



Testing the biogeographical congruence of palaeofloras using molecular phylogenetics: snapdragons and the Madrean–Tethyan flora

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ABSTRACT

Aim The biogeographical congruence hypothesis, that similar spatiotemporal patterns of geographical distribution exist across lineages, is revisited in this study, and biogeographical processes in presumed Madrean–Tethyan plants are investigated by employing phylogenetic analyses.

Location Mediterranean and Californian floristic regions.

Methods The snapdragons (tribe Antirrhineae, Plantaginaceae) are one of the plant groups that best illustrate disjunctions between the New World (14 genera) and the Old World (15 genera). A time-calibrated phylogeny (*ndhF* sequences) and ancestral-area reconstructions were used to test the hypothesis of biogeographical congruence. We estimated support for sister-group relationships together with the probability of temporal congruence of snapdragons and five additional angiosperm groups using a biogeographical approach based on Bayesian inference, parsimony and maximum-likelihood methods.

Results Synchronous divergences of four phylogenetically independent Mediterranean/Californian lineages within Antirrhineae were inferred for the Miocene. This result constitutes the first example of high biogeographical congruence within the same plant group. Analyses of five additional angiosperm groups previously considered exemplars of Madrean–Tethyan disjunctions revealed a total of 10 Mediterranean/Californian sister-group lineages, mostly with Miocene divergence times. In particular, our contrasting biogeographical analysis favoured a prevalent colonization process post-dating the separation of America and Eurasia (Eocene) for at least eight angiosperm lineages.

Main conclusions Explicit testing of the Madrean–Tethyan hypothesis did not support predominant vicariance for Mediterranean/Californian sister groups as previously proposed. Instead, eight Mediterranean/Californian sister-group lineages displayed a Miocene divergence, including considerable biogeographical congruence within Antirrhineae (four independent lineages) and Cistaceae (two lineages).

Keywords

Antirrhineae, Arbutoideae, California, *Cercis*, Cistaceae, disjunct distributions, Geraniaceae, Mediterranean floristic regions, *Platanus*, vicariance.

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INTRODUCTION

Lineages with discontinuous distributions provide ideal systems in which to investigate biogeographical processes. The lure of understanding fragmented distributions has historically led biogeographers to recognize different categories of

intercontinental disjunctions (Raven, 1972; Thorne, 1972). Two main hypotheses underlying disjunct distributions have historically been tested: vicariance (fragmentation of a widespread distribution due to the formation of an isolating barrier) and long-distance dispersal (overcoming of a pre-existing barrier by propagules that give rise to disjunct

populations) (Crisp *et al.*, 2011). Under the vicariance hypothesis, the formation of the barrier coincided with the origin of the disjunction, whereas in the dispersal hypothesis, the barrier is assumed to have pre-dated the disjunction (Morrone & Crisci, 1995; Crisp *et al.*, 2011). Historical biogeography (palaeobiogeography) is concerned not only with barriers and past distributions of particular species groups and their relatives, but also with range shifts and the divergence times of independent lineages.

One of the most intriguing and least studied biogeographical patterns is biogeographical congruence, in which not only do two or more lineages share the same geographical distribution, but this distribution also originated during the same time period (Cunningham & Collins, 1994). In other words, synchronous vicariance or dispersal events reflect shared historical processes. A comparable, but very different, pattern is biogeographical pseudocongruence, which occurs when the common geographical distribution was established at different times, and is thus potentially attributable to different biogeographical processes and conditions (Page, 1990; Cunningham & Collins, 1994; Wen, 1999; Donoghue & Moore, 2003). In order to distinguish between biogeographical congruence and pseudocongruence for lineages that share discontinuous distributions, a temporal framework is required to examine whether disjunctions occurred synchronously and hence may be the result of common processes (congruence), or asynchronously and thus might be attributable to different processes (pseudocongruence) (Donoghue & Moore, 2003).

Fossil occurrences assist in reconstructing distribution patterns over a period of time, but their fragmentary distribution results in many large temporal and spatial gaps. Molecular phylogenetics can provide a reliable spatiotemporal reconstruction of biogeographical events, especially when complemented by chorology, palaeontology and palaeoecology. Therefore, the use of sophisticated molecular phylogenetic methods (see Ronquist & Sanmartín, 2011) has led to detection of an increasing number of coincidental patterns of historical biogeography of animals and plants (Donoghue & Smith, 2004).

Some of the most-studied disjunctions are north temperate discontinuous distributions that include lineages distributed in Asia, Europe and North America (Raven, 1972). The Madrean–Tethyan hypothesis suggests that the formation of two floristic regions (Mediterranean and Californian) that have a summer-drought climate in about the same period of the year (May–September) may be the result of a vicariance process and persistence since the early Tertiary (Palaeogene) (Axelrod, 1975; Raven & Axelrod, 1978). Both the Atlantic Ocean and the summer-humid climate of eastern North America constitute significant present-day dispersal barriers between these two floristic regions. Taxa that occur in both regions have been considered to be representatives of the ancient (early Tertiary) Madrean–Tethyan flora (see Raven & Axelrod, 1978), which consisted of plants in summer-dry areas of the Madrean region (present Sierra Madre Occidental and California in south-western North America) and the Tethys region

(the present Mediterranean basin) (see Takhtajan, 1986). By the later Palaeogene, sclerophyllous vegetation adapted to the expanding dry climate is thought to have formed a dominant, nearly continuous belt across North America and Eurasia (Axelrod, 1975). The taxonomic affinity between plants of the Mediterranean and Californian regions has been hypothesized to reveal a historical signal of ancient Madrean–Tethyan vicariance that has persisted in disjunct Mediterranean-like climates after the opening of the Atlantic Ocean in the Eocene (Thorne, 1972; Axelrod, 1975; Tiffney & Manchester, 2001).

The Madrean–Tethyan vicariance hypothesis has been challenged by a number of molecular phylogenetic studies, primarily in the last two decades (Fritsch, 1996; Manos & Donoghue, 2001). Wen & Ickert-Bond (2009) comprehensively reviewed molecular phylogenies addressing the current disjunction of Madrean–Tethyan plant groups. They reviewed phylogenetic relationships and divergence times between lineages of the two regions and concluded: (1) that vicariance appears to be unlikely given the roughly estimated recent divergence times (< 25 Ma); (2) that convergent evolution may have reinforced morphological and floristic similarity; and (3) that the predominant direction of migration/dispersal was from the Old World to the New World. However, they acknowledged that most of the 14 angiosperm study groups used for the analysis lacked fossil calibrations and an explicit methodology for estimation of divergence times. Preliminary dating results yielded a wide range of divergence times (Eocene to Pleistocene). Likewise, Kadereit & Baldwin (2012) reviewed the same plant groups, and additional data from other groups, and also found that lineages were connected by migrations at different times (but mostly in the Miocene). The question, however, remains as to whether a high degree of biogeographical congruence can be statistically supported for Madrean–Tethyan lineages (Crisp *et al.*, 2011). In particular, to what extent do coincident timings of colonization pre-date the (Eocene) North America–Europe split and therefore constitute a vicariance process of divergence?

It has been suggested that the tribe Antirrhineae (snapdragons) is one of the best candidates to test the biogeographical congruence hypothesis (hereafter BCH) (Raven & Axelrod, 1978), because at least three lineages in the tribe have been found to display a similar Madrean–Tethyan distribution pattern (Vargas *et al.*, 2004). The main objective of this study is therefore to test the spatiotemporal lineage divergence patterns of tribe Antirrhineae in order to account for the origin of the Madrean–Tethyan disjunction and to disentangle biogeographical congruence and pseudocongruence in snapdragons. In addition, to explicitly test the hypothesis of a vicariant origin of the Madrean–Tethyan palaeoflora, we reanalysed existing molecular data for five of the 28 angiosperm groups proposed by Raven & Axelrod (1978). Our working hypothesis is that there is biogeographical congruence within Antirrhineae and with the five additional Madrean–Tethyan angiosperm groups, owing to synchronous divergent events. Finally, we revisit the concept of

biogeographical congruence and propose essential conditions to be met by any group under the BCH.

MATERIALS AND METHODS

Testing the biogeographical congruence hypothesis

Phylogenetic inference can help provide the evidence required to test the BCH for the Madrean–Tethyan flora, specifically: (1) common ancestry of plants involved in the Old/New World disjunction as hypothesized based on taxonomy; (2) an unequivocal sister relationship between Mediterranean and Californian clades; (3) availability of appropriate fossil-based calibration points and estimates of molecular substitution rates for phylogenetic dating; (4) sharing of the same temporal framework of biogeographical events compatible with vicariance or dispersal; and (5) identification of two or more lineages displaying the same biogeographical disjunction and temporal pattern within or across plant groups. In this study, most recent common ancestors (MRCA) and sister-group relationships were inferred based on monophyly of DNA sequences. Molecular clock techniques, coupled with the fossil record, furnished detailed information of the absolute timing of lineage splits. Biogeographical hypothesis testing, based on Bayesian inference, parsimony and maximum likelihood methods, estimated the probability of temporal congruence and directionality of lineage connections (Crisp *et al.*, 2011).

Biogeographical congruence in Antirrhineae

Sampling strategy and DNA sequencing

In the Antirrhineae and most angiosperms, plastid DNA is usually inherited by ovules (Corriveau & Coleman, 1988), and is therefore preferred when reconstructing seed colonization (see Fernández-Mazuecos & Vargas, 2011). A total of 60 accessions of plastid (*ndhF*) sequences were included (see Table S1 in Appendix S1 of Supporting Information), representing 25 of the 27 genera of the tribe Antirrhineae following Sutton's (1988) taxonomic treatment plus two additional genera that have been recognized since (*Pseudomisopates* and *Nanorrhinum*; Güemes, 1997; Ghebrehwet, 2001). Two New World genera (*Epixiphium*, *Holmgrenanthe*) were excluded due to a lack of material; their placement within the *Cymbalaria* clade in a previous internal transcribed spacer (ITS) phylogeny and the congruence between major clades of the ITS and *ndhF* analyses (see below) indicate that excluding these two genera does not significantly affect our analyses (see Fernández-Mazuecos *et al.*, 2013). As a lack of monophyly has rarely been found in Antirrhineae genera (Vargas *et al.*, 2004; Fernández-Mazuecos *et al.*, 2013), only one accession was sampled per genus, although two accessions of the only amphiatlantic genus of Antirrhineae, Old World *Linaria* and New World *Linaria* (formerly *Nuttallanthus*) were included (see Fernández-Mazuecos *et al.*, 2013). As a result, 19 new

sequences of Antirrhineae were generated for this study and eight were retrieved from GenBank. Outgroup taxa were chosen to represent both closely related and more distant families for which reliable fossils are available, and consisted of 33 sequences in GenBank from the order Lamiales, including the families Plantaginaceae, Bignoniaceae, Acanthaceae, Orobanchaceae, Lamiaceae, Verbenaceae, Pedaliaceae, Scrophulariaceae, Gesneriaceae, Calceolariaceae, Oleaceae and Plocospermataceae. See Appendix S2 for a full description of DNA sequencing.

Phylogenetic analysis

The sequences were aligned using MAFFT 6 (Kato & Toh, 2008) with minor adjustments made by visual inspection. Bayesian inference (BI), maximum-likelihood (ML) and maximum-parsimony (MP) analyses were conducted. To determine the optimal model of sequence evolution that best fitted the sequence data (GTR+I+G), the Akaike information criterion (AIC) was implemented using jMODELTEST 0.1.1 (Posada, 2008). See Appendix S2 for a full description of phylogenetic analyses.

Bayesian dating

To estimate divergence times among Lamiales lineages, including the Antirrhineae genera, we analysed the *ndhF* matrix through a relaxed molecular clock approach implemented in BEAST 1.6.1 (Drummond & Rambaut, 2007). No reliable fossils of the Antirrhineae appropriate for molecular calibration have yet been discovered (Martínez-Millán, 2010). We therefore employed a previous molecular estimate and five Lamiales fossils as constraints. All fossils have been considered reliable and proposed as calibration points for molecular dating in previous studies (Besnard *et al.*, 2009; Martínez-Millán, 2010; Thiv *et al.*, 2010). See Table 1 and Appendix S2 for further details.

Ancestral-area reconstructions

Biogeographical reconstructions were conducted delimiting just two areas (Old World/New World), and ancestors were allowed to be present in both. First, we analysed the BEAST output trees using statistical dispersal–vicariance analysis (S-DIVA), an approach to parsimony-based dispersal–vicariance analysis (DIVA; Ronquist, 1997) that accounts for phylogenetic uncertainty. S-DIVA analyses were conducted following the methods of Harris & Xiang (2009) implemented in the program RASP 1.1 (Yu *et al.*, 2011). All outgroup taxa except *Lafuentea* (the sister group to the Antirrhineae) were pruned from the trees. We used 1000 randomly sampled post-burn-in trees from the BEAST run, and the maximum clade credibility tree as the reference tree for summarizing S-DIVA results.

We also performed dispersal–extinction–cladogenesis analysis (DEC; Ree & Smith, 2008), a parametric likelihood-based approach, to reconstruct ancestral distributions. DEC

Table 1 Calibration points, associated fossils and minimum age constraints employed in the relaxed-clock analysis of *ndhF* sequences of Lamiales (including Antirrhineae genera). Nodes are named as in Fig. S1 in Appendix S1.

Fossil (Family/tribe)	Node	Time interval	Minimum age (Ma)	Reference
<i>Fraxinus wilcoxiana</i> (Oleaceae)	A	middle Eocene	37.2	Call & Dilcher (1992)
<i>Catalpa rugosa</i> (Bignoniaceae)	B	early–middle Oligocene	28.4	Reid & Chandler (1926)
<i>Ajuginucula smithii</i> (Lamiaceae)	C	early–middle Oligocene	28.4	Reid & Chandler (1926)
<i>Gratiola tertiaria</i> (Plantaginaceae/Gratioloideae)	D	Miocene	5.3	Łańcucka-Środoniowa (1977)
<i>Plantaginacearumpollis</i> (Plantaginaceae s.str.)	E	middle Miocene	11.6	Nagy (1963)

analysis estimates the most likely geographical distribution of the two daughter lineages following a speciation event. Thus, whereas S-DIVA reports the ancestral range prior to the speciation event, DEC reports how the ancestral range is divided between the two descendants immediately after speciation. DEC analysis was implemented using LAGRANGE 2.0.1 (Ree & Smith, 2008). We set symmetric dispersal between areas, and constant dispersal rates through time, given that the sea barrier between the Old World and the New World formed in the Eocene (Axelrod, 1975) and has remained for the entire duration of diversification in Antirrhineae indicated by the dating analysis (see below).

Temporal congruence

To statistically evaluate the temporal congruence of the ampho-Atlantic disjunctions found across the Antirrhineae, we compared divergence-time estimates for the four Old World/New World lineages across the combined posterior distribution of 72,000 trees taken from the BEAST analysis. Divergence times between Old World and New World lineages of Antirrhineae constitute good estimates of colonization times if we assume that disjunctions are the result of long-distance dispersal (see below). Divergence-time estimates for the four Old World/New World lineages were first compared by inspecting the marginal density distributions of time to the most recent common ancestors (TMRCA) in TRACER 1.4 (Rambaut & Drummond, 2007). We then obtained the posterior probability (PP) of occurrence of each divergence within the boundaries of each geological epoch, as defined by the *International Stratigraphic Chart 2009* (available at <http://www.stratigraphy.org/>). This probability was calculated as the proportion of trees from the posterior distribution where a particular Old World/New World lineage divergence fell into the bounds of a certain geological epoch. Finally, we calculated the PP of temporal congruence of two or more split events as the proportion of trees in which two or more Old World/New World lineage divergences occurred within the bounds of the same geological epoch. In order to provide statistical support for the BCH, PPs above 0.50 were classified into the categories ‘high’ ($0.90 \leq \text{PP} \leq 1.00$), ‘medium’ ($0.75 \leq \text{PP} < 0.90$) and ‘low’ ($0.50 \leq \text{PP} < 0.75$).

In order to test whether the four colonization events of the New World all occurred synchronously within the Miocene (as suggested by previous analyses) with a higher probability than expected by chance, we generated a null

distribution of the timings of the four divergence events. To this end, we produced a distribution of trees with the same topology and the same root age as obtained in the maximum clade credibility (MCC) tree of the Bayesian dating analysis, but allowing the internal branch lengths to vary according to a birth–death model. We fixed the tree topology in BEAST by setting the MCC tree as the starting tree and unselecting all Markov chain Monte Carlo (MCMC) operators that act on the tree model. Analyses were repeated for two different fixed root ages – 69.3 and 105.2 Ma – representing the upper and lower 95% highest posterior density limits of the root age obtained in the MCC tree. For each analysis, we conducted five independent runs of 20 million generations, thus obtaining a total 10,000 trees. We then calculated the percentage of trees that had all four colonization events occurring between 23.03 and 5.33 Ma (Miocene).

Testing the Madrean–Tethyan hypothesis across angiosperm groups

In addition to the Antirrhineae, five of the 28 angiosperm groups considered to support the Madrean–Tethyan hypothesis (Raven & Axelrod, 1978; Kadereit & Baldwin, 2012) were analysed: Arbutioideae (Hileman *et al.*, 2001), *Cercis* (Davis *et al.*, 2002), Cistaceae (Guzmán & Vargas, 2009), Geraniaceae (Fiz-Palacios *et al.*, 2010) and *Platanus* subgen. *Platanus* (Feng *et al.*, 2005). These plant groups were chosen because they fit the assumptions for testing the BCH, i.e. molecular phylogenies based on significant taxonomic sampling and appropriate fossil-based calibration points or molecular substitution rates for phylogenetic tree ultrametrization are available. The five original DNA sequence datasets from published studies were obtained directly from the authors. For each analysis, we used the strategy used for the Antirrhineae (see above, Table S2 in Appendix S1, and Appendix S2).

RESULTS

Biogeographical congruence in Antirrhineae

Phylogenetic analyses

The *ndhF* matrix contained 2086 bp and had 1047 variable sites, of which 686 were parsimony-informative. The phylogenetic analyses revealed that the Antirrhineae constitute a monophyletic group (see Fig. S1 in Appendix S1). All three

phylogenetic analyses (BI, ML and MP) recognized three well-supported lineages formed by Old World/New World sister groups: the *Cymbalaria* lineage (PP = 1; ML-BS = 99.4%; MP-BS = 97%), the *Linaria* lineage (PP = 1; ML-BS = 100%; MP-BS = 100%) and the *Galvezia* lineage (PP = 1; ML-BS = 100%; MP-BS = 100%) (Fig. S1 in Appendix S1; see also Fig. 1). One additional Old World/New World sister clade (the *Sairocarpus* lineage) had lower support (PP = 0.66; ML-BS = 63.2%; MP-BS = 63%) (Fig. S1 in Appendix S1).

Bayesian dating

Standard deviation of the uncorrelated lognormal relaxed clock (0.748) and coefficient of variation (0.783) for rate heterogeneity within our *ndhF* dataset indicated the presence of rate heterogeneity among lineages. Analysis with TRACER 1.4

(Rambaut & Drummond, 2007) confirmed that sample sizes were adequate, with effective sample size (ESS) values above 300 and plots showing equilibrium after discarding the burn-in. The topology of the MCC tree (see Fig. S1a in Appendix S1) was congruent with those of the ML and MP analyses (Fig. S1b–c in Appendix S1). Diversification of the major lineages of Antirrhineae may have occurred in the last 35 Myr, from the Oligocene onwards. Mean ages of the four MRCAs of Old World/New World lineages all fell within the Miocene (Fig. 1), making the possibility of vicariance as an explanation for these disjunctions unlikely.

Ancestral area reconstructions

Biogeographical analyses supported four migrations from the Old World to the New World. In the ancestral range reconstruction using S-DIVA, Old World-to-New World

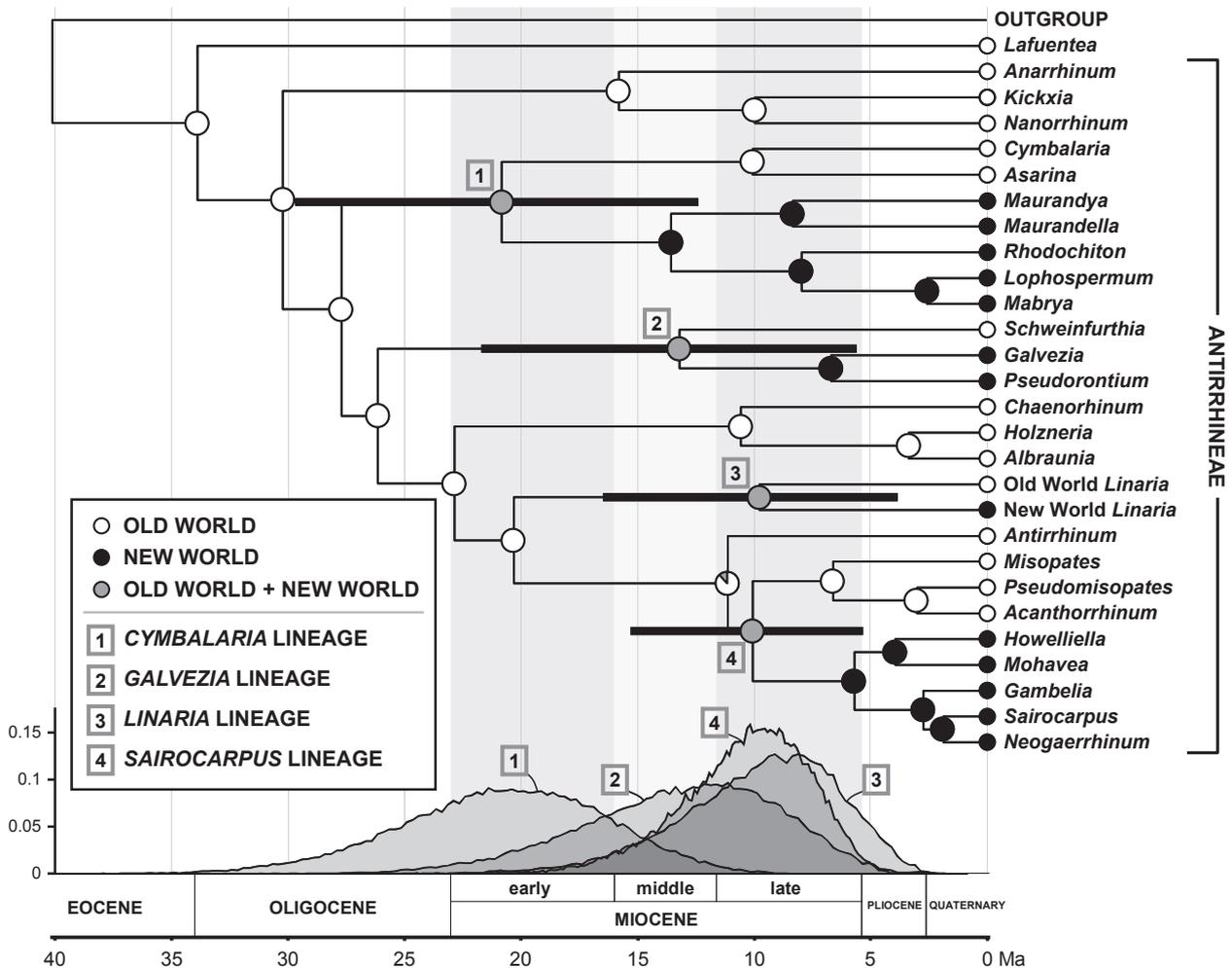


Figure 1 Molecular dating analysis and biogeographical reconstruction of Antirrhineae based on *ndhF* sequences. The maximum clade credibility tree produced by relaxed molecular clock analysis is shown. Outgroup lineages (except *Lafuentea*) have been pruned for clarity. Terminal circles indicate current distributions, and circles or pie charts at nodes represent marginal probabilities for ancestral areas as inferred by S-DIVA analysis. Node bars in black represent the 95% highest posterior density intervals for time to the most recent common ancestors (TMRCA) of four Old World/New World lineages (1–4). The marginal densities of TMRCA of these four lineages are represented along the time-scale.

colonization events were strongly supported for the four lineages: *Cymbalaria* (node 1), *Galvezia* (node 2), *Linaria* (node 3) and *Sairocarpus* (node 4) (see Fig. 1; see also Fig. S2b in Appendix S1). DEC results were also congruent with an Old World-to-New World pattern for the four dispersal events (see Fig. S2a).

Temporal congruence

We compared the posterior distributions of the four TMRCAs (Fig. 1) to assess the temporal congruence of the four disjunction events. High PPs for the Miocene were obtained in the *Sairocarpus* (0.99), *Galvezia* (0.97) and *Linaria* (0.93) lineages (Table 2). For the *Cymbalaria* lineage, the most likely epoch was the Miocene (0.70), followed by the Oligocene (0.29). Within the Miocene, the middle–late subdivision received the highest PP for the *Sairocarpus* (0.96), *Linaria* (0.88) and *Galvezia* (0.74) lineages, whereas the early–middle Miocene received the highest PP (0.69) for the *Cymbalaria* lineage (Table 2). Furthermore, Table 3 shows the probabilities of temporal congruence for Antirrhineae, i.e. the PP that two, three or four TMRCAs occurred within the bounds of the same geological epoch. The Miocene was the only epoch that yielded significant probabilities, including a significant probability (PP = 0.62) of temporal congruence for the four lineages taken together. Interestingly, a medium to high probability value (PP = 0.89) was retrieved when considering only three lineages (*Galvezia*, *Linaria* and *Sairocarpus*). When subdividing the Miocene into three periods in order to narrow down a hypothetical migration period, the middle–late subdivision received the highest probability (PP = 0.63) for temporal congruence of these three lineages (Table 3). Therefore, we interpreted that optimal conditions for Old World/New World divergences within the Antirrhineae were concentrated in a period of about 10 million years (15.97–5.33 Ma).

The proportion of trees with a Miocene colonization to America by the four Antirrhineae lineages (62%; Table 3) was higher than that expected by chance. Indeed, we found that only 12.9% and 20% of trees of the null distributions (fixed root ages of 105.2 and 69.3 Ma, respectively) presented the four colonization events within the Miocene. These results provide further support for the hypothesis of a synchronous colonization of the American continent in the Miocene.

Testing the Madrean–Tethyan hypothesis across angiosperm groups

Our phylogenetic analyses suggested a higher number of Old World/New World lineage divergences than previously considered for these taxonomic groups (Raven & Axelrod, 1978). Our analyses detected 12 angiosperm lineages that include New World and Old World sister sublineages: four in Antirrhineae, one in Arbutoideae, two in *Cercis*, two in Cistaceae, two in Geraniaceae, and one in *Platanus* subgen. *Platanus* (Fig. 2a). Only 10 lineages could, however, be considered

Madrean–Tethyan, because two were found to be very recent (Pliocene–Pleistocene): the eastern American species *Cercis canadensis* is closely related to the Mediterranean *C. siliquastrum*, and the New World species *Erodium texanum* is closely related to Old World *Erodium* lineages (Fig. 2a).

Biogeographical reconstructions for the six datasets indicated an Old World to New World directionality for the Antirrhineae, the two Cistaceae lineages and the basal disjunction of *Cercis* using S-DIVA and DEC analyses (Fig. 2a & Fig. S2). In contrast, a New World to Old World connection was supported for several other lineages (Arbutoideae, *Platanus*; Fig. 2a), and the directionality was equivocal for the *Erodium*–*California* (Geraniaceae) split. In the DEC analyses (see Fig. S2), an Old World to New World dispersal was obtained for Arbutoideae, Cistaceae (one to two events), *Platanus* and *Cercis*, as well as for the four Antirrhineae lineages. Differences between S-DIVA and DEC analyses are due to the different speciation modes modelled by the two methods. In S-DIVA, widespread ancestors are divided at speciation by allopatry, whereas DEC allows a widespread range to be inherited by one of the descendants. In fact, whereas dispersal events were always reconstructed along the branch preceding the MRCA of Old World/New World lineages in S-DIVA (Fig. 2a & Fig. S2), they were frequently reconstructed as an older event in the DEC analysis (see Fig. S2).

The posterior probabilities of the New/Old World splits for each of the five angiosperm groups (in addition to Antirrhineae) are shown in Table 2. In all cases, the divergence-time estimates for each of the Old World/New World divergences are congruent with the estimates obtained in the original publications. For the majority of the angiosperm groups, the Miocene was the epoch with the highest probability of occurrence of Old World/New World divergences (Fig. 2b). The mean probability of the Old World/New World disjunctions for the two datasets, i.e. the six non-Antirrhineae and the four Antirrhineae lineages, was high (0.85 PP) for the Miocene and very low for all other geological epochs (Table 2).

DISCUSSION

Historical biogeography of the six angiosperm groups provided poor support for an ancient (Palaeogene) Madrean–Tethyan flora, which is based on a vicariance hypothesis. Our approach extended the analysis to ten lineages from the six angiosperm groups examined. Interestingly, high levels of biogeographical congruence were found across plant lineages. Nevertheless, most of the intercontinental colonizations postdated the formation of the vicariance barrier of the Eocene (Tiffney & Manchester, 2001).

Synchronous ampho-Atlantic divergences of four independent snapdragon lineages

Our dating analysis demonstrates that Old World/New World lineage divergences of snapdragons (Antirrhineae)

Table 2 The posterior probability of the Old World/New World split of angiosperm lineages within the bounds of the six geological epochs of the Tertiary and five subdivisions of the Miocene.

Lineages	Six geological epochs of the Tertiary ^a						Three subdivisions of the Miocene ^b			Two subdivisions of the Miocene ^c		
	Eocene	Oligocene	Miocene	Pliocene	Pleistocene	Holocene	early Miocene	middle Miocene	late Miocene	early-middle Miocene	middle-late Miocene	
Antirrhineae												
1. <i>Cymbalaria</i>	0.003	0.293	0.703*	< 0.001	< 0.001	< 0.001	0.569*	0.124	0.010	0.693*	0.135	
2. <i>Galvezia</i>	< 0.001	0.018	0.969***	0.013	< 0.001	< 0.001	0.229	0.364	0.376	0.593*	0.740*	
3. <i>Linaria</i>	< 0.001	< 0.001	0.927***	0.072	0.001	< 0.001	0.048	0.220	0.659*	0.268	0.879**	
4. <i>Sairocarpus</i>	< 0.001	0.001	0.991***	0.008	< 0.001	< 0.001	0.036	0.269	0.686*	0.305	0.955***	
Other angiosperm groups												
<i>Platanus orientalis</i> + <i>Platanus racemosa</i>	0.037	0.241	0.707*	0.014	0.001	< 0.001	0.316	0.219	0.172	0.535*	0.392	
<i>Lechea</i>	< 0.001	< 0.001	0.999***	0.001	< 0.001	< 0.001	0.007	0.312	0.680*	0.319	0.992***	
<i>Crocantimum</i> / <i>Hudsonia</i>	< 0.001	< 0.001	0.774**	0.225	< 0.001	< 0.001	0.000	< 0.001	0.774**	< 0.001	0.774**	
<i>Erodium</i> / <i>California</i>	< 0.001	< 0.001	1.000***	< 0.001	< 0.001	< 0.001	1.000***	< 0.001	< 0.001	1.000***	< 0.001	
Arbutoideae Med. vs. N America	0.023	0.387	0.589*	< 0.001	< 0.001	< 0.001	0.584*	0.006	< 0.001	0.589*	0.006	
<i>Cercis</i>	< 0.001	0.210	0.789**	0.001	< 0.001	< 0.001	0.301	0.272	0.216	0.573*	0.489	
Mean	0.006	0.115	0.845**	0.033	< 0.001	< 0.001	0.309	0.179	0.357	0.488	0.536*	

* 0.50 ≤ PP < 0.75; ** 0.75 ≤ PP < 0.90; *** 0.90 ≤ PP.

^aEocene (55.8–33.9 Ma); Oligocene (33.9–23.03 Ma); Miocene (23.03–5.33 Ma); Pliocene (5.33–2.59 Ma); Pleistocene (2.59–0.01 Ma).^bEarly Miocene, 23.03–15.97 Ma; middle Miocene, 15.97–11.61 Ma; late Miocene, 11.61–5.33 Ma.^cEarly-middle Miocene, 23.03–11.61 Ma; middle-late Miocene, 15.97–5.33 Ma.

Table 3 Posterior probabilities (PP) of temporal congruence of two or more Old/New World lineage divergences of Antirrhineae in the six geological epochs of the Tertiary and five subdivisions of the Miocene.

Lineages ^d	Six geological epochs of the Tertiary ^a						Three subdivisions of the Miocene ^b			Two subdivisions of the Miocene ^c	
	Eocene	Oligocene	Miocene	Pliocene	Pleistocene	Holocene	early Miocene	middle Miocene	late Miocene	early–middle Miocene	middle–late Miocene
1,2	< 0.0001	0.0112	0.6868*	< 0.0001	< 0.0001	< 0.0001	0.1154	0.0445	0.0047	0.3887	0.1115
1,3	< 0.0001	0.0002	0.6441*	< 0.0001	< 0.0001	< 0.0001	0.0201	0.0163	0.0076	0.1556	0.1175
1,4	< 0.0001	0.0006	0.6958*	< 0.0001	< 0.0001	< 0.0001	0.0146	0.0196	0.0091	0.1736	0.1302
2,3	< 0.0001	< 0.0001	0.8982**	0.0019	< 0.0001	< 0.0001	0.0153	0.0803	0.2598	0.1754	0.6512*
2,4	< 0.0001	0.0001	0.9605***	0.0001	< 0.0001	< 0.0001	0.0135	0.0981	0.2832	0.2036	0.7119*
3,4	< 0.0001	< 0.0001	0.9190***	0.0007	< 0.0001	< 0.0001	0.0054	0.0757	0.4755	0.1143	0.8418**
1,2,3	< 0.0001	< 0.0001	0.6293*	< 0.0001	< 0.0001	< 0.0001	0.0054	0.0061	0.0038	0.0982	0.0980
1,2,4	< 0.0001	0.0001	0.6792*	< 0.0001	< 0.0001	< 0.0001	0.0048	0.0072	0.0043	0.1124	0.1075
1,3,4	< 0.0001	< 0.0001	0.6372*	< 0.0001	< 0.0001	< 0.0001	0.0020	0.0045	0.0065	0.0578	0.1133
2,3,4	< 0.0001	< 0.0001	0.8904**	< 0.0001	< 0.0001	< 0.0001	0.0019	0.0264	0.2032	0.0786	0.6277*
1,2,3,4	< 0.0001	< 0.0001	0.6225*	< 0.0001	< 0.0001	< 0.0001	0.0006	0.0013	0.0035	0.0389	0.0944

* $0.50 \leq PP < 0.75$; ** $0.75 \leq PP < 0.90$; *** $0.90 \leq PP$.

^aEocene (55.8–33.9 Ma); Oligocene (33.9–23.03 Ma); Miocene (23.03–5.33 Ma); Pliocene (5.33–2.59 Ma); Pleistocene (2.59–0.01 Ma).

^bEarly Miocene, 23.03–15.97 Ma; middle Miocene, 15.97–11.61 Ma; late Miocene, 11.61–5.33 Ma.

^cEarly–middle Miocene, 23.03–11.61 Ma; middle–late Miocene, 15.97–5.33 Ma.

^d1, *Cymbalaria* lineage; 2, *Galvezia* lineage; 3, *Linaria* lineage; 4, *Sairocarpus* lineage.

considerably post-dated the Eocene, and therefore were established after an extensive water barrier (the Atlantic Ocean) had been formed between America and Europe (Tiffney & Manchester, 2001). Therefore, a vicariance process involving four independent splits of snapdragons is not supported by our data. The Bering land bridge between Asia and western North America could have provided a connection for temperate plant lineages into the late Miocene (Hong, 1983). Irrespective of particular geographical routes, our biogeographical reconstruction strongly supported four Old World-to-New World colonization events, followed by allopatric differentiation during the Miocene (Fig. 1). Indeed, the geographical distribution of the sister genus *Lafuentea* and the basal-most lineage of Antirrhineae (the *Anarrhinum* lineage) suggests a primary centre of diversification in the Old World. To our knowledge, this is the first time that biogeographical analyses statistically support a synchronous process of disjunction within a single plant group that is consistent with previous predictions for the BCH (Vargas *et al.*, 2004; Wen & Ickert-Bond, 2009).

The biogeographical congruence hypothesis (BCH) revisited

The process of geographical distribution being shared by two or more lineages that originated during the same period of time is known as biogeographical congruence (Page, 1990; Cunningham & Collins, 1994; Donoghue & Moore, 2003). Three conditions need to be met to reliably support the BCH: (1) similar distributions of taxa in two territories with comparable ecological conditions, e.g. disjunctions of Mediterranean/Californian lineages; (2) common ancestry of

lineages, i.e. sister-group relationships of lineages currently forming part of comparable floras and faunas; and (3) synchrony of divergence times for sister lineages. As more angiosperm groups are found to show similar biogeographical patterns, i.e. multiple lineages exhibit the same disjunction that arose in the same period, greater support is given to biogeographical connectivity (Donoghue *et al.*, 2001; Xiang & Soltis, 2001; Wen & Ickert-Bond, 2009).

Our results do not support the vicariance hypothesis for the Madrean–Tethyan flora, but instead support a process in which multiple colonizations occurred following the establishment of a putative barrier (the Atlantic Ocean) (Crisp *et al.*, 2011). Testing the BCH is also dependent on the length of each geological period. For instance, temporal congruence of the snapdragon lineage divergences is high for the Miocene as a whole, but medium or low when dividing the Miocene into two or three subdivisions, respectively (Table 3). The likelihood of higher or lower biogeographical congruence is therefore closely related to the number of lineages involved and the window of opportunity offered by the duration of each geological period.

Biogeographical congruence also has ecological significance, because it suggests that evolution occurred under similar habitat conditions in two disjunct areas. We found four Antirrhineae disjunctions that fitted into the BCH for the Miocene, and also discovered two lineage splits in the Cistaceae in this geological epoch. In addition, the highest probability estimates for New World/Old World connections in the Geraniaceae and *Cercis* (one lineage each) were also discovered in the Miocene (Fig. 2b). The Miocene is thought to have been a period of expansion for temperate plant groups through northern corridors with similar habitat

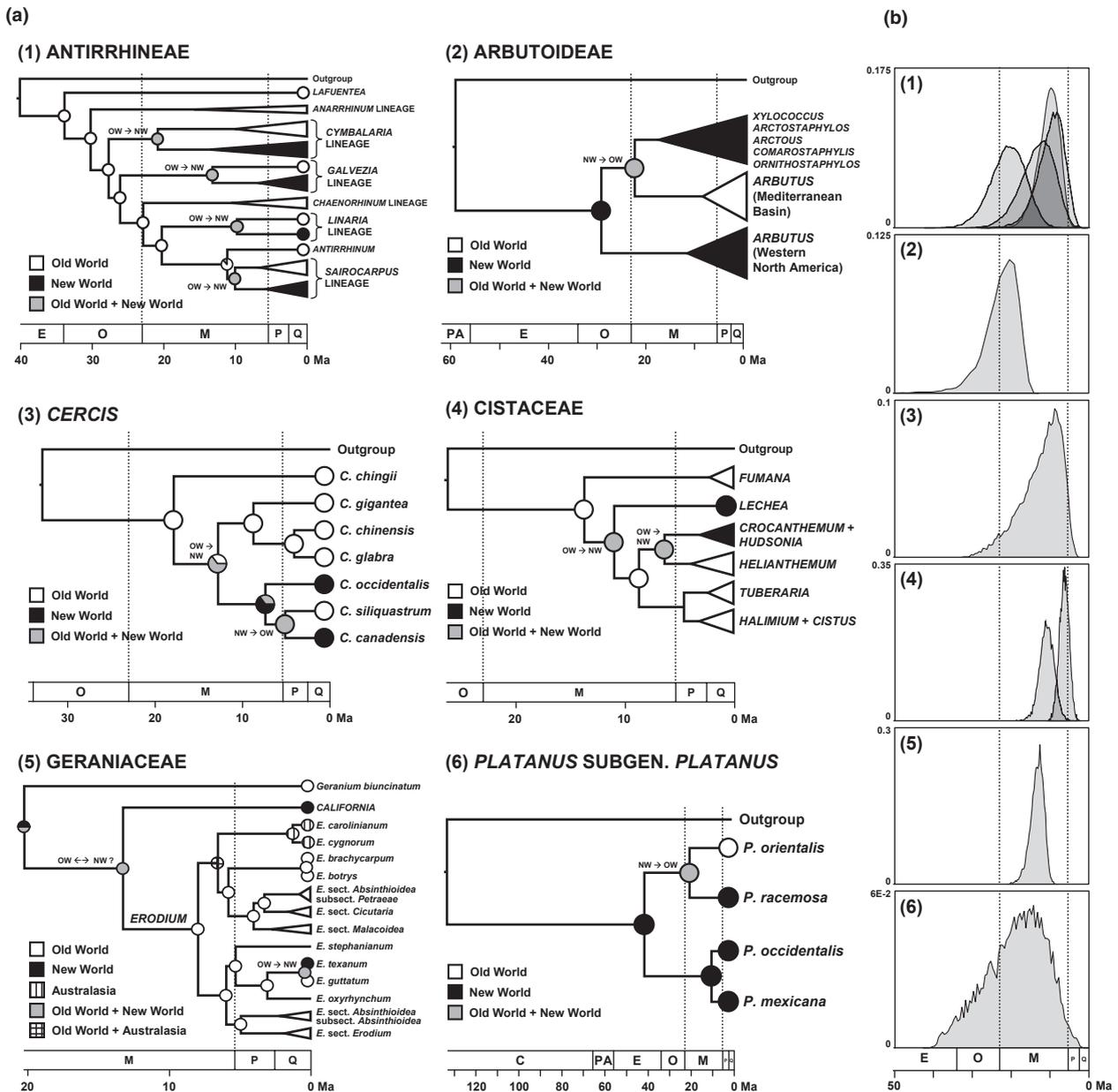


Figure 2 Summarized chronograms and biogeographical reconstructions of six analysed lineages of the Madrean–Tethyan flora. (a) Maximum clade credibility trees produced by relaxed molecular-clock analyses are shown. Mean ages of nodes are represented. Pie charts at nodes indicate marginal probabilities for ancestral areas as inferred by S-DIVA analysis. Shading and hatching of clade triangles represent the ancestral area (marginal probability = 1) of the clade. (b) Marginal densities of TMRCA of Old World/New World clades belonging to the six analysed lineages of the Madrean–Tethyan flora. Two graphs (1, 4) display four and two disjunction nodes in the same plant group. Dotted lines mark the boundaries of the Miocene.

conditions that favoured intercontinental colonization (Grímsson *et al.*, 2007). In contrast, older biogeographical events pre-dating the opening of the oceanic gap between America and Eurasia cannot be ruled out for Arbutoideae (Hileman *et al.*, 2001) and *Platanus* (Feng *et al.*, 2005). Irrespective of specific support for vicariance or long-distance dispersal between America and Eurasia, the question remains as to whether shared ecological conditions in the same (biogeographical congruence) or different (biogeographical pseudocongruence) geological periods have been predomi-

nant in shaping the floras of California and the Mediterranean (Edwards *et al.*, 2007).

Legacy of Tertiary relict floras

Californian and Mediterranean floras appear to have included many closely related plant lineages since the Miocene, well before the onset of Mediterranean climates (Wen & Ickert-Bond, 2009; Kadereit & Baldwin, 2012). More recent connections between populations within species

(*Plantago ovata*, Meyers & Liston, 2008; *Oligomeris linifolia*, Martín-Bravo *et al.*, 2009), and between closely related species (*Erodium* spp., Fiz-Palacios *et al.*, 2010), also provide examples of more recent (Pleistocene) dispersal between Mediterranean floristic regions. These results lead us to conclude that taxonomic treatments constitute a good starting hypothesis regarding Pleistocene biogeographical divergence of lineages at low (intraspecific) taxonomic levels, while supraspecific levels better reflect lineage differentiation during earlier geological epochs of the Tertiary. Indeed, Raven & Axelrod (1978) proposed a Madrean–Tethyan flora primarily based on supraspecific taxa.

A continuous process of plant exchange between other temperate floras of North America and Asia has already been documented (Donoghue *et al.*, 2001; Xiang & Soltis, 2001). A review of 33 dated phylogenies of temperate vascular plants upheld the view that eastern Asia and eastern North America were connected by migrations at different times, but mostly in the Miocene (Donoghue & Smith, 2004). The same is true for Mediterranean/Californian elements, as recently reviewed for 25 angiosperm groups (Kadereit & Baldwin, 2012). Nevertheless, the high number of Mediterranean/Californian disjunctions (10 of the 12 angiosperm disjunctions tested in this paper) that shared a MRCA in the Miocene (Fig. 2), an epoch earlier than the establishment of both Mediterranean climates, is intriguing. The Mediterranean climates are thought to have become established in the Miocene–Pliocene (7–4 Ma; Millar, 2012) in California, and in the Pliocene (2.8 Ma; Suc, 1984) in the Mediterranean basin, when the Miocene floristic connections were already in place (Fig. 2b). Many Mediterranean plants adapted to summer drought are considered to be derived within lineages with earlier adaptations to drought (Millar, 2012). For instance, a drought-adapted Miocene (17–7 Ma) ancestor of Mediterranean–western American lineages has been proposed for *Lonicera* (a genus not included as Madrean–Tethyan in Raven & Axelrod, 1978), potentially conferring pre-adaptation to Mediterranean habitats (Smith & Donoghue, 2010). In conclusion, different degrees of similarity for both the Mediterranean and Californian floras can be estimated in light of floristic merging of ancient (Madrean–Tethyan), old (Miocene) and recent (Mediterranean climates) lineages.

ACKNOWLEDGEMENTS

The authors wish to thank Paul Manos and Lena Hileman for kindly providing datasets for use in this study. Some material was generously provided by J.J. Aldasoro, W.J. Elisens, B. Estébanez, J. Güemes and the herbaria AZ, E, MA, TARI and VAL. We also thank Zeeba Khan for editing the English of this paper, and Peter Linder and the anonymous referees for improving the quality of the manuscript. The study (CGL2009-10031), M.F.-M. (AP2007-01841), I.L. (BES-2010-038044) and B.G. (Juan de la Cierva programme) were supported by the Spanish Ministry of Education. Support was also

received by the Spanish Council of Scientific Research (CSIC: JAEpre) to J.L.B.-P. and by the European Union (Marie Curie IEF fellowship ‘BIRDISLAND’) to L.M.V.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Supplementary Tables S1–S2 including plant material and angiosperm groups exhibiting a Madrean–Tethyan disjunction; and supplementary Figures S1–S2 including phylogenetic relationships of Lamiales and biogeographical reconstructions of the angiosperm groups.

Appendix S2 Supplementary methods on biogeographical congruence in Antirrhineae and testing the Madrean–Tethyan hypothesis across angiosperm groups.

BIOSKETCH

Our research group is broadly interested in evolution, biogeography and molecular systematics of primarily Mediterranean plant groups.

Author contributions: P.V. conceived the idea; E.C. and A.F. performed the lab work; L.M.V., J.L.B.-P., I.L., B.G. and M.F.-M. conducted the analyses; and P.V. wrote the text. All authors contributed intellectually to analytical issues and data interpretation, and all commented on the text.

Editor: Peter Linder