

Syst. Biol. 59(6):705–722, 2010

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DOI:10.1093/sysbio/syq046

Advance Access publication on September 13, 2010

Tracing the Temporal and Spatial Origins of Island Endemics in the Mediterranean Region: A Case Study from the Citrus Family (*Ruta* L., Rutaceae)

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Received 10 September 2009; reviews returned 20 December 2009; accepted 4 May 2010

Associate Editor: Dan Faith

Abstract.—Understanding the origin of island endemics is a central task of historical biogeography. Recent methodological advances provide a rigorous framework to determine the relative contribution of different biogeographic processes (e.g., vicariance, land migration, long-distance dispersal) to the origin of island endemics. With its complex but well-known history of microplate movements and climatic oscillations, the Mediterranean region (including the Mediterranean basin and Macaronesia) provides the geographic backdrop for the diversification of *Ruta* L., the type genus of Rutaceae (citrus family). Phylogenetic, molecular dating, and ancestral range reconstruction analyses were carried out to investigate the extent to which past geological connections and climatic history of the Mediterranean region explain the current distribution of species in *Ruta*, with emphasis on its island endemics. The analyses showed that *Ruta* invaded the region from the north well before the onset of the Mediterranean climate and diversified in situ as the climate became Mediterranean. The continental fragment island endemics of the genus originated via processes of land migration/vicariance driven by connections/disconnections between microplates, whereas the oceanic island endemics were the product of a single colonization event from the mainland followed by in situ diversification. This study emphasizes the need for an integrative, hypothesis-based approach to historical biogeography and stresses the importance of temporary land connections and colonization opportunity in the biotic assembly of continental fragment and oceanic islands, respectively. [Continental fragment islands; geologic history; historical biogeography; Mediterranean region; oceanic islands; paleoclimate; *Ruta*.]

Historical biogeography has evolved from a mostly pattern-oriented exercise to an integrative, model-based discipline. The past 10 years have witnessed a resurgence of biogeographic studies that strive to integrate information from phylogenies, fossils, molecular dating, the geologic record, and paleoclimatic reconstructions. In particular, the following have become instrumental in setting up sound historical biogeography hypotheses: 1) the establishment of a temporal framework (Hunn and Upchurch 2001; Donoghue and Moore 2003); 2) the a priori incorporation of explicit models pertaining to relevant biological and earth processes into biogeographic analysis (Ree et al. 2005; Sanmartín et al. 2008; Ree and Sanmartín 2009); and 3) the shift in focus from the vicariance-versus-dispersal dualism to a more quantitative assessment of the contribution of different historical processes, including geodispersal and extinction, to current patterns of distribution (Crisci et al. 2003; Lieberman 2003; Lomolino et al. 2006; Upchurch 2008).

The integration of different kinds of data/models into biogeographic analysis introduces more variables into the reconstruction of biogeographic scenarios, often producing an increase in uncertainty with respect to the inferences being made (Conti et al. 2004; Heads 2005; Rutschmann et al. 2007; Clark et al. 2008; Nylander et al. 2008; Ho and Phillips 2009). A crucial step in minimizing uncertainty, and a prerequisite for sound analyses in historical biogeography, is the choice of a group of organisms for which 1) a robust phylogenetic framework is present; 2) reliable fossils for molecular clock calibration are available; 3) obvious traits facilitating long-distance

dispersal (hereafter, LDD) are absent; and 4) the geology and paleoclimate of the area housing them are well understood. The focal area (the Mediterranean region) and taxon (*Ruta* L.) selected for the present study satisfy these requirements.

With its complex but well-known history of microplate movements and climatic oscillations, the Mediterranean region provides the geographic backdrop for the diversification of *Ruta*, the type genus of Rutaceae (citrus family). As currently circumscribed, *Ruta* includes nine species of perennial herbs: *R. angustifolia*, *R. chalepensis*, *R. montana*, and *R. graveolens* exhibit a circum-Mediterranean distribution; *R. corsica* is endemic to Corsica and *R. lamarmorae* to Sardinia; *R. pinnata*, *R. oreojasme*, and *R. microcarpa* are endemic to the Canary Islands (Townsend 1968; Bramwell and Bramwell 2001; Bacchetta et al. 2006; Fig. 1). The limits of the geographic distribution of *Ruta* broadly correspond to the limits of the Mediterranean region, and the genus often occurs in association with elements characteristic of the Mediterranean vegetation (e.g., *Ulex*, *Quercus*, *Pistacia*; Bonet 1992). Thus, the biogeographic history of *Ruta* has broad implications for the assembly of the Mediterranean flora. A recent phylogenetic analysis of *Ruta* and closely related genera ascertained the monophyly of the genus (Salvo et al. 2008). Additionally, the paleontological record of Rutaceae is quite rich (Gregor 1989) and *Ruta* produces capsules and seeds without any obvious adaptations for wind dispersal (Engler 1896; Engler 1931), two factors that contribute to making *Ruta* an ideal genus for a biogeographic analysis.

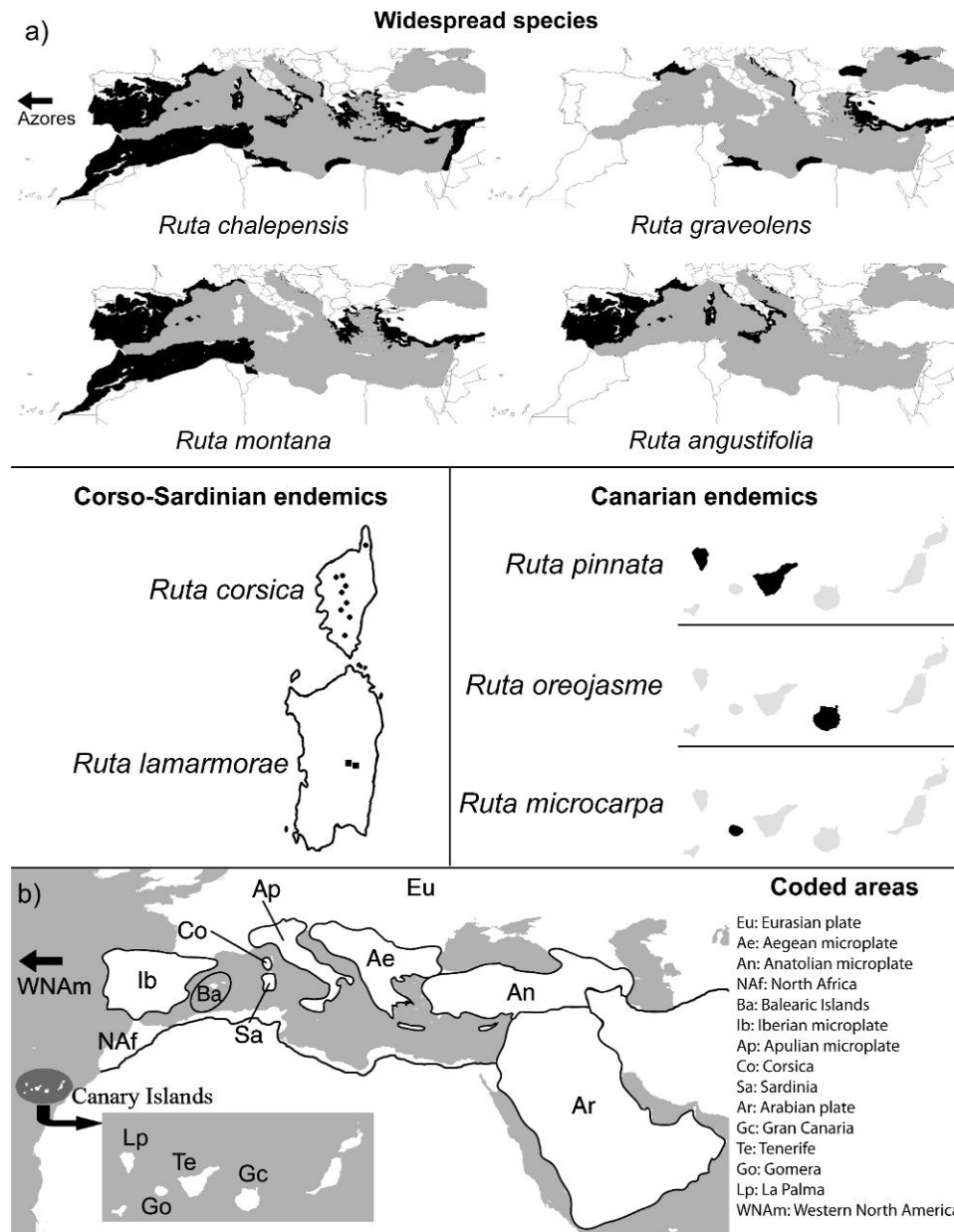


FIGURE 1. Distribution of *Ruta* and selected areas: (a) geographic distribution of the 9 species of *Ruta*; (b) areas selected for the ancestral range reconstruction analyses, as defined by [Mansion et al. \(2008\)](#). Distribution of *R. corsica* and *R. lamarmorae* taken from [Bacchetta et al. \(2006\)](#).

The evolution of diversity in *Ruta* has not been examined in a temporal framework, nor has its current distribution been analyzed in the context of the geologic history and paleoclimate of the Mediterranean basin. Most importantly, the occurrence of five out of nine species endemic to either continental fragment (Corsica and Sardinia) or oceanic (the Canary archipelago) islands offers a unique opportunity for comparing modes of biogeographic evolution in these two kinds of islands. The main difference between the biogeographic mechanisms that can be invoked to explain the origin of the five island endemics is that the colonization of the oceanic Canary Islands could have been achieved

exclusively via LDD, whereas colonization of the continental fragment islands of Corsica and Sardinia could have been effected via both LDD and land migration ([Emerson 2002](#); [Cowie and Holland 2006](#); [Lomolino et al. 2006](#); [Sanmartín et al. 2008](#)). Below we summarize the geologic history, main paleoclimatologic features, and hypotheses on the origin of the flora of the focal area, with special emphasis on Corsica, Sardinia, and the Canary Islands.

With its 22,500 species and about 13,000 endemics, the Mediterranean region—comprising the Mediterranean basin and the Macaronesian islands ([Quézel 1985](#))—has been recognized as a hot spot of biodiversity ([Médail](#)

and Quézel 1997; Myers et al. 2000). A large component of the Mediterranean basin formed during the Cenozoic, simultaneously with the ongoing convergence of Africa with respect to Europe (Dercourt et al. 1986; Dewey et al. 1989; Rosenbaum et al. 2002b). The present landlocked configuration of the basin resulted from the collision of the Arabian plate with stable Eurasia in the middle Miocene, which led to the closure of the connection between the Tethys Sea and the Indian Ocean (Krijgsman 2002; Garfunkel 2004). In the early Miocene (23–16 Ma) and beyond, the basin experienced subtropical conditions, with little seasonal change in temperature and relatively high levels of summer rainfall (Thompson 2005). In the middle Miocene (16–14 Ma), seasonal contrasts in the temperature regime started to develop, leading to the establishment of the current Mediterranean climatic rhythm (summer drought) in the Pliocene (3–2 Ma; Suc 1984; Thompson 2005). The Pleistocene (1.8–0.01 Ma) witnessed the alternation of warmer and colder conditions during the glacial cycles, while increased aridity and gradual warming marked the Holocene (0.01 Ma to present).

Both the beginning of a trend toward increasing aridification in the Mediterranean region (9–8 Ma) and the onset of the Mediterranean climate (around 3–2 Ma) are believed to have had a big impact on the composition and structure of the Mediterranean flora (Suc 1984; Ivanov et al. 2002; Thompson 2005; Van Dam 2006; Lo Presti and Oberprieler 2009). The former event led to the progressive replacement of the tropical elements of the Mediterranean flora (e.g., mangroves, Taxodiaceae) with sclerophyllous plant communities; the latter event caused coastal forests to disappear and xerophytic taxa (e.g., *Olea*, *Quercus ilex*-type, *Pistacia*) to expand (Suc 1984; Thompson 2005). Early workers temporally divided the floristic elements of the Mediterranean region into two main groups, depending on whether they were believed to have originated before or after the development of such climate (Pons and Quézel 1985; Thompson 2005).

A series of events likely to have profoundly influenced the biogeography of the Mediterranean basin concerns the geologic history of the continental fragment islands of Corsica and Sardinia (Caccone et al. 1994; Palmer 1998; Caccone and Sbordoni 2001; Ketmaier et al. 2003, 2006; Fochetti et al. 2004). Before the early Oligocene, these two islands were situated adjacent to current southern France, forming a continuous geologic entity (part of the so-called Hercynian massif) that was subsequently fragmented into microplates that dispersed throughout the western Mediterranean (Alvarez et al. 1974; Rosenbaum et al. 2002a). These tectonic fragments included the Tuscan archipelago (in Italy), the Balearic Islands, the internal parts of the Betic-Rif *cordillera* (in Spain and Morocco, respectively), the Kabylies (in Algeria), and the Calabro-Peloritan massif (in southern Italy). According to tectonic reconstructions, in the late Oligocene (30–28 Ma) the Balearic–Kabylies microplate and the Corso-Sardinian–Calabro-Peloritan microplate separated from the east-

ern part of the proto-Iberian peninsula (Alvarez et al. 1974; Dewey et al. 1989; Rosenbaum et al. 2002a). At around 25 Ma, the Balearic–Kabylies microplate started to rotate clockwise until the Balearic Islands reached their current position (around 21 Ma) and then became detached from the Kabylies terrane, which continued to drift toward North Africa. At the same time, the Corso-Sardinian–Calabro-Peloritan microplate moved eastward, with a counterclockwise rotation of approximately 30°, until it collided with the western side of the Apulian microplate around 20–18 Ma (Cherchi and Montadert 1982; Deino et al. 2001; Rosenbaum et al. 2002a; Speranza et al. 2002). These two microplates remained connected until the opening of the North Tyrrhenian Sea in the Tortonian (around 9 Ma; Rosenbaum and Lister 2004). The Calabro-Peloritan block finally split off from the Corso-Sardinian (C-S) microplate around 5 Ma, reaching its current position as the southern-most tip of the Italian peninsula during the last 2 myr (Rosenbaum et al. 2002a). During the Messinian salinity crisis (MSC; 5.96–5.33 Ma), an event characterized by the dramatic drying and salinity increase of the Mediterranean Sea (Robertson and Grasso 1995; Krijgsman 2002), the C-S microplate was most likely linked with the southwestern Alps and present-day Tuscany (Italy) to the north and with North Africa to the south (Rögl and Steininger 1983; Rouchy and Saint-Martin 1992; Robertson and Grasso 1995; Gover et al. 2009).

The tectonic separation of Corsica from Sardinia began 15 Ma and was complete by 9 Ma (Alvarez 1972, 1976; Alvarez et al. 1974; Bonin et al. 1979; Orsini et al. 1980; Cherchi and Montadert 1982), although episodic contacts between these two islands persisted until very recently, in particular during the MSC (Rögl and Steininger 1983; Gover et al. 2009) and the Last Glacial Maximum (LGM; around 0.02 Ma; Lambeck et al. 2004; Lambeck and Purcell 2005). At present, the two islands are situated in the middle of the western Mediterranean basin and are separated by a narrow (11 km) and shallow (less than 60 m deep) water channel, the Strait of Bonifacio.

Corsica and Sardinia have been identified as one of the 10 areas with the highest biodiversity in the Mediterranean (Médail and Quézel 1997), with about 4300 plant species, including around 340 listed endemics and subendemics (Arrigoni 1979; Gamisans and Jeanmonod 1993). A long tradition of floristic studies on the C-S flora has revealed affinities with the Pyrenees, Provence, southern Spain, the Balearic Islands, Liguria, Calabria/Sicily, and North Africa (Moris 1837–1859; Barbey 1885; Briquet 1910; Braun-Blanquet 1926; Contandriopoulos 1962; Bocquet et al. 1978; Jeanmonod and Gamisans 1987). Five main scenarios have been advanced to explain the origin of the C-S endemic flora: 1) the split between the C-S microplate and the proto-Iberian peninsula (30–28 Ma), causing vicariant speciation in taxa that inhabited both areas before the split (Gamisans 1975); 2) subsequent land connections between the C-S microplate and adjacent areas, which facilitated the entering of floristic elements into the

two islands (Braun-Blanquet 1926; Contandriopoulos 1962); 3) the MSC (5.96–5.33 Ma), which, through the mesh of land corridors present in the Mediterranean basin at that time, allowed a “veritable explosion in migrations” across the basin, affecting also Corsica and Sardinia (Bocquet et al. 1978, p. 277); 4) the Quaternary glacial cycles, which, due to the supposed connections between the C-S block and present-day Provence/Liguria/Tuscany at that time, enhanced biotic exchange (Mariotti 1990); and 5) LDD, first proposed by Engler (1879) to explain the origin of the Corsican flora. These scenarios, however, were based on floristic studies that lacked an explicit spatiotemporal framework and sometimes used the distribution of organisms to infer past paleogeographical settings, rather than vice versa.

The Canary Islands, in the Atlantic Ocean, off the coast of Africa, are part of the Macaronesian phyto-geographical region, which also includes the Azores, Madeira, the Salvage Islands, and the Cape Verde Islands (Sunding 1979). These islands were formed in an east–west progression, starting with Fuerteventura (around 20 Ma) and ending with El Hierro (around 1 Ma; Carracedo et al. 1998; Anguita and Hernán 2000). The Canaries have a Mediterranean climate, generally similar to that of Corsica and Sardinia (Whittaker and Fernandez-Palacios 2007), and are floristically the richest islands of Macaronesia, with around 680 endemic species (Reyes-Betancort et al. 2008). Interest in the phylogenetic origin of the Canarian biota has significantly increased in recent years (Juan et al. 2000; Conti et al. 2004; Kim et al. 2008; Sanmartín et al. 2008). From a temporal point of view, the endemic flora of the Canary Islands has been viewed either as the relict of a formerly widespread subtropical flora that covered southern Europe and North Africa during the Tertiary or as a relatively younger flora (Emerson 2002; Carine 2005). In any case, because the Canary Islands are a volcanic archipelago and were thus never connected to the mainland, LDD represents the only possible mode of colonization of the archipelago (Cowie and Holland 2006; Sanmartín et al. 2008). Different LDD scenarios have been proposed for the colonization of these islands, invoking, at one extreme, a single LDD event to the older island, followed by progressive colonization of the younger islands in a stepping-stone fashion, or, at the other extreme, multiple LDD events in a sequence that is independent from the order of island emergence (Juan et al. 2000; Cowie and Holland 2006; Sanmartín et al. 2008).

By adopting an integrative approach, including phylogenetic, molecular dating, and ancestral range reconstruction analyses, in the context of the geologic history and paleoclimate of the Mediterranean region, we ask the following: 1) Was the origin and diversification of *Ruta* concomitant with the onset of the Mediterranean climate, and did its ancestor evolve in situ or in areas neighboring the Mediterranean region?; 2) Does the fragmentation of the Hercynian massif explain the origin of the C-S lineage?; 3) Does the formation of

the Strait of Bonifacio explain the divergence between the two Corsican and Sardinian endemic species, respectively?; and 4) Does the colonization of the Canary Islands by *Ruta* conform to a stepping-stone mode of island diversification, and was the origin of the three Canarian endemics concomitant with the onset of the Mediterranean climate?

MATERIALS AND METHODS

Taxon Sampling

All extant species of *Ruta* were sampled (Townsend 1968; Bramwell and Bramwell 2001; Bacchetta et al. 2006). The sampling of *Ruta*'s most closely-related taxa was guided by careful examination of taxonomic (Engler 1896, 1931), phytochemical (Da Silva et al. 1988), and phylogenetic studies (Chase et al. 1999; Scott et al. 2000; Poon et al. 2007; Groppo et al. 2008; Salvo et al. 2008) on Rutaceae and included *Thamnosma*, *Boenninghausenia*, *Haplophyllum*, *Cneoridium*, and representatives of subfamily Aurantioideae. Because available fossils have been linked with extant taxa that are distantly related to *Ruta* (see below), species representative of the phylogenetic diversity of Rutaceae, including the fossil-bearing taxa, were sampled according to the family-level phylogenetic analysis of Groppo et al. (2008). Where possible, two species per genus were included for the fossil-bearing taxa, in order to discriminate between their crown and stem nodes for fossil calibration purposes (see Magallón 2004). The final matrix contained 48 accessions, including 44 from Rutaceae and two each from Meliaceae and Simaroubaceae, which served as outgroups (Gadek et al. 1996; Muellner et al. 2007). Included material, voucher information, sources, and GenBank/EBI accession numbers are listed in online Appendix 1.

DNA Sequences and Phylogenetic Analyses

Three chloroplast markers that provided sufficient resolution at our level of investigation and allowed unequivocal alignments were chosen: the coding region of the *matK* gene, amplified using primers 1F and 1R (Sang et al. 1997); the *rpl16* intron, amplified using primers F71 and R1516 (Baum et al. 1998); and the intergenic spacer between the *trnL* (UAA)3' exon and *trnF* (GAA) (from hereafter, *trnL-trnF* intergenic spacer), amplified with primers e and f (Taberlet et al. 1991). DNA extraction and polymerase chain reaction amplification and sequencing followed the methods described in Salvo et al. (2008). Sequences were edited and assembled using Sequencher 4.2 software (Gene Codes Corp., Ann Arbor, MI). Base positions were individually double-checked for agreement between the complementary strands. All sequences were visually aligned in MacClade 4.06 (Maddison P.G. and Maddison D.R. 2000). Regions of ambiguous alignment were excluded from the analysis. Gap positions were treated as missing data, unequivocally aligned gaps being coded as presence/absence of characters with the software GapCoder

(Young and Healy 2003) and then added as binary characters to the data matrix.

Three data partitions were defined, corresponding to the three loci of the chloroplast genome examined in this study. We are aware of the important issue of partitioning in multilocus molecular studies and of the problems of under- and overparameterization (Nylander et al. 2004; Brown and Lemmon 2007); however, exploratory analyses using different partitioning schemes did not have a noticeable impact on the resulting chronograms. The individual partitions were initially analyzed separately to establish whether any strongly supported (i.e., >85 bootstrap percentage [BP]) clades were incongruent among the respective trees. Because no such incongruence was detected, the sequences of the three loci were combined in a single data set. The combined matrix, which is available at TreeBASE (<http://purl.org/phylo/treebase/phyloids/study/TB2:S10145>), was then analyzed using maximum parsimony (MP), maximum likelihood (ML), and Bayesian Inference (BI). Parsimony analyses were conducted using PAUP*4.0b10 (Swofford 2001). All changes were treated as unordered and equally weighted. Tree search was performed using the following protocol: 1) a heuristic search was carried out with 1000 replicates of random taxon addition sequence and 10 trees held at each step, and tree bisection–reconnection (TBR) branch swapping on best trees only, with no more than 100 trees saved per replicate; 2) the best trees found in (1) were then used as starting trees for a second heuristic search using TBR branch swapping until all swapping options were explored and saving multiple trees. The STEEPEST DESCENT option was used in both (1) and (2). Relative support for each node obtained by MP was assessed using bootstrap resampling (Felsenstein 1985). The following protocol was employed: heuristic search, 1000 bootstrap replicates, 100 random addition sequence replicates with three trees held at each step, and TBR swapping with STEEPEST DESCENT and saving no more than 10 trees per replicate.

ML analyses were performed using RAxML (Stamatakis 2006). The General Time Reversible + Gamma model of nucleotide substitution was used, with a separate model for each data partition. Support values for nodes in the phylogenetic tree were obtained by analyzing 1000 pseudoreplicate data sets generated through bootstrap sampling from the original alignment.

Bayesian inference was performed in MRBAYES v3.1.2 (Huelsbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003), after determining the model of evolution most suitable for each individual chloroplast DNA (cpDNA) region with the Akaike Information Criterion (Akaike 1974) in ModelTest 3.06 (Posada and Crandall 1998). The General Time Reversible + Gamma (GTR+G) model of nucleotide substitution was selected for all three regions. Two independent runs with four Monte Carlo Markov chains (MCMCs, one cold and three incrementally heated) run for 5×10^6 generations, with trees sampled every 1000th generation, were per-

formed. Each chain used a random tree as starting point and the default temperature parameter value of 0.2. The first 25,000 sampled trees were discarded as “burn in” after checking for stability on the log-likelihood curves and after visual inspection of the split (clade) frequencies using the software AWTY (Wilgenbusch et al. 2004). The remaining trees were used to build a 50% majority rule consensus tree.

Selection of Fossils for Calibration

Rutaceae are well represented in the fossil floras of North America, Europe, and Asia, extending back into the latest Cretaceous. The most comprehensive revision of the fossil record of the family was carried out by Gregor (1989), who used morphological characters of the seeds' testa, inner anatomy, and surface in order to differentiate fossil taxa and determine affinities with extant genera. The oldest certain Rutaceae fossil is *Rutaspermum biornatum* (around 65 Ma; Maastriichtian of Walbeck, Germany; Knobloch and Mai 1986), followed by fossils belonging to the extant genera *Zanthoxylum*, *Euodia*, *Acronychia*, *Toddalia*, and *Fagaropsis* (Gregor 1989). Fossils of *Ruta* have not been found. Four fossils accompanied by detailed morphological descriptions, dates associated with the geologic interval of their collection locality, and well-supported affinities with modern taxa were selected: *Euodia lignita* Tiffney (Tiffney 1980, 1994), *Toddalia excavata* (Chandler) Gregor (Gregor 1979), *Ptelea enervosa* H.V. Smith (Smith 1938), and *Skimmia tortonica* Palamarev and Usunova (Palamarev and Usunova 1970). Detailed information on these fossils is presented in online Appendix 2.

Molecular Dating Analyses

In order to investigate the degree of substitution rate variation among lineages, a likelihood-ratio test (LRT) was performed using PAUP*. First, the best model of evolution for the combined matrix without gap coding was selected using ModelTest. Second, the parameters describing the selected model were used to compute the likelihood score of the Bayesian 95% majority rule consensus tree with and without enforcing substitution rate constancy. Because the LRT rejected rate constancy, molecular dating analyses were carried out using two Bayesian methods that allow for variation of substitution rate among tree branches. The first method, which assumes rate autocorrelation between neighboring branches, was implemented in MULTIDIVTIME (Thorne et al. 1998; Kishino et al. 2001; Thorne and Kishino 2002). The second method, where different models of among-lineage rate variation can be used and priors on calibrations can be modeled with parametric distributions, was implemented in BEAST v1.4.8 (Drummond et al. 2006; Drummond and Rambaut 2007).

MULTIDIVTIME analyses were carried out using the following protocol. After having estimated branch lengths and their variance–covariance matrix with the

BASEML and ESTBRANCHES programmes, contained in the PAML 4 (Yang 2007) and Multidistribute v. 9/25/03 (Thorne et al. 1998; Thorne and Kishino 2002) packages, respectively, we performed MCMC searches in MULTIDIVTIME. Four prior distributions were specified in units of 10 myr: the mean of the ingroup's age (rttm) was set to 6.5 because the oldest reliable fossil of Rutaceae was dated to 65 Ma (Knobloch and Mai 1986); the standard deviation of the ingroup's age (rttmsd) was set to 6.0 because an ingroup age older than the appearance of tricolpate pollen in the fossil record (around 125 Ma; Sanderson and Doyle 2001; Anderson et al. 2005) was deemed unrealistic; the mean (rtrate) and standard deviation (rtratesd) of the rate at the root node were set to 0.0096 substitutions/site/10 Ma by dividing the median of the distances between the ingroup root and the tips by rttm; the mean (brownmean) and standard deviation (brownstd) of the Brownian motion parameter ν were set to 0.23 units, so that $\text{brownmean} \times \text{rttm} = 1.5$. Finally, the age of a larger clade to which the group belongs (bigtime) was set to 12.5, referring again to the emergence of tricolpate pollen in the fossil record. Internal calibrations were set up using the four fossils mentioned above: the upper (younger) bound of the geologic interval in which each fossil was found represented the minimum age constraint. An age of 1.597 was assigned to the stem node of *Euodia*, an age of 3.72 to the node subtending *Toddalia*, an age of 1.1608 to the stem node of *Ptelea*, and an age of 0.7246 to the node subtending *Skimmia*. The Bayesian 50% majority rule consensus tree obtained with MRBAYES was used as the input tree. The MCMC was run for 10^6 generations and sampled every 100^{th} generation, after an initial burn-in period of 10^5 generations. Two separate runs were conducted to check for convergence on similar posterior distributions. To assess the effect of choice of priors on resulting age estimates, we performed sensitivity analyses by selecting different root prior distributions and by repeating all analyses with a fully resolved input tree, the maximum a posteriori (MAP) tree inferred with MRBAYES (online Appendix 3).

BEAST simultaneously estimates phylogenetic relationships and nodal ages. The age estimates are calibrated through the specification of prior age distributions for certain nodes in the tree, guided by independent information from the fossil record. Because the oldest fossilized representative of a clade corresponds to the minimum age of that clade, parametric distributions can be modeled around fossil calibration points, in which the probability of a clade being younger than its oldest fossil drops immediately to zero, but the probability of it being older than its oldest fossil decays more gradually (Ho and Phillips 2009). Calibrations using the four fossils described above were modeled with a lognormal distribution, where 95% of the prior weight fell within the geologic interval in which each fossil was discovered (online Appendix 2). For *Euodia lignita* (33.9–15.97 Ma), the parameters of the lognormal calibration prior were hard minimum bound 15.97, mean 2.1933,

and standard deviation 0.4214. For *Toddalia excavata* (40.4–37.2 Ma), the parameters were hard minimum bound 37.2, mean 0.47, and standard deviation 0.4215. For *Ptelea enervosa* (15.97–11.608 Ma): hard minimum bound 11.608, mean 0.7798, and standard deviation 0.4214. And for *Skimmia tortonica* (11.608–7.246 Ma): hard minimum bound 7.246, mean 0.7798, and standard deviation 0.4214. The prior age of the root was also modeled with a lognormal distribution with the following parameters: hard minimum bound 65, mean 2.3, and standard deviation 0.7. The mean of this distribution corresponded to the oldest, reliable Rutaceae fossil and the 1% lower tail to the appearance of tricolpate pollen in the fossil record (see above). After having selected a GTR substitution model with 4 gamma categories, an uncorrelated, lognormal, relaxed-clock model and a Yule prior on the tree, five independent runs of 10^7 generations each, sampling every 1000^{th} generation, with a burn-in of 10^6 generations each, were conducted. The post-burn-in trees from each run were combined, and a maximum clade credibility tree was computed. Similarly to MULTIDIVTIME, sensitivity analyses were carried out to assess the impact of different root priors and prior distributions for fossil calibrations on the posterior distribution of age estimates (online Appendix 3).

Because the node to which the fossil *Ptelea enervosa* was attached received weak support, in terms of both posterior probability and BP values (Fig. 2), MULTIDIVTIME and BEAST analyses were repeated omitting this fossil calibration.

Ancestral Range Reconstruction Analyses

To infer ancestral areas and geographic speciation scenarios for *Ruta* and its sister group, we implemented a dispersal–extinction–cladogenesis (DEC) model of range evolution, consisting of 15 component areas, using the program Lagrange (Ree and Smith 2008). The areas, shown in Fig. 1, were taken from Mansion et al. (2008), who defined them on the basis of continental plates or microplates identified in tectonic reconstructions of the Mediterranean basin (Stampfli et al. 1991; Rögl 1999; Krijgsman 2002; Rosenbaum et al. 2002a; Meulenkamp and Sissingh 2003). Species ranges were defined by presence–absence coding (online Appendix 4). The DEC model describes ancestor–descendant transitions between geographic ranges by processes of dispersal (range expansion), local extinction (range contraction), and cladogenesis (range subdivision/inheritance) and defines the likelihood of species-range data arrayed at the tips of a phylogenetic tree as a function of rates of dispersal and local extinction (see Ree and Smith 2008). Fifteen areas yield a set of $2^{15} = 32,768$ theoretically possible geographic ranges (area subsets), many of which were excluded from consideration based on the biological implausibility of their spatial configurations (e.g., wide disjuncts). We defined biologically plausible ranges as those that were “contiguous”: that is, represented connected nodes in an area adjacency graph. We also discarded ranges larger

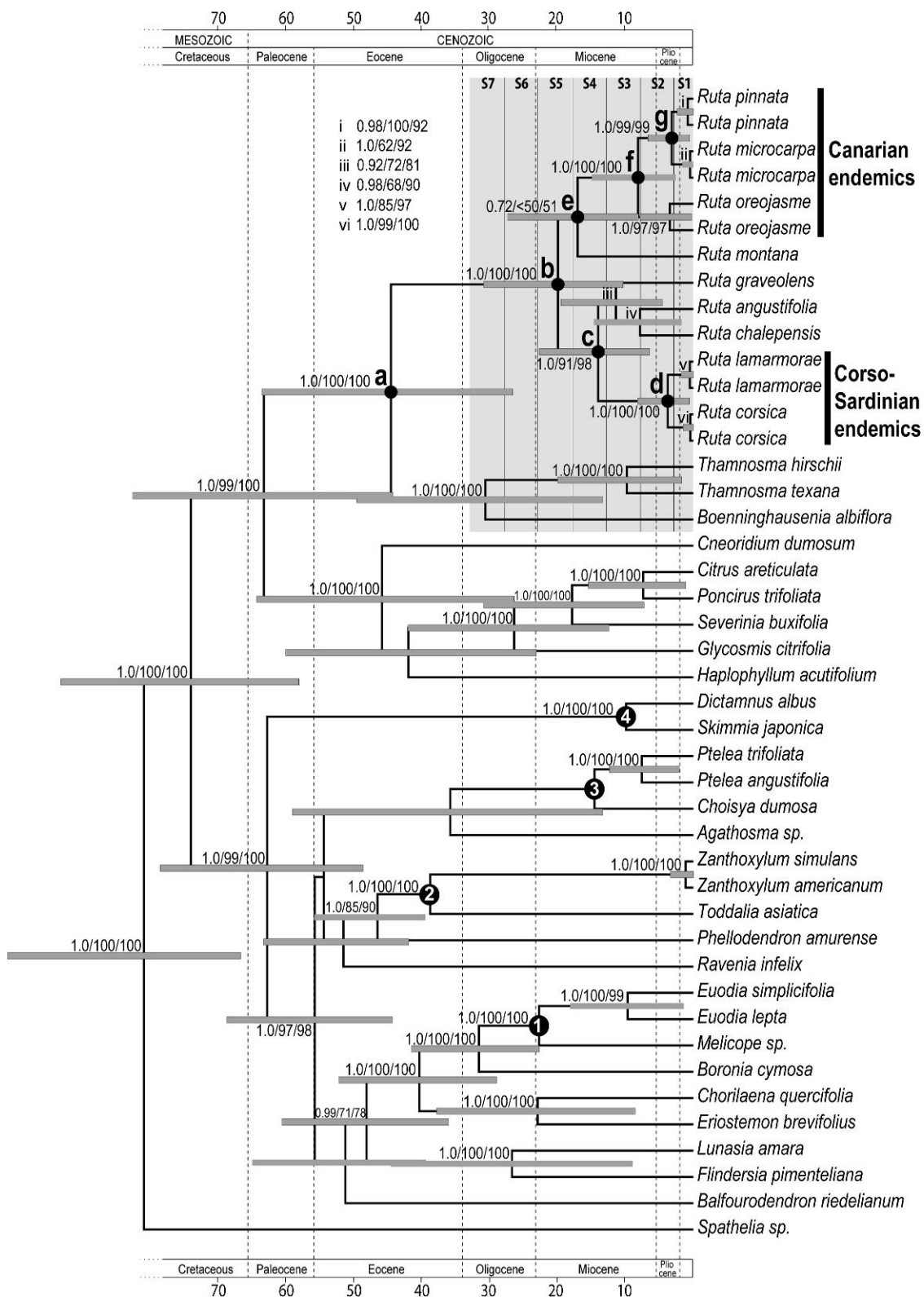


FIGURE 2. Chronogram inferred with BEAST. Maximum clade credibility tree with mean nodal ages and 95% HPD intervals indicated by gray bars. Nodes “a” to “f” indicate nodes of interest; nodes “1” to “4” indicate fossil constraints. Values next to branches represent Bayesian Posterior Probabilities/Maximum Parsimony Bootstrap Percentages/Maximum Likelihood Bootstrap Percentages. The gray rectangle shows the 7 time slices (“S1” to “S7”) used for the biogeographic analyses. The outgroup is not shown.

than two areas in size that were not subsets of observed species ranges (e.g., *R. montana*, with five areas, and *R. chalepensis*, with 10 areas). The motivation for this step was to further reduce the dimensions of the model's transition matrix, thus increasing its computational feasibility. The final number of ranges in the analysis was 412.

Following the principles described in [Ree and Smith \(2008\)](#), we constructed temporal constraints on rates of dispersal between areas based on paleogeographic reconstructions of area position through time ([Carracedo et al. 1998](#); [Anguita and Hernán 2000](#); [Dercourt et al. 2000](#); [Scotese 2001](#); [Rosenbaum et al. 2002a](#); [Meulenkamp and Sissingh 2003](#)). These constraints were implemented as a series of seven time slices. For each, we constructed a matrix of scaling factors (between zero and one) for the dispersal rate between areas according to their geographic position, interpreting greater distances and/or the extent of geographic barriers (e.g., sea straits, mountain chains) as being inversely proportional to the expected rate. All time slices together spanned the past 32.5 myr, with the most recent slice being 2.5 myr in duration, and the rest 5 myr each (Fig. 2 and online Appendix 5).

Analyses using the temporally constrained DEC model were performed on: 1) the maximum clade credibility tree inferred with BEAST; 2) the chronogram inferred with MULTIDIVTIME using the 50% majority rule consensus tree, obtained with MRBAYES, as input tree; and 3) the chronogram inferred with MULTIDIVTIME using the MAP tree, obtained with MRBAYES, as input tree. For each tree, "global" rates of dispersal and local extinction were first estimated without conditioning on any particular ancestral areas at internal nodes. Then, these rates were used to find the ML values of ancestral range, and subdivision-inheritance scenarios at each internal node on the tree. Each node was considered in isolation, without conditioning on any other ancestral states.

RESULTS

Phylogenetic Analyses

The combined cpDNA matrix included a total of 150 sequences, of which 66 were newly generated (GenBank accession numbers are reported in online Appendix 1). The MP analysis yielded 52 most parsimonious trees of 2815 steps, with a consistency index of 0.716 and a retention index of 0.839. The strict consensus of these trees was topologically identical to the 50% majority rule consensus tree found with BI and to the maximum clade credibility tree inferred with BEAST (Fig. 2).

The topology of the BEAST tree was generally well supported in terms of both BP (obtained from both MP and ML analyses) and PP (obtained from the MRBAYES analysis) values, with 12 nodes with BP values <85 and 7 nodes with PP values <0.95, out of 43 nodes (Fig. 2). As in [Salvo et al. \(2008\)](#), both the C-S endemics—*R. corsica* and *R. lamarmorae*—and the Canarian endemics—

R. pinnata, *R. oreojasme*, and *R. microcarpa*—formed strongly supported clades (nodes d and f; BP = 100, PP = 1; Fig. 2). Most importantly, the nodes of interest (nodes a to g, Fig. 2) and the calibration nodes (nodes 1 to 4, Fig. 2) received high support in terms of both BP and PP values, except for node e (i.e., the split between the Canarian endemics and their sister group) and node 3 (i.e., the node to which the fossil *Ptelea enervosa* was attached).

Molecular Dating Analyses

The two separate runs for each MULTIDIVTIME analysis converged on similar posterior distributions. The mean nodal ages and confidence intervals (CIs) inferred for the nodes of interest are shown in Table 1. The sensitivity analyses demonstrated that only the choice of "bigtime" prior affected the resulting age estimates but with only very small effects on the nodes of interest (online Appendix 3).

The BEAST analyses found a high level of substitution rate variation across the sampled sequences, as indicated by the marginal posterior probability of the coefficient of variation of rates: mean = 0.986; 95% highest posterior density (HPD) interval = (0.758, 1.228). Additionally, no evidence of rate autocorrelation between neighboring branches was detected, as indicated by the marginal posterior probability of rate covariance (mean = -0.0151; 95% HPD interval = (-0.177, 0.177)), although this statistic has been found to have relatively low power ([Moore and Donoghue 2007](#); [Ho 2009](#)). The nodal heights and 95% HPD intervals inferred with BEAST are graphically shown in Fig. 2; for the nodes of interest, these values are reported in Table 1. The sensitivity analyses indicated that the choice of different parametric distributions for the calibration nodes did not have an effect on the resulting mean nodal heights and 95% HPD intervals (online Appendix 3). The specification of a root prior, instead, yielded younger mean nodal heights and narrower 95% HPD intervals, as compared with analyses carried out without specifying such a prior (online Appendix 3). We decided to conduct the final BEAST analysis implementing a root prior because this produced narrower 95% HPD intervals and because we did not see any reason why available estimates of the age of the root (i.e., Rutaceae) from the fossil record ([Knobloch and Mai 1986](#)) and from previous phylogenetic findings ([Muellner et al. 2007](#)) should have been excluded. As in the MULTIDIVTIME analysis, the analysis performed omitting the fossil *Ptelea enervosa* yielded similar age estimates to the analysis carried out including this fossil.

Generally, the mean nodal ages estimated with MULTIDIVTIME were older than those inferred with BEAST (black regression line; online Appendix 6). However, when we compared both mean nodal ages (grey regression line; online Appendix 6) and CIs (Table 1) for the nodes within *Ruta* only, the results of both dating methods converged on similar values.

TABLE 1. Results of molecular dating and ancestral range reconstruction analyses

Node of interest	Description	BEAST analysis, mean nodal age (CI)	MULTIDIVTIME analysis, mean nodal age (CI)	Lagrange analysis with BEAST chronogram, ancestral range	Lagrange analysis with MULTIDIVTIME chronograms, ancestral range
a	Origin of <i>Ruta</i>	44.5596 (26.6484–63.5337)	46.2206 (32.0645–62.0346)	Eu + WNAm	Eu
b	Initial diversification of <i>Ruta</i>	19.9653 (10.3709–30.8809)	18.4885 (8.9494–32.3782)	Eu + Ae + An + Ba + Ib + Ap + Co + Sa + Ar + NAF	Eu + Ae + An + Ba + Ib + Ap + Co + Sa + Ar + NAF
c	Origin of the C-S lineage	14.0183 (6.4570–22.6956)	15.1908 (7.0254–27.6679)	Eu + Ae + An + Ba + Ib + Ap + Co + Sa + Ar + NAF	Co
d	Split between <i>R. corsica</i> and <i>R. lamarmorae</i>	3.7575 (0.5616–8.2029)	3.0826 (0.4987–8.6193)	Sa + Co	Sa + Co
e	Origin of the Canarian endemics	17.0426 (8.1353–27.3673)	16.8132 (7.9005–29.9693)	NAf	NAf
f	Split between <i>R. oreojasme</i> and the rest	8.1351 (2.6191–14.9420)	6.9065 (2.2322–15.7054)	Go	Go
g	Split between <i>R. pinnata</i> and <i>R. microcarpa</i>	3.1749 (0.5541–6.6831)	2.7843 (0.4336–8.1470)	Te + Go	Te + Go

Notes: The nodes of interest refer to Figure 2. CI = confidence interval: for BEAST, this refers to the 95% HPD intervals; for MULTIDIVTIME to the 95% credibility intervals. For area abbreviations, see Figure 1.

Ancestral Range Reconstruction Analyses

For each node in each of the three chronograms inferred using MULTIDIVTIME and BEAST, Lagrange estimated the likelihoods of all possible ancestral range reconstructions (online Appendix 7). We used the ML values for further analysis, acknowledging that estimation error associated with statistical uncertainty leaves the door open to alternative interpretations. Lagrange analyses of the BEAST chronogram produced a higher likelihood score and global dispersal rate than analyses of the MULTIDIVTIME chronograms (online Appendix 7). Across trees, reconstructions were identical for the initial diversification of *Ruta*, the split between *R. corsica* and *R. lamarmorae*, the origin of the Canarian endemics, the split between *R. oreojasme* and the rest, and the split between *R. pinnata* and *R. microcarpa* but differed for the origin of *Ruta* and the C-S lineage (Table 1). However, in the last two cases, the ancestral areas inferred with the MULTIDIVTIME trees were always a subset of the areas inferred with the BEAST tree; hence, biogeographic scenarios were based on the reconstructions obtained with the latter tree. Generally, nodes that are separated by long branches, such as those inferred with MULTIDIVTIME, correspond to a more restricted range because longer branches allow more time for dispersal events, which can widen the range, to happen (see Smith 2009).

DISCUSSION

The Ancestor of Ruta Invaded the Mediterranean Region from the North before the Onset of the Mediterranean Climate

The molecular dating and ancestral range analyses suggested that *Ruta* diverged from its sister group, comprising the East Asian *Boeninghausenia* and the disjunct Afro-American *Thamnosma*, in the middle Eocene, in an area comprising Eurasia and western North America (Fig. 3: node a, I, A; Table 1). At that time, the Bering land bridge (Marincovich and

Gladenkov 2001) might have allowed the ancestor of *Ruta* to attain such a distribution. In the middle Eocene, southern Europe was still an archipelago and the Atlantic and Indian Oceans were connected via the Tethys Sea (Rögl 1999; Fig. 3A). When *Ruta* started to diversify, in the early Miocene, the paleogeographic setting of the developing Mediterranean basin had dramatically changed (Fig. 3: node b, I, B; Table 1). Two key tectonic events occurred between the middle Eocene and early Miocene, which might have facilitated the major southward range expansion of the genus from Eurasia to the margins of the proto-Mediterranean basin, as suggested by the ancestral range reconstruction analyses (Table 1). The progressive accretion of the microplates located between the Paratethys and Tethys Seas (Fig. 3A) caused the formation of a more-or-less continuous landmass, extending from the proto-Iberian peninsula to Asia Minor, in the late Oligocene-early Miocene, roughly between 25 and 20 Ma (Fig. 3B; Rögl 1999; Meulenkamp and Sissingh 2003). This new geological configuration allowed biotic exchange between the eastern and western proto-Mediterranean basin, resulting in the circum-Mediterranean distribution of many terrestrial organisms (e.g., butterflies, crane flies, scorpions, frogs, newts, beetles, Asteraceae, *Cyclamen*; Steininger et al. 1985; Oosterbroek and Arntzen 1992; Palmer and Cambefort 2000; Sanmartín 2003; Oberprieler 2005; Mansion et al. 2008; Micó et al. 2009; Yesson et al. 2009). This landmass might have facilitated land migration of the ancestor of *Ruta* around the proto-Mediterranean basin. Around 20 Ma, the collision of the African and Arabian plates with the Anatolian microplate, resulting in the interruption of the Tethys Sea, caused the formation of a land corridor between Africa and Eurasia across Arabia and Asia Minor (Hallam 1981; Rögl 1999; Krijgsman 2002; Lomolino et al. 2006). This almost-continuous land corridor, which was crucial for the Eurasian-African faunal exchange during the early Miocene (Coryndon and Savage 1973; Steininger et al. 1985), likely facilitated

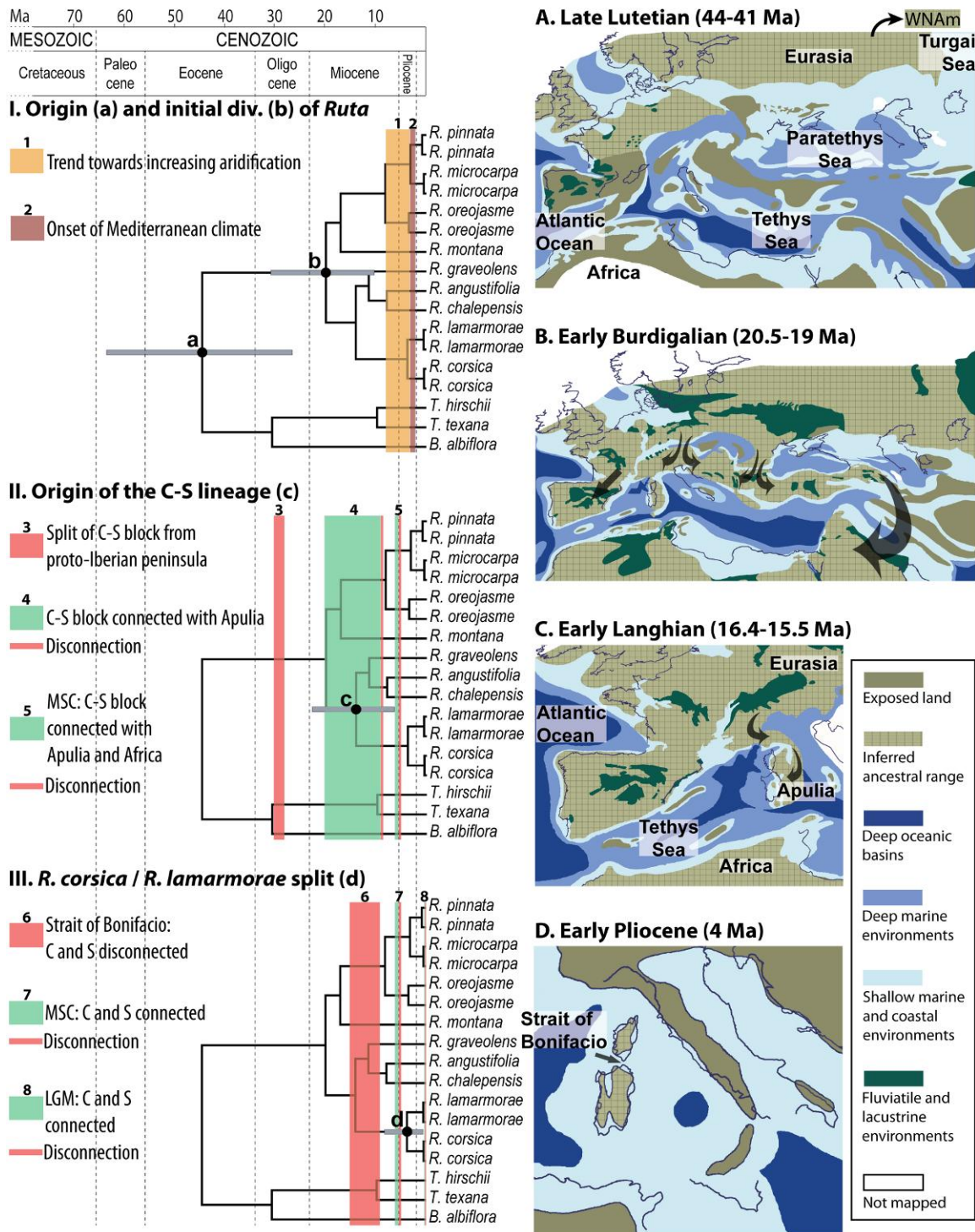


FIGURE 3. Biogeographic scenarios for *Ruta* and its C-S endemics. On the left, dated phylogeny for *Ruta* and its sister group (taken from Fig. 2) showing I, the origin (node a) and initial diversification (i.e., div.; node b) of *Ruta*; II, the origin of the C-S lineage (node c); and III, the split between *R. corsica* (endemic to Corsica, C) and *R. lamarmorae* (endemic to Sardinia, S; node d). Vertical, colored bars represent the time windows of climatic/geologic events hypothesized to have affected the biogeography of *Ruta*: (1) beginning of a trend toward increasing aridification = 9–8 Ma (Ivanov et al. 2002; Van Dam 2006); (2) onset of the Mediterranean climate = ~3–2 Ma (Suc 1984; Thompson 2005); (3) split between the C-S microplate and the proto-Iberian peninsula = 30–28 Ma (Alvarez et al. 1974; Rosenbaum et al. 2002a); (4) C-S block connected with Apulia, then disconnected from the Apulian microplate = 20–9 Ma (Cherchi and Montadert 1982; Rosenbaum et al. 2002a; Speranza et al. 2002; Rosenbaum and Lister 2004); (5 and 7) MSC = 5.96–5.33 Ma (Gover et al. 2009); (6) formation of the Strait of Bonifacio = 15–9 Ma (Alvarez et al. 1974; Cherchi and Montadert 1982); (8) LGM = 0.02 Ma (Lambeck et al. 2004; Lambeck and Purcell 2005). On the right, paleo-maps associated with the nodes of interest, with corresponding letter. Maps A, B, and C were modified from Dercourt et al. (2000); map D was modified from Cavazza et al. (2004).

the expansion of the ancestor of *Ruta* from Eurasia, through the Arabian plate, to North Africa (Fig. 3B).

The temporal envelopes for the origin of *Ruta* (node a) and its subsequent invasion of the Mediterranean basin (Fig. 3: node b, I; Table 1) precede both the trend toward increasing aridification, starting 9–8 Ma (Ivanov et al. 2002; Van Dam 2006), and the onset of the Mediterranean climate (Suc 1984; Thompson 2005). Although the onset of Mediterranean-type climates has been shown to trigger radiations in some plant groups (e.g., *Pelargonium*, Cape region, Bakker et al. 2005; *Drosera*, southern Australia, Yesson and Culham 2006), no temporal overlap between this climatic event and diversification has been detected in others (e.g., *Anthemis*, Mediterranean region, Lo Presti and Oberprieler 2009; *Protea*, Cape region, Barraclough and Reeves 2005). Perhaps, in the latter instances, the filtering of elements from pre-existing regional species pools and the arrival of others by dispersal from surrounding regions might have prevented the in situ, climatically driven diversification of lineages (Donoghue 2008). In fact, it has been suggested that the filtering of elements from the ancient geofloras that spread across the Northern Hemisphere during the Tertiary (Tertiary geofloras; Wolfe 1975, 1978; Hickey et al. 1983; Tiffney 1985) played a crucial role in the assembly of the Mediterranean floristic diversity (Thompson 2005; Ackerly 2009). For example, Mai (1989) concluded that the elements characteristic of the Mediterranean sclerophyll vegetation originated in the Tertiary geofloras. In particular, taxa such as *Quercus* and *Pistacia*, commonly associated with *Ruta* (Bonet 1992), were derived from the mixed mesophytic forest, a deciduous forest that formed in the Northern Hemisphere during the Oligocene due to the mixing of two Tertiary geofloras, the boreotropical forest and the Arcto-Tertiary geoflora (Tiffney 1985; Mai 1989). Interestingly, the inferred ancestral area for the origin of *Ruta* (Fig. 3I, A) overlaps with the proposed range of the mixed mesophytic forest both in space and in time (Kvacek et al. 2006; Kürschner and Kvacek 2009). Unfortunately, the poor fossil record of *Ruta* (Gregor 1989) fails to provide any empirical evidence for or against the inferred northern origin of the genus.

In summary, the results of our integrated molecular dating and ancestral range reconstruction analyses indicate that *Ruta* originated ex situ (i.e., outside of the Mediterranean region), in a large area comprising Eurasia and western North America, well before the onset of the Mediterranean climate (Fig. 3I, A). At that time, the Northern Hemisphere was covered predominantly by the boreotropical forest (Axelrod 1975; Mai 1989; Thompson 2005). It then expanded its range southward, invaded several landmasses situated around the forming Mediterranean basin (Fig. 3I, B), and diversified in situ as the climate changed from subtropical to Mediterranean. Such diversification was probably driven by the geologic complexity of the region, characterized by the appearance and disap-

pearance of barriers to dispersal, as found for other Mediterranean groups (Palmer and Camberfort 2000; Sanmartín 2003; Oberprieler 2005; Thompson 2005; Mansion et al. 2008).

The Origin of the C-S Lineage Is Better Explained by the Separation between the C-S and Apulian Microplates in the Miocene than by the Fragmentation of the Hercynian Massif in the Oligocene

Island endemics have been traditionally divided into two groups: paleo-endemics and neo-endemics (Favarger and Contandriopoulos 1961; Stebbins and Major 1965; Bramwell 1972; Mansion et al. 2009). The former are ancient lineages, often relict elements of once-widespread groups, which are geographically and taxonomically isolated from their closest extant relatives and show little geographic variation; the latter are more recently evolved and exhibit geographical and taxonomic proximity to their closest extant relatives (Cardona and Contandriopoulos 1979; Thompson 2005). Due to their narrow distribution—being present in only a few, isolated mountains of Corsica and Sardinia—and their morphological distinctness, as compared with other congeneric species (Bacchetta et al. 2006), *R. corsica* and *R. lamarmorae* have long been considered as relictual, paleo-endemic species (Cardona and Contandriopoulos 1979; Arrigoni 1983; Thompson 2005), their origin being linked to the Oligocene separation of the C-S microplate from the proto-Iberian peninsula (Gamisans 1975).

The molecular dating and ancestral range reconstruction analyses indicated that the ancestor of the two C-S endemics and their widespread sister clade occurred in a broad area, ranging from Eurasia to North Africa, during the middle Miocene (Fig. 3: node c, II, C; Table 1). At that time, the C-S microplate was connected to the Apulian microplate (Fig. 3C; Cherchi and Montadert 1982; Deino et al. 2001; Rosenbaum et al. 2002a; Speranza et al. 2002; Rosenbaum and Lister 2004), suggesting an invasion of the former via land migration through the latter. The subsequent separation between these two landmasses might have driven the allopatric divergence of the ancestor of *R. corsica* and *R. lamarmorae* from the mainland relatives. A visual inspection of the chronogram (Fig. 3II) indicates that the time window associated with the connection between the C-S and Apulian microplates is much larger than the time windows associated with other events. This is the result of the uncertainty surrounding this tectonic event (Robertson and Grasso 1995). In particular, the formation of the Tyrrhenian Sea, which terminated the above-mentioned connection, is one of the most complicated aspects of the geologic history of the Mediterranean basin because it is the result of the interaction of 3 mountain chains—the Alps, the Apennines, and the Maghrebides (Selli 1985). Nevertheless, the envelope of uncertainty surrounding the above-mentioned connection overlaps with the CI for the age of the split

between the ancestor of the C-S endemics and their sister clade (Fig. 3II), strongly suggesting that both processes of land migration through the temporary C-S block/Apulia land corridor and allopatric speciation driven by the disruption of such corridor played a role in the origin of the C-S endemic lineage. These results emphasize the role of land connections between the C-S microplate and adjacent areas during the migration of the C-S block toward its current position in the assembly of the C-S flora, as first suggested by Braun-Blanquet (1926).

Mansion et al. (2009) proposed an explicit spatio/temporal approach to discriminate between island paleo- and neo-endemics. If a speciation event postdated the formation of either an oceanic or continental fragment island, resulting in the in situ divergence of the island endemic from its closest relative, then the island endemic was classified as "neo-endemic." If the speciation event is driven by or precedes island formation, the island endemic was classified as "paleo-endemic," that is, either a surviving element of the ancestral continental biota on the island or an immigrant that might have originated ex situ and colonized the island after its formation via LDD. With respect to continental fragment islands, it is important to pinpoint the geologic event that led to the separation from the continent, which can be used as temporary boundary for the classification of the islands' endemic species as either paleo- or neo-endemic. Although the connection of Corsica and Sardinia with the proto-Iberian peninsula has been known for a relatively long time (Alvarez et al. 1974; Boccaletti and Guazzone 1974), the subsequent geologic events involving the interaction of the two islands with the Apulian microplate have been elucidated only recently and are more controversial (Rosenbaum and Lister 2004; Rosenbaum et al. 2008). Thus, biogeographers have traditionally focused on the fragmentation of the Hercynian massif (30–28 Ma) to classify the C-S endemic flora into paleo- or neo-endemic species (e.g., Gamisans 1975; Cardona and Contandriopoulos 1979), rather than on subsequent tectonic events. However, because the CI for the divergence between the C-S endemic lineage and its closest relatives overlaps with the temporal window of the separation between the C-S and Apulian microplates (Fig. 3II), the continental fragment island endemics can still be considered paleo-endemics, but in relation to this later geologic event, rather than the initial splitting of the C-S microplate from the proto-Iberian peninsula.

The Split between the Corsican and Sardinian Endemics Is Better Explained by Events Associated with the MSC than by the Formation of the Strait of Bonifacio

Salvo et al. (2008) conjectured that the divergence between *R. corsica* and *R. lamarmorae* might have been caused by vicariance driven by the geologic formation of the Strait of Bonifacio in the middle Miocene (15–9 Ma; Alvarez et al. 1974; Bonin et al. 1979; Orsini

et al. 1980; Cherchi and Montadert 1982). However, subsequent climatic events might have also caused such divergence, for example, the end of the MSC (Rögl and Steininger 1983; Gover et al. 2009) and the end of the LGM (Lambeck et al. 2004; Lambeck and Purcell 2005), which marked the re-flooding of the Strait of Bonifacio.

The ancestral range reconstruction analyses inferred a potential area of distribution for the ancestor of *R. corsica* and *R. lamarmorae* comprising Corsica and Sardinia (Fig. 3: node d, III, D; Table 1), thus congruent with a scenario of vicariant speciation. However, the molecular dating analyses estimated an age of 4–3 Ma for the split between the two species (Fig. 3III; Table 1). This estimate and its CI postdate the formation of the Strait of Bonifacio, indicating that the divergence between the two C-S endemics was not associated with this tectonic event. Instead, the CI for the split between *R. corsica* and *R. lamarmorae* overlaps with the end of the MSC (5.33 Ma), when the flooding of the Mediterranean basin caused the renewed separation between the two islands (Fig. 3III).

The MSC has been repeatedly invoked to explain current distributional patterns of different Mediterranean taxa (Bocquet et al. 1978; Palmer and Cambefort 2000; Sanmartín 2003; Mansion et al. 2009). The drying out and re-flooding of the Mediterranean basin is a plausible mechanism by which barriers to terrestrial dispersal were removed and created, respectively (Yesson et al. 2009). Altaba (1998), however, cast doubt on the biogeographical significance of this event because 1) the paleogeography of the Mediterranean basin during the MSC is still unclear (Meijer and Krijgsman 2005), 2) dispersal across a hot, saline desert may have been difficult for most organisms, and 3) most biogeographic studies of the MSC have found a link between differentiation in terrestrial taxa and the onset of the MSC, whereas such differentiation should be connected with the refilling of the basin.

The two C-S endemics are restricted to the main mountainous areas of Corsica and Sardinia, growing above 1000 m.a.s.l. (Fig. 1). Moreover, they are very specialized in their habitat requirements, occurring mainly on siliceous substrates and amid the *Carici-Genistetea lobelii* plant community (Bacchetta et al. 2006). With these facts in mind, an interesting line of future research concerns the reconstruction of ancestral ecological niches, by using the techniques of phyloclimatic modeling (Yesson and Culham 2006), in order to determine which parts of the inferred ancestral range (i.e., Corsica and Sardinia) might have been environmentally suitable.

The Colonization of the Canary Islands by the Canarian Clade of Ruta Does Not Conform to a Stepping-Stone Model and Its Origin Predated the Onset of the Mediterranean Climate

The phylogenetic analyses showed that the Canarian endemics—*R. pinnata*, *R. oreojasme*, and *R. microcarpa*—form a monophyletic group (Fig. 2: node f), as in the

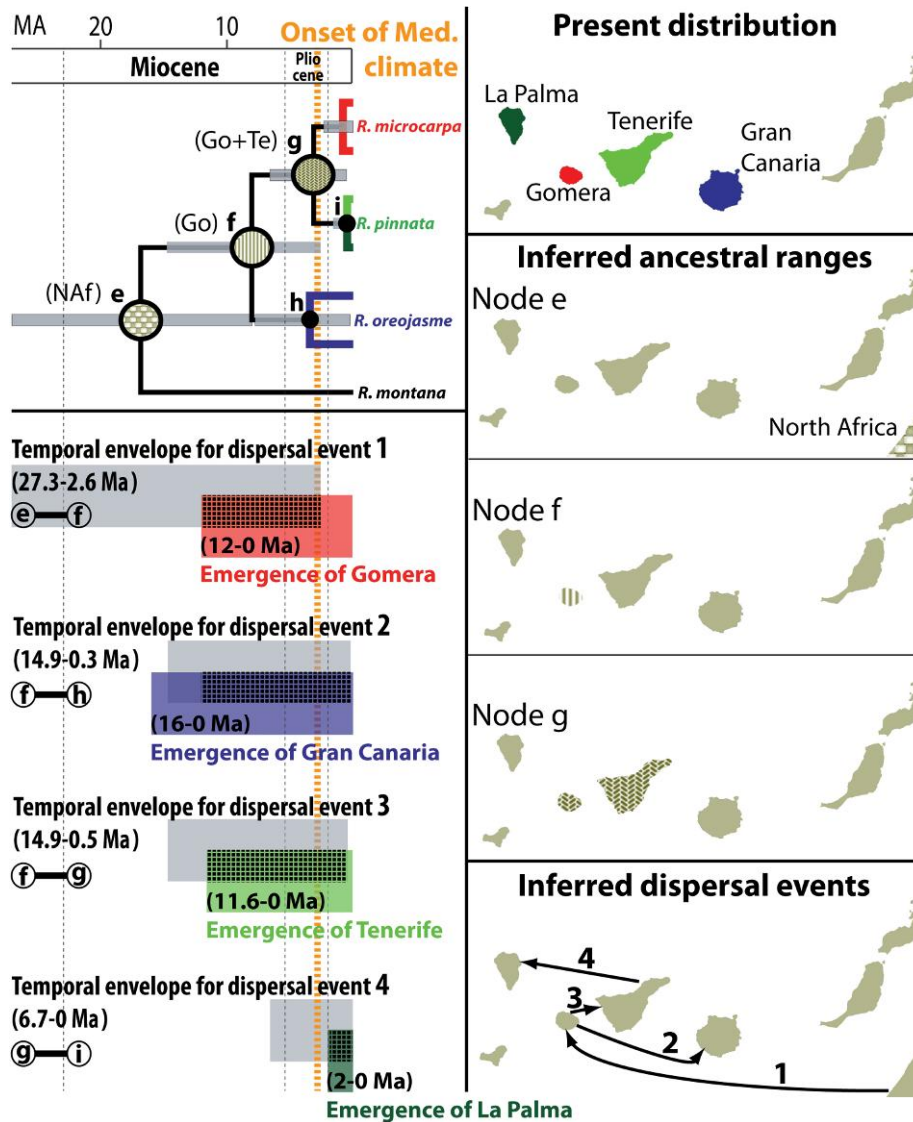


FIGURE 4. Biogeographic scenario for the Canary Islands clade of *Ruta*. Right, top: present distribution of the three Canary endemics. Right, middle: maps of the Canary Islands associated with nodes e, f, and g, showing ancestral ranges reconstructed with Lagrange (reported also on the tree on the left; Table 1). Right, bottom: inferred, most-parsimonious number of dispersal events, indicated by arrows. Left, top: dated phylogeny for the Canary endemics and their sister group; node “e”: split between the Canary endemics and *R. montana*; node “f”: split between *R. oreojasme* and the rest; node “g”: split between *R. microcarpa* and *R. pinnata*; node “h”: crown node of *R. oreojasme*; node “i”: crown node of *R. pinnata*. Left, bottom: gray rectangles: temporal envelopes for dispersal events 1–4, inferred along internodes connecting nodes e and f, f and h, f and g, and g and i, respectively, spanning CIs of nodes bracketing internodes (Table 1); colored rectangles: time intervals between island emergence (oldest estimate) and present time; hatched pattern: most likely time window for island colonization resulting from overlap between temporal envelopes for dispersal events and island emergence. Dashed orange line: onset of Mediterranean climate (~3 Ma; Fernández-Palacios et al. 2008). Ages of the Canary Islands from Carracedo et al. (1998) and Anguita and Hernán (2000).

majority of Canary genera so far investigated (Cavazza et al. 2004), indicating that they were the product of a single colonization event into the islands (Fig. 4). The ancestral range reconstruction analyses inferred North Africa to be the source area of such colonization event (Fig. 4: node e; Table 1), as commonly found in other groups (Cavazza et al. 2004). Due to the dynamic geologic history of the Canary archipelago (Whittaker et al. 2008), biogeographic scenarios involving numerous interisland dispersal events, also

to islands that are no longer emerged, are possible. The most parsimonious interpretation of the inferred ancestral areas and present distribution of the Canary endemics requires a minimum of four dispersal events: one from North Africa (the ancestral area inferred for node e) to Gomera (the ancestral area inferred for node f); one from Gomera to Gran Canaria; one from Gomera to Tenerife (one of the ancestral areas inferred for node g); and one from Tenerife to La Palma (Fig. 4, right; Table 1).

By integrating evidence from the molecular dating results and the geologic history of the Canary Islands, we are able to add a temporal dimension to the hypothesized dispersal events. Because these events are inferred along internodes, the temporal uncertainty associated with the nodes bracketing the internodes in question has to be taken into account. For example, we hypothesize a temporal envelope for dispersal event 1, which was inferred along the internode connecting nodes e and f, spanning the lower bound of the CI of node e (27.3 Ma) and the upper bound of the CI of node f (2.6 Ma; Fig. 4, left; Table 1). We proceed in a similar fashion for the inferred dispersal events 2, 3, and 4 (Fig. 4). Overlap between island emergence (Carracedo et al. 1998; Anguita and Hernán 2000) and temporal envelopes for each dispersal event strongly suggests that the most likely time windows of island colonization were 12–2.6 Ma for Gomera, 14.9–0.3 Ma for Gran Canaria, 11.6–0.5 Ma for Tenerife, and 2–0 Ma for La Palma (Fig. 4, left). Because Gomera was the source area for the colonization of Gran Canaria, dispersal event 2 could not have predated dispersal event 1.

Three main modes of species diversification in the Canary Islands have been identified: 1) stepping-stone, with a single colonization event from the mainland followed by colonization events proceeding from older to younger islands; 2) multiple independent colonization events from the mainland, followed by within-island speciation; and 3) interisland colonization between similar ecological habitats (Sanmartín et al. 2008). For the Canarian endemics of *Ruta*, only one colonization event from the mainland was inferred and, hence, mode of diversification (2) can be ruled out.

Because Gran Canaria is older, and closer to the mainland, than Gomera (Carracedo et al. 1998; Anguita and Hernán 2000) and because the sequence of splitting events within the Canarian clade is congruent with the order of island formation, a stepping-stone mode of island colonization seemed plausible. However, Gomera, and not Gran Canaria, was inferred as the ancestral range for node f with both BEAST and MULTIDIVTIME chronograms, suggesting that Gomera was the first island to be colonized by *Ruta* and casting doubt on a stepping-stone biogeographic scenario (Fig. 4). This counterintuitive result might depend on the uncertainty surrounding the Lagrange reconstructions (online Appendix 7; see also Ree and Smith 2008) and the complexity of the underlying temporally constrained DEC model (e.g., Clark et al. 2008). Therefore, our proposed pathways for the colonization of the Canary Islands should be viewed as a starting hypothesis that can be tested in future studies based on expanded infraspecific and interisland taxon sampling.

R. pinnata, *R. oreojasme*, and *R. microcarpa* are confined to the same vegetational zone, thermophilous scrubland (Bramwell and Bramwell 2001), which is a recent ecosystem, believed to have originated concomitantly with the onset of the Mediterranean climate (~3 Ma; Fernández-Palacios et al. 2008). For this reason, these

species were assumed to have originated together with the onset of such climate. However, the inferred temporal windows for the initial invasion of the Canary archipelago and subsequent island colonizations (i.e., except for La Palma) precede the onset of the Mediterranean climate (Fig. 4, bottom left) and overlap with a time when the Canary Islands were mainly covered by laurel and pine forests (Fernández-Palacios et al. 2008). This suggests that: 1) when *Ruta* colonized the Canary Islands, the islands' vegetation was very different than at present; 2) in order to persist under the novel Mediterranean climate/vegetation, *Ruta* likely changed its ecological requirements; 3) the filtering of the Canarian endemics into thermophilous scrublands after the onset of the Mediterranean climate occurred in parallel in different islands; and 4) interisland colonization between similar ecological habitats cannot be readily endorsed because it does not take into account the different time windows inferred for the colonization of the different islands. Again, the application of ecological niche modeling approaches within a phylogenetic framework might help to elucidate some of these issues.

CONCLUSIONS

Our study demonstrates that the integration of different sources of information from phylogenetics, molecular dating, ancestral range reconstruction, and geologic/paleoclimatic models is indispensable for explaining biogeographic patterns. Additionally, the clear formulation of a hypothesis-based framework at the onset of the research helps to avoid the construction of a posteriori biogeographic scenarios. With respect to island biogeography theory (MacArthur and Wilson 1967; Whittaker and Fernández-Palacios 2007; Whittaker et al. 2008), our study stresses the importance of temporary land connections in the biotic assembly of continental fragment islands and of determining discrete time windows of island colonization in order to better understand distributional patterns in oceanic islands (Carine 2005; Kim et al. 2008).

The paleogeographic and paleoclimatic settings in which biodiversity evolved should be carefully incorporated in biogeographic studies, as implemented in recently developed approaches to ancestral range reconstruction (i.e., Lagrange; Ree and Smith 2008). Inferred areas, however, can only indicate the maximum possible extent of the distribution of an ancestor. To achieve more realistic estimates of ancestral distributions, methods for the projection of ancestral ecological niches into paleoclimatic and paleogeographic configurations need to be further developed (Yesson and Culham 2006; Evans et al. 2009). Within *Ruta*, the application of niche modeling tools will be fundamental to achieve a more complete understanding of the relative roles of geologic versus climatic factors in speciation and of niche conservatism versus niche evolution in shaping distributional patterns in the Mediterranean region (Donoghue 2008; Ackerly 2009).

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.sysbio.oxfordjournals.org/>.

FUNDING

This work was supported by the Swiss National Science Foundation grant no. 3100A0-105495 to E.C. and the Institute of Systematic Botany of the University of Zürich. Additional financial support from the Georges-und-Antoine-Claraz-Schenkung, the Kommission für Reisestipendien der Schweizerischen Akademie der Naturwissenschaften (SANW), and the Equal Opportunity Office of the University of Zurich are gratefully acknowledged.

ACKNOWLEDGMENTS

The authors would like to thank B. Keller, J. de Vos, S. Manafzadeh, and L. Granato for their help and comments on an early draft; G. Bacchetta, A. Bertini, J. M. Fernández-Palacios, W. Krijgsman, A. Antonelli, and S. Magallón for stimulating discussions; G. Mansion for help in the field; M. Pirie for help with r8s and BEAST; J. Fuertes-Aguilar and A. Santos-Guerra for material from the Canary Islands; L. Hugot for collecting permits in Corsica; B. Seitz and E. Benetti for help in the library; Jack Sullivan, Dan Faith, Isabel Sanmartín, one anonymous reviewer, and Deborah Ciszek for constructive criticism on the manuscript.

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