediterranean plant groups by means of phylogenetic approaches related to ecological preferences [but see 18].
Significant shrub components in the European-African Mediterranean ecosystems (e.g., “maquis”, “garrigue”) belong to Cistaceae (Tuberrara, Halimium, Cistus). Cistus is a genus of 21 frutaceous and suffrutaceous shrub species with a predominantly Mediterranean distribution [19], except for five species endemic to the Canary Islands (Table 1). Previous phylogenetic studies revealed the separation of the Cistus-Halimium lineage and identification of two major natural groups: one of purple-flowered Cistus species (hereafter the purple-flowered lineage) and other containing the white-flowered species of Cistus, plus the pinkish-flowered C. parviflorus (hereafter the white-flowered lineage) [20,21,22]. Moreover, the white-flowered lineage is divided in two groups; one containing C. clusi and C. mumbyi species (hereafter the C. clusi group) and other containing the rest of the white-flowered Cistus species (9), plus C. parviflorus (hereafter the C. salviifolius group) (Fig. 1). Despite the two lineages (the C. clusi and the C. salviifolius groups) are inhabiting the Mediterranean basin, the C. salviifolius group has undergone higher differentiation and displays greater variation in leaf trichome density, size, shape and tissue thickness than do the C. clusi group. These properties influence the resistance to drought stress and solar irradiance [14]. Indeed, ecological analyses of leaf morphological and physiological characters in dry environments [23,24] appear to be related to speciation of Mediterranean plants.

In this study, we used a molecular phylogenetic approach of DNA sequence data, sampled from both the nuclear (ITS, ncpGS) and the plastid (trnL-trnF, trnK-matK, trnS-trnG, rbcL) genomes, to test the explicit hypothesis of adaptive radiation. We first explored single ancestry in the Cistus-Halimium complex and differentiation in short periods of time by means of phylogenetic and molecular clock analyses [1,25]. To test evolution in Mediterranean conditions, we chose a lineage exclusive to the Mediterranean basin (C. salviifolius lineage). Phenotype-environment correlation was further conducted to infer the role of ecological and vegetative characteristics [26,27,28] involved in speciation of this group.

### Results

#### Phylogenetic analyses

The characteristics of the six sequence data sets are summarized in Table 2. MP analysis using Finch parsimony resulted in 104 shortest trees of length 1317 steps (Fig. 1) for the combined sequence matrix. The consistency index (CI) for these trees was 0.82 and the retention index (RI) was 0.80. The BI tree displayed similar topology (except for the Halimium umbellatum position) and support values. Plastid and nuclear datasets yielded a similar phylogenetic pattern, although plastid sequences provided a more resolved tree (results not shown). In addition to strong (99% BS, 94 PP) support for the monophyly of the Cistus-Halimium complex, parsimony and Bayesian consensus trees were consistent at different places: (1) Cistus species were not monophyletic; (2) Cistus species were divided in two lineages, one of purple-flowered species (except C. parviflorus) (100% BS, 100 PP) and other of white-flowered species plus C. parviflorus (97% BS, 100 PP); (3) Cistus crispus was the sister-group of the rest of purple-flowered species (100% BS, 100 PP); and (4) a sister-group relationship existed between the C. clusi group (100% BS, 100 PP) and the rest of the white-flowered species plus C. parviflorus (81% BS, 100 PP). Halimium umbellatum appears to be related to the white-flowered lineage in the Bayesian analysis, but not in the MP analysis (Fig. 1).

#### Evaluating patterns of trait evolution

The range of inter-specific variation in leaf morphology and ecological requirements is shown in Table 3. Character reconstruction of three morphological and three ecological characters mapped on the Bayesian consensus tree (Fig. 2) using MacClade optimization and Bayesian inference to investigate patterns of evolution. The most relevant results from the historical reconstructions are following described:

1. **Leaf shape (Fig. 2A).** The character state reconstruction showed linear or linear-lanceolate to elliptic leaves as a plesiomorphic state. Ovate-lanceolate and ovate shapes evolved twice in the C. salviifolius lineage.

2. **Labdanum secretion (Fig. 2B).** The character was equivocal in most of the C. salviifolius lineage because, in part, of missing data from two species (C. mumbyi, C. pouzolzii). A medium percentage (5-10%) of secretion per unit leaf dry weight was, however, traced as the most likely ancestral state.

3. **Upper leaf pubescence (Fig. 2C).** The character was revealed as very homoplastic within the C. salviifolius lineage. Despite the reconstruction was equivocal tracing the state at some nodes, independent acquisition (up to three times) of a denseomentum is interpreted. Shifts between glabrous and subglabrous leaves appeared dynamic.

4. **Soil (Fig. 2D).** The historical reconstruction traced silicolous soils as the ancestral state for the C. salviifolius lineage. It was noteworthy that the only two species inhabiting basic (C. parviflorus) and ultrabasic (C. albanicus) soils within this lineage are sister species.

5. **Insolation conditions (Fig. 2E).** Character optimization was equivocal reconstructing the ancestral state in the C. salviifolius lineage. Two sister species groups underwent a dramatic change in insolation conditions (C. parviflorus-C. albanicus; C. populifolius-C. pouzolzii). Although ancestral character states were poorly optimised for insolation conditions, reversal to high solar exposure (helioxerophyllous) was unequivocally acquired for C. parviflorus.

6. **Environment (Fig. 2F).** A high frequency in habitat change was found in the C. salviifolius lineage. Similar environments were shared in a few groups with (C. pouzolzii, C. populifolius) or without (C. psilosepalus, C. parviflorus) sister relationships. In contrast, three habitats were occupied by four closely-related species (C. laurifolius, C. psilosepalus, C. parviflorus, C. albanicus) suggesting a dynamic habitat change in the course of evolution of the C. salviifolius lineage.

The BayesTraits analysis of trait evolution was used to test reconstruction uncertainty. Table S2 reports ratedev settings and mean values (±95% confidence intervals) of the log-likelihood and posterior distributions of the rate of coefficients obtained from the reversible jump (RJ) MCMC analysis. The mean of the Bayesian posterior probabilities of each character state at every node (nine nodes) are provided in Table 4 and Fig. 2. The 95% confidence intervals of the posterior probabilities were all lower than ±0.004. The Bayesian results mostly supported the MP (MacClade) optimization. Particular points of disagreement between both analyses were: (1) subglabre leaves at the root of the tree in the MP analysis whereas the Bayesian probability (0.52) was higher for glabre leaves; (2) ancestral states at node 2 were reconstructed as linear and subglabre leaves in the MP analysis, while the Bayesian approach estimated a higher probability for linear-lanceolate (0.68) and glabre leaves (0.68) states to be ancestral; (3) the historical reconstruction using the MP optimization traced Quercus suber/ilex and Pinus woodlands as the ancestral state at node 4 (C. bidanif- C. salviifolius), while Quercus suber/ilex woodlands showed the highest posterior probability (0.38); (4) subhelioxerophyly condition was ancestral at node 5 in the MP optimization but
Table 1. List of species used in the phylogenetic analysis.

| Taxon | Distribution | Locality/source | Voucher |
|-------|--------------|-----------------|---------|
| Cistus L. | | | |
| Cistus albanicus E.F. Warb. ex Heywood | Albania, Greece | Cultivated | R. G. Page 8cBGA04 (MA) |
| Cistus albidus L | Iberia, S France, N Italy, N Africa, Corsica, Sardigna | Spain, Madrid, Aldea del Fresno | P. Vargas 25PV03 (MA) |
| Cistus chinamadensis Bañares et Romero | La Gomera, Tenerife (Canary Islands) | Canary Islands, La Gomera | Á. Fernández & J. Leralta 44BGA04 (MA) |
| Cistus clusii Dunal subsp. clusii | Spain, Italia, N Africa, Sicily | Spain, Málaga, Mijas | C. Navarro et al. (MA61671) |
| Cistus clusii Dunal subsp. multiflorus Demoly | Balea Island, SE Iberia Peninsula | Spain, Balear Islands, Mallorca, Sa Rápita | P. Vargas 209PV04 (MA) |
| Cistus creticus L. | Mediterranean Basin | Greece, Olympus | B. Guzmán 58BGA04 (MA) |
| Cistus crispus L. | Iberia, S France, N Italy, N Africa, Corsica, Sicily | Spain, Córdoba, Posadas | B. Guzmán 99BGA04 (MA) |
| Cistus heterophyllus Desf. | SE Spain, N Africa | Morocco, Beni-Hadifa | B. Guzmán 28BGA04 (MA) |
| Cistus horrens Demoly | Gran Canaria (Canary Islands) | Canary Islands, Gran Canaria, Ayacata | B. Guzmán 109BGA04 (MA) |
| Cistus ladanifer L. subsp. africanus | S Spain, N Africa | Morocco, Targuis | B. Guzmán 78BGA03 (MA) |
| Cistus ladanifer L. subsp. ladanifer | S France, Iberia, N Africa, Cyprus | Spain, Madrid, Boadilla del Monte | B. Guzmán 29BGA04 (MA) |
| Cistus ladanifer L. subsp. sulcatus | S Portugal | Portugal, Sagres | B. Guzmán 13BGA04 (MA) |
| Cistus laurifolius L. | N Africa, Iberia, France, Italy, Corsica, Turkey | Spain, Jaén, Sierra de Segura | R. G. Page 149BGA04 (MA) |
| Cistus libanotis L. | Portugal, S Spain, Argelia | Spain, Córdoba | B. Guzmán 35BGA04 (MA) |
| Cistus monspeliensis L. | Mediterranean Basin, Canary Islands | Portugal, Sagres | O. Filippi 4BGA04 (MA) |
| Cistus munbyi Pomel | Algeria, Morocco | Morocco | R. G. Page 8BGA04 (MA) |
| Cistus ochreatus C. Sm. ex Buch | Gran Canaria (Canary Islands) | Canary Islands, Gran Canaria | P. Escobar 48/05 (MA) |
| Cistus osbeckiifolius Webb ex Christ | Tenerife (Canary Islands) | Canary Islands, Tenerife | O. Filippi 68BGA04 (MA) |
| Cistus parviflorus L. subsp. major (Dunal) Heywood | Iberia, N Morocco | Portugal, Ourique | P. Vargas 5PV03 (MA) |
| Cistus populinus L. subsp. populifolius | N Africa, S France | Spain, Ávila, Arenas de San Pedro | R. G. Page 8BGA04 (MA) |
| Cistus pouzolzii Delile | Algeria, N Morocco, France | France | P. Vargas 7PV03 (MA) |
| Cistus psilosepalus Sweet | Iberia, France | Spain, Ávila, Arenas de San Pedro | P. Vargas 6PV03 (MA) |
| Cistus symphytifolius L. | Mediterranean Basin | Spain, Ávila, Arenas de San Pedro | B. Guzmán 143BGA04 (MA) |
| Cistus laurifolius L. | Canary Islands, Gran Canaria, Ayacata | | |
| Cistus ladanifer L. subsp. africanus | | | |
| Cistus ladanifer L. subsp. ladanifer | | | |
| Cistus ladanifer L. subsp. sulcatus | | | |
| Cistus laurifolius L. | | | |
| Cistus libanotis L. | | | |
| Cistus monspeliensis L. | | | |
| Cistus munbyi Pomel | | | |
| Cistus ochreatus C. Sm. ex Buch | | | |
| Cistus osbeckiifolius Webb ex Christ | | | |
| Cistus parviflorus Lam. | | | |
| Cistus populinus L. subsp. major (Dunal) Heywood | | | |
| Cistus pouzolzii Delile | | | |
| Cistus psilosepalus Sweet | | | |
| Cistus symphytifolius L. | | | |

Fumana (Dunal) Spach

Fumana thymifolia (L.) Spach ex Webb | Mediterranean Basin | Portugal, Ferrierias | B. Guzmán 53BGA04 (MA) |

Halimium (Dunal) Spach

Halimium atlanticum Humbert & Maire | N Africa | Morocco, Tazzeke | RDG14/2006/5 |
| Halimium atriplicifolium (Lam.) Spach | Spain, N Morocco | Spain, Granada, Sierra Nevada | P. Vargas 120PV04 (MA) |
| Halimium calycinum (L.) K. Koch | Iberia, NW Morocco | Portugal, Cabo Sardão | B. Guzmán 49BGA04 (MA) |
| Halimium halimifolium (L.) Willk. halimifolium | Iberia, Morocco | Spain, Málaga, Marbella | A. Segura (MA580185) |
| Halimium lasianthum (Lam.) Spach lasianthum | SW Iberia, N Morocco | Spain, Málaga | P. Vargas 3PV06 |
| Halimium lasiocyclus (Boiss. & Reut.) Gross ex Engl. subsp. rhipheum (Pau & Font Quer) Maire | N Africa | Morocco, Bab-Berred | P. Escobar 665/04 (MA) |
| Halimium ocyoides (Lam.) Willk. | Iberia Peninsula, N Morocco | Portugal, Coimbra | R. G. Page 158BGA04 (MA) |
| Halimium umbellatum (L.) Spach | Mediterranean Basin | Spain, Madrid, Tres Cantos | P. Vargas 71BGA04 (MA) |

Helianthemum Mill.

Helianthemum squarnatum (L.) Dum. Cours. | Iberia, N Africa | Cultivated | B. Guzmán 70BGA04 (MA) |

Tuberaria Dunal

Tuberaria guttata (L.) Fourn. | W Europe, Mediterranean Basin, Canary Islands | Portugal, Vila do Vispo | B. Guzmán 44BGA04 (MA) |

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the submesophyllous condition displayed the highest posterior probability (0.70); (5) scrub vegetation was the ancestral state at node 9 (C. parviflorus-C. albanicus) using the MacClade optimization, while Abies cephalonica woodlands displayed the highest posterior probability (0.42).

Bayesian analysis of correlated evolution

Table 5 shows the log-Bayes factor calculations and significance following the scale of Bayes factor test presented by Kass & Raftery [29]. The evolution of leaf traits was not closely associated with ancestral changes in environment and insolation conditions. There

Table 2. Characteristics of each of the DNA sequence regions used in the phylogenetic analysis of Cistaceae and the white-flowered Cistus.

|                  | trnS-trnG | trnL-trnF | trnK-matK | rbcL | ITS  | ncpGS |
|------------------|-----------|-----------|-----------|------|------|-------|
| **Cistaceae**    |           |           |           |      |      |       |
| **Length (bp)**  |           |           |           |      |      |       |
| Total aligned length | 1084     | 516       | 1403      | 1404 | 697  | 402   |
| Length range - ingroup | 617–824  | 399–461   | 1302–1357 | 1403–1404 | 644–650 | 340–452 |
| Length range - outgroup | 158–684  | 377–422   | 1301–1316 | 1404 | 585–654 | 318   |
| **Number of characters** |         |           |           |      |      |       |
| Total included   | 713       | 516       | 1403      | 1379 | 697  | 402   |
| Variable/parsimony-informative | 148/54   | 128/52    | 280/108   | 103/44 | 203–69 | 86/17 |
| Mean G+C content | 21%       | 33%       | 33%       | 43%  | 65%  | 40%   |
| Maximum sequence divergence (GTR) | 17.92%   | 14.1%     | 14.08%    | 4.11% | 20.37% | 35.4%  |
| Sequence evolution model (Akaike Test) | GTR+G     | GTR+G     | GTR+G     | GTR+I | GTR+H+G | HKY+G |
| **White-flowered Cistus plus C. parviflorus** |   |           |           |      |      |       |
| No. of variable/parsimony-informative characters | 45/25     | 28/11     | 33/12     | 20/10 | 75/33 | 25/8  |
| Maximum sequence divergence (GTR) | 1.90%     | 3.15%     | 0.85%     | 0.74% | 4.21% | 3.11% |
| Sequence evolution model (Akaike Test) | GTR+H     | F81+H     | GTR       | HKY  | HKY+H+G | HKY+G |
Bayes factor = \text{leaf shape} (\log\text{-Bayes factor} = 2) was evidence against a correlated evolution of insolation conditions to leaf pubescence and environment [30], estimates of barely correlated evolution have a strong evidence against correlated evolution of insolation conditions to leaf pubescence and environment

was evidence against a correlated evolution of insolation conditions to leaf shape (log-Bayes factor = −1.5) and labdanum secretion (log-Bayes factor = −1.8). Additionally, barely evidence against correlated evolution for leaf pubescence and environment has been found (log-Bayes factor = −0.1). In contrast, barely correlated evolution was suggested between three pairs of variables: leaf shape/environment (log-Bayes factor = 0.7), labdanum secretion/environment (log-Bayes factor = 0.8) and leaf pubescence/insolation conditions (log-Bayes factor = 0.6). As already discussed elsewhere for organism radiations [30], estimates of barely correlated evolution have a strong evolutionary significance considering short tree branches.

Haplotype analysis of the white-flowered Cistus lineage

Sequence length of the white-flowered Cistus lineage was 417–461 bp for \text{tnrS-trnG}, 561–585 for \text{trnL-trnF}, 1309–1357 for \text{trnK-matK} and 1378–1379 for \text{rbcL} (Table 2). The combined data of plastid sequences for 10 species (13 taxa) of the C. \text{salicifolius} lineage distinguished only 12 substitution-based haplotypes (Table S3). Haplotypes were exclusive to a single species or subspecies (Table S3), except for one for both C. \text{ladanifer} subspp. \text{ladanifer} and \text{sulcatus}. TCS constructed a single, star-like network (Fig. 3) displaying no loops. This analysis is congruent with a multiple lineage divergence pattern from ancestral haplotypes, as expected in a radiation.

### Table 3. Morphological and environmental characteristics of the white-flowered Cistus lineage. Data were taken from Grosser [75], Martin & Guinea [76], Dansereau [77], Warburg [78], Demoly and Montserrat [79], Greuter [80], Gülz et al. [49]** and own observations.

| Soil | Climate conditions | Altitude (m) | Insolation conditions*, environment | Leaf shape (length × width in mm) |
|------|-------------------|--------------|-------------------------------------|----------------------------------|
| C. albanicus | serpentes mesic, Mediterranean mountain | 1000–1500 | submesophyllous, Abies cephalonica woodlands | elliptic (3–5 × 0.8–1.5) |
| C. clusi | calcicolo dry to semi-arid, Mediterranean coast | 0–1500 | heliophyllous, bushy vegetation | linear (10–25 × 1–2) |
| C. ladanifer | silicous dry, Mediterranean | (0) 300–1000 (1500) | subheliophyllous, degraded Quercus suber/ilex woodlands | linear-lanceolate (40–80 × 6–21) |
| C. laurifolius | silicous, mesic, Mediterranean mountain | 1900 (400–2800) | submesophyllous, degraded Q. pyrenaica/ligustica and Pinus woodland | ovate-lanceolate (40–90 × 17–30) |
| C. libanotis | silicous, sandy dry, Mediterranean coast | 0–500 (1200) | subsciophyllous, degraded Pinus halepensis/ pinea and Quercus suber woodlands | linear (22–40 × 2–5) |
| C. monspeliensis | silicous, dry, Mediterranean | 0–800 (1200) | subheliophyllous, degraded Quercus suber/ilex and Pinus woodlands | linear-lanceolate (15–45 × 2–7) |
| C. munbi | calcicolo Mediterranean coast | 0–100 | heliophyllous, degraded Quercus suber/ilex and Pinus woodlands | linear (6–30 × 1–4) |
| C. parvifolius | calcicolo dry, Mediterranean coast | 0–600 | heliophyllous, scrub vegetation | ovate (15–30 × 7–27) |
| C. populifolius | silicous, dry, Mediterranean | 200–1500 | submesophyllous, degraded Quercus suber and Pinus woodlands | ovate-lanceolate (50–95 × 25–55) |
| C. pouzolzi | silicous, dry, mountain Mediterranean | 800–1800 | subheliophyllous, degraded Quercus suber/ilex and Pinus woodlands | lanceolate-elliptic (20–31 × 4–11) |
| C. psilosepalus | silicous, humid, woodlands of Atlantic influence | 0–800 (1100) | submesophyllous, scrub vegetation | lanceolate-elliptic (30–65 × 10–23) |
| C. salviifolius | silicous, Mediterranean and Eurosiberian regions | 0–1800 | subheliophyllous/submesophyllous, degraded woodlands of many types | ovate (8–18 × 7–12) |

| Leaf margin | Leaf venation | Leaf surface and texture | Labdanum secretion** | Leaf non-secretarial trichomes** |
|-------------|--------------|--------------------------|---------------------|--------------------------------|
| Upper surface | Lower surface |                           |                     |                                |
| C. albamicus | flat | reticulate | smooth, soft | 1.0 | long, single, stellate | glabre |
| C. clusi | revolute | uni-nerve | smooth, coriaceous | 6.0 | subglabre with tuft of single hairs | dense tomentum of stellate |
| C. ladanifer | flat | Pinnate | smooth, coriaceous | 12.5 | glabre | dense tomentum of stellate |
| C. laurifolius | slightly crispate | Parallel | smooth, coriaceous | 13.5 | glabre | dense tomentum of single and stellate (deciduous) |
| C. libanotis | revolute | uni-nerve | smooth, coriaceous | 6.1 | subglabre, stellate | dense tomentum of stellate |
| C. monspeliensis | flat, slightly revolute | Parallel | smooth, coriaceous | 10.7 | subglabre, stellate | dense tomentum of minute stellate |
| C. munbi | revolute | uni-nerve | smooth, coriaceous | – | subglabre | dense tomentum of stellate |
| C. parvifolius | flat | Parallel | smooth, coriaceous | 1.2 | dense tomentum, stellate | dense tomentum of stellate |
| C. populifolius | flat | Pinnate | smooth, coriaceous | 5.6 | glabre | glabre |
| C. pouzolzi | crispate | Parallel | rough, coriaceous | – | dense tomentum, single and stellate | dense tomentum of single and stellate |
| C. psilosepalus | flat | reticulate | smooth, soft | 2.0 | subglabre, stellate | stellate |
| C. salviifolius | slightly crispate | Pinnate | rough, coriaceous | 0.5 | stellate | stellate |

Note: 1 values from 16 leaves. 2% per unit leaf dry weight.

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Estimates of divergence times

Results of the dating analysis are shown in Table 6 and Fig. 4. In general, the data indicated a Pliocene-Pleistocene (2.11 ± 0.87 Ma) divergence between the basal-most Halimium and Cistus-Halimium groups, followed by a Pleistocene differentiation of the major clades of the latter group. An ancestor shared by Halimium umbellatum and Cistus appeared to have diverged after the Pliocene-Pleistocene boundary (1.47 ± 0.35 Ma). Short branch lengths may reflect a rapid divergence process in the white-flowered lineage. An early divergent lineage of C. clusii and C. mumbyi (C. clusii lineage) at 1.04 ± 0.25 Ma was followed by differentiation of 10 species (C. salviifolius lineage) in the Mid Pleistocene (0.88 ± 0.22 Ma). The average per-lineage species diversification rate for the C. salviifolius lineage was 1.46–2.44 species per million years.

Discussion

An adaptive radiation comprises a group of species that inhabit a variety of environments, differ in morphological and other traits important in utilizing these environments, and are descended from a common ancestor that rapidly speciated over a short period of time [1]. Available phylogenetic and ecological evidence suggests
that the \textit{C. salviifolius} lineage of 10 white-flowered species meets the four criteria to strictly test adaptive radiation: common ancestry, rapid speciation, phenotype-environment relationships and trait utility [1,4].

Monophyly of the white-flowered \textit{Cistus} lineage is strongly supported irrespective of phylogenetic methods and DNA sequences used (Fig. 1). Accordingly, the 12 white-flowered species form a well-defined natural group and fulfill the common ancestry condition.

The concept of rapid speciation is not very well defined, even though a considerable number of species is needed [1]. Asymmetry between sister clades in their number of descendant species is one of the operational standards to distinguish speciation bursts from stochastic background rates [31]. Compared with the two taxa included in the \textit{C. clusi} lineage, the remaining taxa (13) form a sister group (\textit{C. salviifolius} lineage) and can be considered as a significant burst. In fact, asymmetries between both lineages can also be inferred in a temporal pattern. After the split of the most common recent ancestor of the two lineages (1.04±0.25 Ma), a relatively long period of time was necessary to bring about limited (2) extant species in \textit{C. clusi} lineage, in contrast to the 10 species generated in the \textit{C. salviifolius} group (Fig. 4). Alternatively, rapid radiation is also interpreted as high rates of differentiation in comparison to those of flowering plants. The estimated rate of diversification in the \textit{C. salviifolius} lineage was significantly higher (1.46–2.44 species per million years) compared to the median rate of diversification of angiosperm families (0.12 species per million years), with a maximum of 0.39) [32] and to that found in the Andean Valeraniaceae [33], and similar to the explosive radiation described for Andean \textit{Lupinus} [34]. Rapid diversification in the \textit{C. salviifolius} lineage was already predicted by a combination of different sources of evidence prior to performing explicit analysis of radiation: (1) lack of resolution and low support values depicted mainly in the parsimony-based tree because of a low number of

### Table 4. Mean of posterior probabilities of Bayesian inference character state evolution of successive iterations (9,000,000) by RJ MCMC (see text) for six characters.

| Character                  | Root         | Node 1       | Node 2       | Node 3       | Node 4       | Node 5       | Node 6       | Node 7       | Node 8       | Node 9       |
|----------------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Leaf shape                 | 0.42/0.42/0.08/0.07 | 0.23/0.49/0.28 | 0.52/0.36/0.12 | 0.77/0.17/0.06 | 0.32/0.31/0.16/0.21 | 0.21/0.10/0.22/0.14/0.11/0.13/0.09 |
| Labdanum secretion         | 0.09/0.00/0.01/0.00 | 0.05/0.89/0.06 | 0.01/0.98/0.01 | 0.00/0.99/0.01 | 0.99/0.00/0.00/0.00 | 0.96/0.01/0.01/0.01/0.01/0.00/0.00 |
| Leaf pubescence            | 0.18/0.58/0.07/0.07 | 0.30/0.34/0.36 | 0.68/0.19/0.13 | 0.98/0.01/0.01 | 0.05/0.45/0.17/0.33 | 0.06/0.09/0.33/0.18/0.12/0.14/0.08 |
| Insolation conditions      | 0.03/0.86/0.06/0.05 | 0.25/0.22/0.43 | 0.75/0.10/0.15 | 0.98/0.01/0.01 | 0.02/0.51/0.02/0.45 | 0.06/0.09/0.40/0.19/0.12/0.06/0.08 |
| Environment                | 0.08/0.60/0.09/0.23 | 0.32/0.14/0.54 | 0.66/0.09/0.25 | 0.89/0.07/0.04 | 0.06/0.76/0.06/0.12 | 0.09/0.09/0.13/0.38/0.13/0.09/0.09 |

The 95% confidence intervals of the posterior probabilities were all less than 0.004. In bold character state evolution as traced in MacClade optimization (Fig. 2). Particular points of disagreement between Bayesian and the MacClade optimization are underlined. Node codes as in Fig. 2A.

Note: Values in the table reflect estimates based on the averaging over 1000 Bayesian trees.

- Leaf shape: linear/linear-lanceolate to elliptic/ovate-lanceolate/ovate.
- Labdanum secretion: 0–5/5–10/10–15% per unit leaf dry weight.
- Leaf pubescence: glabre/subglabre/dense tomentum.
- Soil: silicolous/calcicolous/serpentin.
- Insolation conditions: helioxerophyllous/subheliophyllous/subsciophyllous/submesophyllous.

### Table 5. Calculations for log-Bayes factor tests in favour of a dependent model. In the final column, we followed the Bayes factor test [29] in our interpretation of the log-Bayes factor.

| Character                  | Log-harmonic mean | log-Bayes factor | Significance |
|----------------------------|-------------------|-----------------|-------------|
| Leaf shape/environment     | 0.18              | 0.01            | barely in favour |
| Leaf shape/insolation      | 0.00              | −0.08           | against     |
| Labdanum secretion/insolation | 0.14             | 0.21            | barely in favour |
| Leaf pubescence/insolation | 0.11              | 0.23            | barely in favour |

Note: Mean calculated from 9,000,000 iterations values.

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polymorphisms [20], which is overcome by increasing the number of DNA substitutions (this paper); (2) short branch lengths and low pairwise sequence divergence (Fig. 4, Table 2); (3) low resolution at the core of the haplotype network [35] (Fig. 3).

In addition to evidence for common ancestry and rapid diversification, the fit of the diverse phenotypes observed in a lineage with their environment is necessary in prediction of adaptive radiations [1]. Our character reconstruction suggests that shifts in leaf features allowing the colonization of different habitats have been related with specific speciation events (Fig. 2). Acquisition of diverse leaf features is associated with recent lineage splits, and thus closely related taxa exhibit different leaf morphologies [26]. Our character state optimization reveals that the most common recent ancestors of four sister species diversified in different environmental conditions (Fig. 2D, 2E, 2F) by means of shifts in leaf shape (Fig. 2A) and leaf pubescence (Fig. 2C). In addition, transition in leaf labdanum secretion is observed in the Bayesian inference (Table 4; Fig. 2B). Trends of correlated evolution between leaf traits and at least one ecological trait (environment, insolation conditions) have been found (Table 5).

The barely correlated evolution found in three morphological/ecological traits indicates that shifts in environmental conditions must parallel evolutionary changes in Cistus leaf morphology as a whole and not in individual leaf features. Experimental studies testing correlated evolution of all leaf traits should be further performed to analyse compensatory effects (trade-off). Alternatively to sister species approaches, another strong indication of the adaptive value of a trait is when phylogenetically separate, but ecologically similar, species converge or show parallel patterns of variation along similar ecological gradients [36]. Multiple leaf morphological character-states studied across the white-flowered lineage (shape, labdanum secretion, pubescence) have been independently acquired at least twice (Fig. 2).

Evidence that some morphological and/or physiological traits of species are particularly useful is the fourth necessary condition to support the most strict concept of adaptive radiation: trait utility [1]. The adaptive implications of leaf size and shape differences are well documented [37,38]. In absence of explicit experimental studies (plant translocation, common garden conditions) for all species involved in this adaptive radiation [39], the body of knowledge for particular traits is analysed. Although our six DNA sequence data set rendered certain phylogenetic uncertainty for some sister species relationships because of moderate support (Fig. 1), the most plausible hypothesis allows assessing low character-state reconstruction uncertainty of leaf morphological utility using MacClade optimization and BayesTraits analysis of trait evolution. Leaf size and shape are implicated in important aspects as thermoregulation [40,41], efficiency of water use [23,42], photosynthetic potential [43], branching and rooting strategies [44], among others. Moreover, comparative studies have revealed the existence of well-marked ecological and leaf morphological trends [45]. Small leaf size (specifically narrow leaves) are generally favoured under high exposure and/or low water availability as they help to maintain favourable leaf temperature and improves water use efficiency [42,44]. Indeed, small-leaved species are concentrated at the high exposure end on south-facing slopes in Mediterranean garrigue and Californian chaparral [46]. Although our character reconstruction hypothesis indicates dynamic shifts of leaf shapes, the ancestral state (narrow leaves) appears to have evolved early into linear-lanceolate to elliptic, and then into ovate (plus ovate-lanceolate) leaves independently four times. In fact, helioxerophyllous species (C.
Table 6. Penalized Likelihood (bootstrapping of 100 trees) molecular clock estimates of ages for constrained and unconstrained nodes.

| Node | Mean age (Ma) | SD (Ma) | Maximum age (Ma) | Minimum age (Ma) |
|------|---------------|---------|------------------|------------------|
| A (11) | 9.65          | 2.21    | 11.00            | 0.58             |
| B (5.3) | 4.87          | 1.10    | 5.30             | 0.21             |
| 1      | 2.11          | 0.87    | 4.93             | 0.14             |
| 2      | 1.01          | 0.31    | 1.99             | 0.06             |
| 3      | 1.78          | 0.45    | 2.74             | 0.12             |
| 4      | 1.25          | 0.32    | 1.80             | 0.07             |
| 5      | 0.53          | 0.15    | 0.83             | 0.03             |
| 6      | 0.30          | 0.09    | 0.49             | 0.02             |
| 7      | 0.15          | 0.06    | 0.33             | 0.006            |
| 8      | 1.56          | 0.38    | 2.32             | 0.09             |
| 9      | 0.80          | 0.21    | 1.17             | 0.05             |
| 10     | 0.52          | 0.14    | 0.78             | 0.03             |
| 11     | 0.19          | 0.07    | 0.33             | 0.01             |
| 12     | 0.04          | 0.02    | 0.13             | 0.002            |
| 13     | 0.05          | 0.06    | 0.21             | 0.000            |
| 14     | 0.04          | 0.02    | 0.13             | 0.003            |
| 15     | 1.47          | 0.35    | 2.09             | 0.08             |
| 16     | 1.04          | 0.25    | 1.41             | 0.06             |
| 17     | 0.23          | 0.09    | 0.43             | 0.01             |
| 18     | 0.09          | 0.04    | 0.20             | 0.003            |
| 19     | 0.88          | 0.22    | 1.22             | 0.06             |
| 20     | 0.82          | 0.20    | 1.13             | 0.05             |
| 21     | 0.72          | 0.18    | 0.97             | 0.04             |
| 22     | 0.17          | 0.06    | 0.34             | 0.009            |
| 23     | 0.04          | 0.02    | 0.11             | 0.0003           |
| 24     | 0.65          | 0.16    | 0.89             | 0.04             |
| 25     | 0.45          | 0.12    | 0.71             | 0.02             |
| 26     | 0.06          | 0.04    | 0.21             | 0.002            |
| 27     | 0.61          | 0.23    | 0.91             | 0.00             |
| 28     | 0.31          | 0.23    | 0.67             | 0.00             |
| 29     | 0.28          | 0.28    | 0.71             | 0.00             |

Nodes A and B are assigned a maximum age (indicate in parentheses) as derived from palynological studies [66,67]. Letters and numeric codes for each node of the phylogeny of Cistaceae correspond to those shown in Fig. 4. Ma = million years ago; SD = Standard deviation.

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clusii, C. mumbyi, C. libanotis) show ancestral linear revolute leaves while submesophyllous species with broad, flat leaves inhabit shadier environments (C. laurifolius, C. populifolius, C. psilosepalus) (Table 3, Fig. 2). Leaf shape is not, however, the only phenotypic trait associated with adaptation to dry conditions. Leaf pubescence is reported to be an adaptation to sunnier and hotter environments by reducing transpiration, increasing the probability of water uptake by leaves, maintaining favourable leaf temperature, and protecting against UV-B radiation responsible for photosynthetic inhibition [47,48]. Accordingly, a combination of leaf traits appears to constrain species of Cistus. Sister species within the white-flowered Cistus lineage have different leaf traits related to xeric environments. The ovate leaves of C. parviflorus are unsuitable for xeric environments are protected by a dense tomentum of stellate hairs. The same is true at a lower extent in C. salviifolius. In addition, leaves can be highly reflective in the visible spectrum by covering the upper surface with labdanum, and then decreasing transpiration [49]. The high leaf secretion of resins (labdanum) in the linear-lanceolate leaves of C. monspeliensis and C. ladanifer may confer a trade-off compared to the narrower leaves of C. clusii, C. mumbyi, and C. libanotis, which display linear leaves and lower labdanum concentration (Fig. 2). Further studies are needed to pinpoint whether combination of multiple leaf strategies are equally fit in dry, Mediterranean habitats suffering from dry hot summers and high solar radiation.

In summary, the evolutionary history of the 10 species (13 taxa) of the C. salviifolius lineage fits into utilization of the niche space in a novel manner far after the Mediterranean climate establishment [50]. A Mediterranean Cistus ancestor with linear, medium labdanum content and glabrous or subglabrous leaves may have spawned new lines of evolution exploiting six pre-existing Mediterranean habitats. Multiple leaf strategies were successfully essayed in the course of speciation to occupy particular environments and become part of the dominant element in the Mediterranean scrub. As far as we know, this is the first documented plant group involved in an adaptive radiation process in the Mediterranean region.

Materials and Methods

Sample strategy and DNA sequencing

A total of 36 individuals representing the 21 species of Cistus, one of Fumana, eight of Halimium, one of Helianthemum and one of Tubera was sequenced for four plastid (trnL-trnF spacer, trnS-trnG spacer, trnK-matK spacer, rbcL exon) and two nuclear (ITS, ncpGS) DNA regions (Table 1; Table S1) to perform phylogenetic analyses and estimate divergence times of Cistus and related lineages. In addition, a data set comprising only the white-flowered Cistus species (plus C. parviflorus) was used to infer character evolution, correlated evolution and haplotype analyses.

Standard primers were used for amplification of the ITS region [51 for 17SE,52 for ITS4], the trnL-UAA-trnF(GAA) [53], the trnK-matK [trnK-3914F and matK-1470R, 54] and the trnS-GCU-trnG (UGC) [55] spacers. The rbcL exon was amplified in two overlapping segments using the following primer combination: 1F-724R and 1229F-1460R [56]. A portion of the glutamine synthetase (ncpGS) was amplified for the first time in 11 Cistus species with the universal primers Gscp687f and Gscp856r [57]. To ensure a homogeneous amplification reaction we design two 24-nucleotide-long primers specific for amplifying and sequencing Cistus species (CIS-687F 5’GTAGCTGGAATCAACATCAGTG3’, CIS-856r: 5’GCTTGTTCAGTATTCTGTCG3’). After 1–3 min pretreatment at 94°C, PCR conditions for amplification were: 24—39 cycles of 1 min at 94°C, 30 s-1 min at 50–55°C and 1–4 min at 72°C (for details see 19). PCR primers were used for cycle sequencing of the spacers, the rbcL exon and the ncpGS gene while the ITS 5 and ITS 4 [52] primers were used for cycle sequencing the ITS region. Additionally, due to mononucleotide repeat stretches (poly-T, poly-A) the internal primer trnS-GpolTf: 5’TTAGATTCTATTTACATTCT3’ was used to sequence the trnS-trnG spacer in the purple-flowered species. Sequenced data were assembled and edited using the program Seqed (Applied Biosystems, California). The limits of the regions were determined by position of flanking primers. IUPAC symbols were used to represent nucleotide ambiguities.

Molecular analyses

Phylogenetic analyses. Maximum Parsimony (MP) and Bayesian Inference (BI) analyses were performed on a combined
Figure 4. Phylogenetic chronogram of the *Cistus-Halimium* complex based on the Bayesian consensus tree. Fossil calibration points are indicated on the tree. Shaded area delineates the establishment of the Mediterranean climate 2.8 million years ago [50]. Geological timescales are shown both at the top and the bottom. Photographs illustrate diversity in leaf morphology of the white-flowered *Cistus* species (only subsp. *ladanifer* of *C. ladanifer*, subsp. *clusii* of *C. clusii* and subsp. *populifolius* of *C. populifolius* are illustrated). Species insolation conditions [77] are plotted on the right side of the tree (○, heliophyllum and subheliophyllum; ▲, subsciophyllum and submesophyllum).

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molecular data set of \text{trnL-trnF}, \text{trnS-trnG}, \text{trnK-matK}, \text{rbcL}, \text{ITS} and \text{ncp60} sequences. Sequences were aligned using Clustal X 1.82b [50], with further adjustments by visual inspection. All parsimony analyses were conducted using Fitch parsimony [59] with equal weighting of all characters and of transitions/transversions. Heuristic searches were replicated 1000 times with random taxon-addition sequences, tree-bisection-reconnection (TBR) branch swapping, the options MulTrees and Steepest Descent in effect and holding 10 trees per replicate. Internal support was assessed using 100,000 bootstrap (BS) replicates [fast stepwise-addition, 60].

To determine the simplest model of sequence evolution that best fits the sequence data, the Hierarchical Likelihood Ratio Test (hLRRT) and Akaike Information Criterion (AIC) were implemented using MrModeltest 1.1b [61,62] in each data set. A Bayesian Inference analysis (BI) was conducted in MrBayes 3.0b4 [63] using two identical searches with two million generations each (four MCMC, chain temperature = 0.2; sample frequency = 100). In both runs probabilities converged at the same stable value after generation 100,000 approximately. 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.

**Molecular dating and diversification rates.** Divergence dates were estimated for nodes of the Bayesian consensus tree. To check the constancy of substitution rates we used the Langley and Fitch (LF) test [64]. We rejected the null hypothesis of constant rate (\(\chi^2 = 5204.26; \text{d.f.} = 54\)) and, then, divergence times were estimated using the r8S 1.71 program [65] with a Penalized Likelihood (PL) approach. Penalized Likelihood 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 to estimate the rate smoothing parameter (measure of the rate variation and autocorrelation of rates from clade to clade). The rate smoothing with the lowest crossvalidation score was selected and the dating procedure was repeated with the following parameters: collapse; num_time_guesses = 5 and num_restarts = 5. Crossvalidation suggested 10 as the best smooth parameter. Branching order and branch lengths from 100 Bayesian trees sampled every 10,000 generations after stationary were analyzed to obtain means and standard deviations of clade ages [34]. To convert relative divergence times into absolute time units we used two maximum-age fossil constraints. Palynological studies identified \text{Hibbanthone} pollen in Upper Miocene formations (11 Ma) from France [66] and \text{Tubera}na pollen in Pliocene formations (5.3 Ma) from Germany [67].

Species diversification rates, assuming an equal rate of random speciation Yule model, were calculated using the formula \text{SR} = [(\log(N) - \log(N_0)) / T] [34,68,69], where \(N\) is the total number of extant species in the clade of interest, \(N_0\) is the initial species diversity, usually taken as 1, and \(T\) is the inferred age of the clade (million years). Upper and lower standard deviations of age estimates were used in calculations of speciation rates.

**Character evolution.** Patterns of evolution of six key traits (leaf shape, leaf labdananum secretion, leaf pubescence, soil requirements, insolation conditions, habitat) were explored in the white-flowered \textit{Cistus} lineage using the Bayesian consensus tree (calculated using the same parameters as above). Optimizations were performed in MacClade 4.06 [70] assuming Fitch Parsimony, equal weighting of all characters, transitions among all states equally probable and treating characters as unordered. Character states were determined from literature and personal observations. Samples of \textit{Cistus crispus} and \textit{C. heterophyllus} were used as outgroup sequences.

In addition, to account for values of phylogenetic mapping uncertainty, probabilities of ancestral states for the six traits were estimated individually using the BayesMultiState program, contained in the BayesTraits 1.0 package [71], under MonteCarlo Markov Chain (MCMC) method and allowing transitions between character states in both directions. To reduce the autocorrelation of successive samples, 1000 trees were drawn from the distribution of 1.9\times10^6 trees, which equates to sampling every 1900th generation of the chains used in the phylogenetic analysis. As suggested in BayesMultiState manual, to reduce some of the uncertainty and arbitrariness of choosing prior in MCMC studies, we used the hyperprior approach, in concrete the reversible-jump (RJ) hyperprior with a gamma prior (mean and variance seeded from uniform distributions on the interval 0 to 10). Preliminary analyses were run to adjust the rateprior parameter until the acceptance rates of proposed changes was around 20–40%. Using rateprior settings (Table S2), we ran the RJ MCMC analyses for each trait three times independently for 1.0\times10^7 iterations, sampling every hundredth iteration to produce 90,000 sampled points and discarding the first 1,000,000 iterations. All runs gave mostly the same results and we report one of them here. We use the “Aclnode” command to find the proportion of the likelihood associated with each of the possible states at each node.

**Testing correlated evolution.** We modelled correlated evolution of discrete binary traits (leaf shape/insolation conditions, leaf shape/habit, labdananum secretion/insolation conditions, labdananum secretion/habit, leaf pubescence/insolation conditions, leaf pubescence/habit) on 1000 Bayesian trees using the BayesDiscrete program, contained in the BayesTraits 1.0 package [71] and the same parameters described above. The method compares the statistical likelihood of a model in which two binary traits are allowed to evolve independently on the tree, with a model in which the two traits are allowed to evolve in a correlated fashion. Evidence for correlated evolution arises if the dependent or correlated model shows significantly better fit to the data than the independent model. As the independent and dependent models are estimated by MCMC, their goodness of fit is compared using the log-Bayes Factor test: \(2\log[\text{harmonic mean(dependent model)}] - \log[\text{harmonic mean (independent model)}]\)

We used one sample per species of the white-flowered lineage given the monophyly of all species [72]. As binary traits are required, we coded traits as followed: leaf shape, 0 linear to elliptic, 1 ovate-lanceolate to ovate; labdananum secretion, 0 zero to eight percent, 1 nine to fifteen; upper leaf pubescence, 0 glabre to subglabre, 1 dense tomentum; insolation conditions, 0 helioxerophyllous to subphelioxerophyllous, 1 subphelioxerophyllous to submesophyllous; environment, 0 bushy and scrub vegetation, 1 woodlands.

**Haplotype data analysis.** Sequences of plastid DNA (\text{trnL-trnF}, \text{trnK-matK} \text{trnS-trnG} and \text{rbcL}) were combined to analyze relationships among the white-flowered \textit{Cistus} (plus \textit{C. parviflorus}) plastid haplotypes. We used the software TCS 1.21 to infer plastid haplotype ancestry [73]. The program implements a statistical parsimony approach using the algorithm described in Templeton et al. [74] 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.

**Supporting Information**

**Table S1** GenBank accession numbers. Found at: doi:10.1371/journal.pone.0006362.s001 (0.10 MB DOC)

**Table S2** Bayesian inference of trait evolution of successive iterations of the chain (9,000,000) in the white-flowered \textit{Cistus}
lineage by reversible jump Markov chain Monte Carlo. Means± confidence intervals (95%) of the log-likelihoods (Lh) and rate coefficients are shown.

Found at: doi:10.1371/journal.pone.0006362.s002 (0.07 MB DOC)

Table S3
Found at: doi:10.1371/journal.pone.0006362.s003 (0.20 MB DOC)

References

1. Schluter D (2000) The ecology of adaptive radiation. New York: Oxford University Press Inc.
2. Cavalli-Sforza LS, Menozzi P, Piazza A (1994) The history and geography of human genes. Princeton, New Jersey: Princeton University Press.
3. Baldwin BG, Robichaux RH (1995) Historical biogeography and ecology of the Hawaiian silversword alliance (Asteraceae): new molecular phylogenetic perspectives. In: Wagner WL, Funk VA, eds. Hawaiian biogeography: evolution on a hotspot archipelago, Washington, D.C.: Smithsonian Institution Press, 317–337.
4. Meirmehg B, Abele T, Brauchl C, McAlary J, Perez de Paz PL, et al. (2006) Molecular evidence for adaptive radiation of *Alcea rosea* (Malvaceae) on the Canary Islands as inferred from chloroplast and nuclear DNA sequences and DAPC fingerprint data. Mol Phylogenet Evol 41: 566–578.
5. Hodges SA (1997) Rapid radiation due to a key innovation in columbines (Ranunculaceae). In: Givnish TJ, Sytsma KJ, eds. Molecular evolution and adaptive radiation. New York, USA: Cambridge University Press, pp. 391–405.
6. MacArthur RH, Wilson EO (1967) The theory of island biogeography. Princeton, New Jersey: Princeton University Press.
7. Baldwin BG, Crawford DJ, Francisco-Ortega J, Santos-Guerra A (1996) A common origin for woody plants. Am Nat 140: 421–446.
8. Baldwin BG, Robichaux RH (1995) Historical biogeography and ecology of the Hawaiian silversword alliance (Asteraceae): new molecular phylogenetic perspectives. In: Wagner WL, Funk VA, eds. Hawaiian biogeography: evolution on a hotspot archipelago, Washington, D.C.: Smithsonian Institution Press, 317–337.
9. Kim S-C, Crawford DJ, Francisco-Ortega J, Santos-Guerra A (1996) A common origin for woody plants. Am Nat 140: 421–446.
10. Reinthal PN, Meyer A (1997) Molecular phylogenetic tests of speciation models in the Canary Islands as inferred from chloroplast and nuclear DNA sequences and DAPC fingerprint data. Mol Phylogenet Evol 41: 566–578.

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Author Contributions

Conceived and designed the experiments: PV. Performed the experiments: BG MDL. Analyzed the data: BG PV. Wrote the paper: BG PV.
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40. Ehleringer JR, Clark C (1988) Evolution and adaptation in *Evolvulus* (Asteraceae). In: Gotlieb LD, Sudholt K, eds. Plant evolutionary biology. New York: Chapman and Hall Ltd, 221–248.
49. Gull P, Herrmann H, Hanget K (1996) Leaf trichomes in the genus *Cistus*. Flora 191: 83–104.
50. Suet J, Bertini A, Combrouxie-Nebout N, Diniz F, Leroy S, et al. (1995) Structure of West Mediterranean vegetation and climate since 5.3 Ma. Acta Zool Cracov 38: 3–16.
51. White T, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelldelf D, Sninsky J, White T, eds. PCR protocols: a guide to methods and applications. San Diego: Academic Press, 315–322.
52. Sun V, Skinner DZ, Liang GH, Hulbert SH (1997) Phylogenetic analysis of *Sugum* and related taxa using Internal Transcribed Spacer of nuclear ribosomal DNA. Theor Appl Genet 98: 26–32.
53. Taberlet P, Gofl L, GP, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Plast Biol 17: 1105–1109.
54. Johnson LA, Soltis DE (1994) *atpB* DNA and phylogenetic reconstruction in Saxifragaceae s. str. Syst Bot 19: 143–156.
55. Hamilton M (1999) Four primers pairs for the amplification of chloroplast intergenic regions with intraspecific variation. Mol Ecol 8: 521–523.
56. Savolainen V, Chase MW, Hoot SB, Morton CM, Soltis DE, et al. (2000) Phylogenetics of flowering plants based on combined analysis of plastid *ndhF* and *rbcL* gene sequences. Syst Biol 49: 306–362.
57. Emswiller E, Doyle J J (1999) Chloroplast-expressed Glutamine synthetase (ncpGS): Potential utility for phylogenetic studies with an example from *Oxalis* (Oxalidaceae). Mol Phylogenet Evol 12: 310–319.
58. Thompson JD, Gibson TJJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25: 4876–4882.
59. Swoford D (2002) PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland, MA: Sinauer Associates.
60. Mort ME, Soltis PS, Soltis DE, Malvey M (2000) Comparison of three methods for estimating internal support on phylogenetic trees. Syst Biol 49: 169–171.
61. Nylander JAA (2002) MrModeltest v1.0b. Uppsala: Department of Systematic Zoology, Uppsala University.
62. Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817–818.
63. Rouhani F, Huchtenbeek JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.
64. Magallon SA, Sanderson MJ (2005) Angiosperm divergence times: The effect of genes, codon positions, and time constraints. Evolution 59: 1653–1670.
65. Sanderson MJ (2002) Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. Mol Biol Evol 19: 101–109.
66. Naud G, Suet JP (1975) Contribution à l’étude paléohistorique des Côtières (Ardeche). Première analyses polliniques dans les alluvions sous-basaltique et interbasaltiques de Mirabel (Miocène supérieur). Bull Soc Géol France 17: 620–627.
67. Menke B (1976) Pliozane und älterezeitare Sporen- und pollenflora von Schleswig-Holstein. Geologisches Jahrbuch Reihe A 32: 3–197.
68. Kendall DG (1953) Stochastic processes and population growth. J Roy Stat Soc B 11: 230–264.
69. Moran PA (2000) Estimation methods for evolutive processes. J Roy Stat Soc B 13: 141–146.
70. Maddison WP, Maddison DR (1992) MacClade: Analysis of Phylogeny and Character Evolution, version 3.01. Sunderland, MA: Sinauer Associates.
71. Page M, Meade A (2007) BayesTraits:Version 1.0.
72. Gottlieb LD, Subodh KJ, eds. Plant evolutionary biology. New York: Chapman and Hall Ltd, 221–248.
73. Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9: 1657–1659.
74. Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132: 619–633.
75. Grosser W (1903) Cistaceae. In: Engel A, ed. Das Pflanzenreich. Berlin: Breitkopf & Hartel, 161p.
76. Warburg EF (1968) *Cistus albanicus*. In: Castroviejo S, Aedo C, Cirujano S, Moore DM, Valentine DH, eds. Flora Europaea. Cambridge: Cambridge University Press, 282–285.
77. Dansereau P (1958) Notes sur les Cistes. III: Les conditions de la distribution de *Cistus*. Bull Soc Geographique. C R Seances Soc Biogeogr 304: 22–25.
78. Sukkoveev S, Arbo G, Cirjano S, Lainz M, Montserrat P, et al. eds. Flora iberica. Madrid: Consejo de Investigaciones Científicas, 319–337.
79. Greuter W (1996) Proposal to conserve the name *Cistus albanicus* (Cistaceae). Taxon 45: 715–716.