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ORIGINAL ARTICLE

The historical biogeography of *Scabiosa* (Dipsacaceae): implications for Old World plant disjunctions

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ABSTRACT

Aim To reconstruct the temporal and biogeographical history of Old World disjunctions in *Scabiosa* (Dipsacaceae) and the timing of diversification in the Mediterranean Basin, in order to evaluate the importance of biogeographical and climatological history (particularly the onset of a mediterranean climate) in shaping *Scabiosa* distributions.

Location Europe and the Mediterranean Basin, southern Africa and eastern Asia.

Methods This study uses maximum-likelihood and Bayesian phylogenetic analyses of chloroplast DNA (*atp*B–*rbc*L, *trn*L–*trn*F, *trn*S–*trn*G, *psb*A–*trn*H) and nuclear ribosomal DNA [internal transcribed spacer (ITS) and external transcribed spacer (ETS)] from 24 out of *c.* 37 ingroup taxa, BEAST molecular dating, and the dispersal–extinction–cladogenesis method (LAGRANGE) to reconstruct ancestral geographical ranges and the timing of diversification of the major clades of *Scabiosa*.

Results Biogeographical and divergence time reconstructions showed that *Scabiosa* originated during the Miocene and diversified in Europe, followed by independent movements into Asia and Africa. Several of the major clades were inferred to have radiated sometime between the late Miocene and early Pleistocene, a timeframe that encompasses the onset of the mediterranean climate in Europe. More recent middle–late Pleistocene radiations in the Mediterranean Basin and southern Africa have played a large role in *Scabiosa* diversification.

Main conclusions Members of *Scabiosa* appear to have capitalized on adaptations to montane and/or dry conditions in order to colonize similar habitats in different biogeographical regions. The formation of the East African Rift mountains is potentially of great importance in explaining the southward migration of *Scabiosa*. The initial diversification of *Scabiosa* in Europe during the Miocene is not consistent with the initiation of the mediterranean climate, but may instead be associated with increased aridity and the retreat of subtropical lineages during this time. However, the radiation of some of the major subclades within *Scabiosa* may have been associated with an emerging mediterranean climate. More recent and rapid radiations in both the Mediterranean Basin and southern Africa highlight the probable importance of Pleistocene climate fluctuations in *Scabiosa* diversification.

Keywords
Biogeographical disjunctions, biogeography, Dipsacaceae, divergence times, diversification, historical biogeography, Mediterranean, Miocene, *Scabiosa*, southern Africa.

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INTRODUCTION

A remarkable characteristic of angiosperm biogeography is the intercontinental disjunct distributions of closely related species (Thorne, 1972; Raven & Axelrod, 1974). An understanding of the origin and evolution of these patterns provides insight into the assembly of floras and the biogeographical processes behind current distributions of plant diversity. Some disjunct patterns are more common than others (Thorne, 1972), highlighting the potential importance of particular climatological and geological events in shaping patterns of biodiversity. In the Old World, a Europe–eastern Asia disjunction pattern is thought to have resulted from vicariance of a once widespread Cenozoic flora (e.g. Thorne, 1972; Wood, 1972; Tiffney, 1985a,b; Wen, 1999; Milne & Abbott, 2002) as a result of Pliocene climatic cooling and aridification followed by Quarternary glaciations (Webb & Bartlein, 1992; Willis *et al.*, 1999). In addition, disjunct distributions between southern Africa and Eurasia are found in a number of groups (Goldblatt, 1978), with dispersal from south to north the most common explanation (Caujapé-Castells *et al.*, 2001; Goldblatt *et al.*, 2002; Coleman *et al.*, 2003; Calviño *et al.*, 2006; Galley *et al.*, 2007; del Hoyo *et al.*, 2009; Sanmartín *et al.*, 2010; Désamoré *et al.*, 2011; but see McGuire & Kron, 2005). Migration may have been via the East African Rift mountains after their formation in the Pliocene and/or the result of long-distance dispersal (e.g. Levyns, 1964; Coleman *et al.*, 2003; Galley *et al.*, 2007). An alternative explanation of the African–Eurasian disjunctions is that they were caused by the fragmentation of a once widespread Cenozoic flora (Quézel, 1978; Andrus *et al.*, 2004).

Scabiosa L. (Dipsacaceae) is characterized by a triple Old World disjunction (Asia – Europe – southern Africa) and is an ideal group with which to investigate the competing hypotheses regarding the timing and origin of the disjunctions outlined above. Members of *Scabiosa* occur in Europe, primarily in the Mediterranean Basin (five species and two species complexes that include *c.* 14 taxa; Jasiewicz, 1976), Asia (*c.* 12 species), and eastern and southern Africa (*c.* 8 species). The distribution of *Scabiosa*, and Dipsacaceae in general, is somewhat unusual in that, unlike related clades in the Dipsacales, it apparently never made it to the New World except as introduced weeds. Moreover, *Scabiosa* is the only lineage in Dipsacaceae with significant radiations in Europe, Asia and southern Africa. *Scabiosa* contains *c.* 23 species and two taxonomically difficult species complexes: *S. columbaria* s.l. containing *c.* nine taxa, and *S. ochroleuca* s.l. containing *c.* five taxa (Jasiewicz, 1976). However, the number of reported taxa in these complexes varies and is often much higher (Verlaque, 1986). *Scabiosa* species typically have narrow

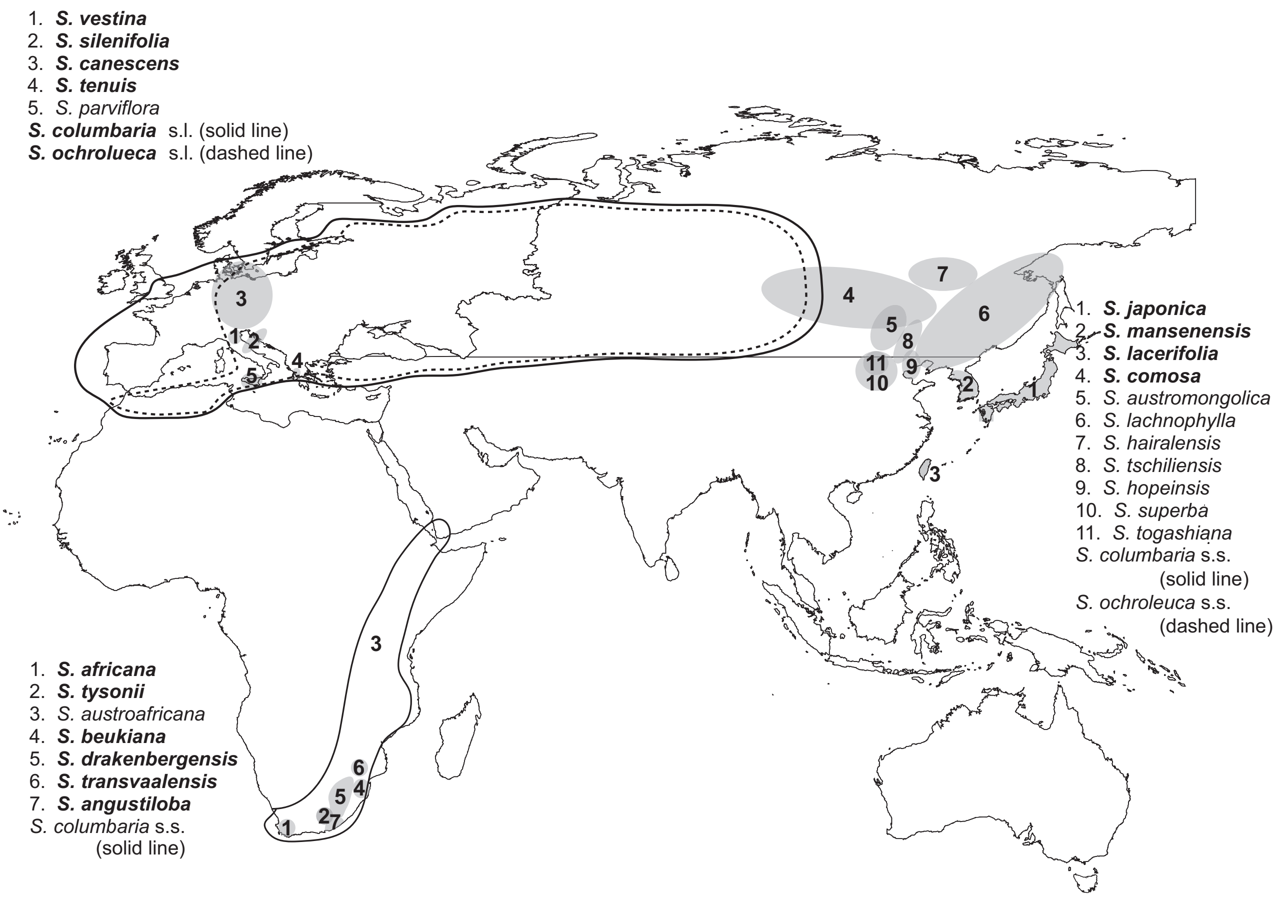

Figure 1 Distribution map of *Scabiosa* adapted from Verlaque (1986). Species included in this study are in bold.

distributions, with the remarkable exception of the widespread *S. ochroleuca* L. s.s and *S. columbaria* L. s.s., which cover an immense region encompassing most of the range of *Scabiosa* (Fig. 1). In all three regions, most taxa occur in montane or steppe habitats, with several European species occurring in dry, rocky soils in the Mediterranean Basin.

Scabiosa contains mostly bisexual, out-crossing, insect-pollinated diploid perennials, with the exception of two annual species that occur in lowland regions of the Mediterranean (*S. tenuis* Spruner and *S. parviflora* Desf.). Like other Dipsacaceae, members of *Scabiosa* have capitate inflorescences and an epicalyx – a novel organ that subtends the calyx and functions in seed germination, protection and dispersal (Ehrendorfer, 1965a,b; Verlaque, 1984; Mayer, 1995; Donoghue *et al.*, 2003; Caputo *et al.*, 2004). The epicalyx and calyx together form the dispersal unit, and members of *Scabiosa* are thought to be adapted for both wind dispersal and dispersal by epizoochory (adhesive animal dispersal; Ehrendorfer, 1965a; Caputo *et al.*, 2004), the latter of which might permit long-distance dispersal (Fischer *et al.*, 1996; Venable *et al.*, 2008). The monophyly of *Scabiosa* is well established, and previous phylogenetic analyses of Dipsacaceae have confidently resolved it as sister to *Sixalix* Raf. within the Scabioseae (Fig. 2; Caputo *et al.*, 2004; Avino *et al.*, 2009; Carlson *et al.*, 2009). Previous studies of Dipsacaceae phylogeny have included relatively few members of *Scabiosa*, and therefore phylogenetic relationships within *Scabiosa* have remained unclear.

Most European *Scabiosa* taxa occur in the Mediterranean Basin, a 'hotspot' of plant diversity (Myers, 1990; Cowling *et al.*, 1996; Médail & Quézel, 1997). The remarkable diversity in this region has frequently been linked to increased diversification associated with the evolution of

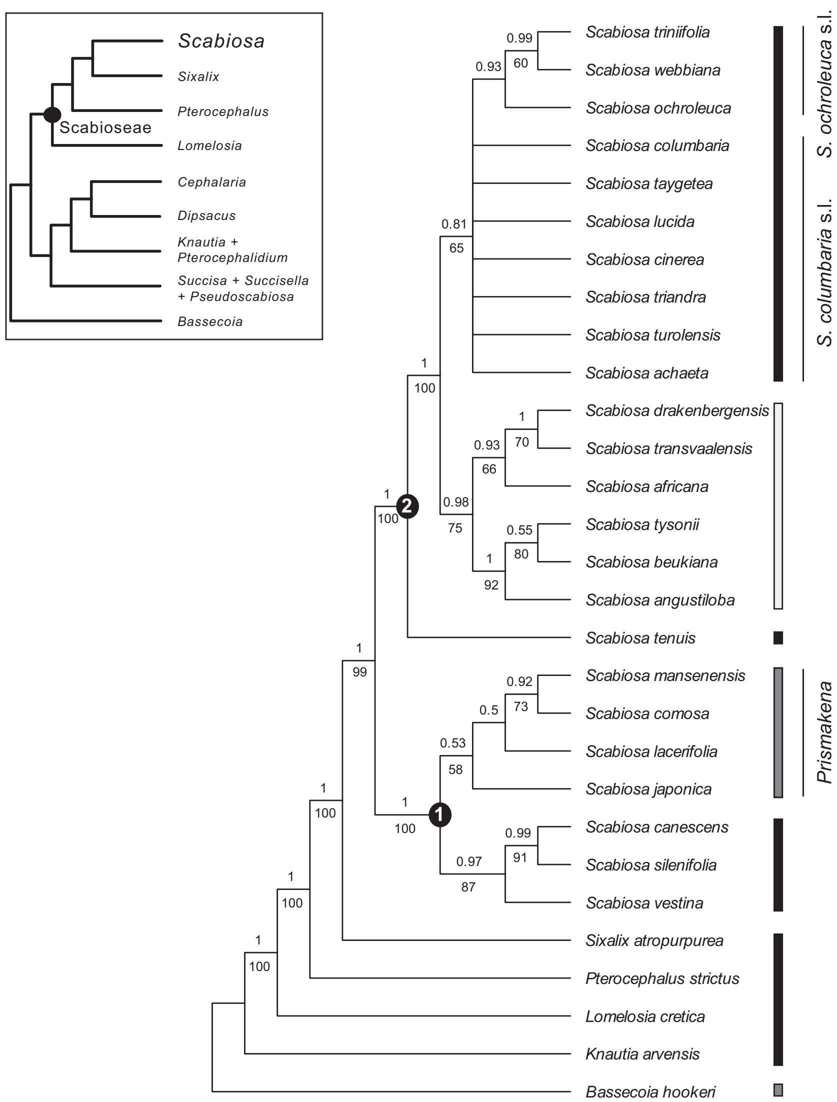

Figure 2 Majority-rule (50%) consensus tree for *Scabiosa* produced from the Bayesian analysis. Support values are indicated above and below the nodes (Bayesian posterior probabilities above, maximum-likelihood bootstraps below). Clades 1 and 2 are numbered, and clade names used in the text are indicated. Black bars indicate European taxa, the light grey bar indicates African taxa and the dark grey bar indicates Asian taxa. A summary of Dipsacaceae relationships, showing the position of *Scabiosa*, is shown in the inset (Carlson *et al.*, 2009).

summer drought during the Pliocene, which led to a seasonal mediterranean climate (Suc, 1984); however, studies of Mediterranean clades that incorporate molecular dating have shown varied results. Diversification in different Mediterranean groups is inferred to have occurred before, during and after the initiation of a mediterranean climate (e.g. Fritsch, 1996; Hileman *et al.*, 2001; Yesson & Culham, 2006; Guzmán *et al.*, 2009; Yesson *et al.*, 2009; Valente *et al.*, 2010), with several groups reported to have experienced multiple bouts of diversification at different times throughout the Neogene (e.g. Coleman *et al.*, 2003; del Hoyo *et al.*, 2009; Lo Presti & Oberprieler, 2009; Roquet *et al.*, 2009; Salvo *et al.*, 2010). These results highlight the need for a more nuanced explanation for the evolution of this flora that reflects the biogeographical and climatic history of the region.

The goal of this study is to assess the origin(s) and timing of the intercontinental disjunctions in *Scabiosa.* We also aim to investigate the initiation of diversification in Asia, Africa and Europe, particularly as it relates to the onset of a mediterranean climate in Europe during the Pliocene. We based phylogenetic analyses on DNA sequences from six gene regions: the chloroplast markers *atp*B–*rbc*L, *trn*L–*trn*F, *trn*S–*trn*G, *psb*A–*trn*H, and the nuclear ribosomal internal transcribed spacer (ITS) and external transcribed spacer (ETS). Temporal evolution in *Scabiosa* was estimated using a Bayesian divergence time analysis (BEAST), using fossil calibrations reported in the literature. Lastly, biogeographical patterns were investigated using a maximum-likelihood-based dispersal–extinction–cladogenesis model for geographical range evolution (LAGRANGE).

MATERIALS AND METHODS

Sampling and sequences

Scabiosa consists of *c.* 23 species and two species complexes with uncertain taxonomic boundaries: *S. columbaria* s.l. containing *c.* nine taxa and *S. ochroleuca* s.l. containing *c.* five taxa (Jasiewicz, 1976). For this study, 24 taxa were sampled, including seven members of *S. columbaria* s.l. and three members of *S. ochroleuca* s.l., and all major biogeographical regions were represented. For rooting purposes, five species within Dipsacaceae [*Bassecoia hookeri* V. Mayer & Ehrendorfer, *Knautia arvensis* (L.) Coult., *Lomelosia cretica* (L.) Greuter & Burdet, *Pterocephalus strictus* Boiss. & Hohen. and *Sixalix atropurpurea* (L.) Greuter & Burdet] were included based on previous phylogenetic studies (Avino *et al.*, 2009; Carlson *et al.*, 2009). For divergence time estimation, six additional outgroups were sampled from relatives in the Valeriana clade of the Dipsacales (Donoghue *et al.*, 2001): *Triplostegia glandulifera* Wall ex DC, *Valeriana officinalis* L., *Centranthus rubber* (L.) DC, *Nardostachys jatamansi* DC, *Patrinia triloba* Miq. and *Morina longifolia* Wall. This allowed us to include key fossil calibration points (see below).

Sequence data were collected from herbarium specimens, silica-preserved field collections and GenBank (see Appendix S1 in Supporting Information). Total genomic DNA was extracted using a Qiagen DNeasy tissue kit (Qiagen, Valencia, CA, USA), or a modified version using beta-Mercaptoethanol and proteinase-K for herbarium specimens (Wurdack *et al.*, 2004). Six gene regions were amplified and sequenced using standard primers [*trn*L–*trn*F region (Taberlet *et al.*, 1991); *atp*B–*rbc*L region reverse primer (Manen *et al.*, 1994) and forward primer (Carlson *et al.*, 2009); *trn*S^{UGA}–*trn*G^{GCG} (Shaw *et al.*, 2005); *psb*A–*trn*H (Sang *et al.*, 1997); ITS (White *et al.*, 1990); ETS (Baldwin & Markos, 1998; Markos & Baldwin, 2001)]. Standard polymerase chain reaction (PCR) protocols were used to amplify these regions, and the PCR products were cleaned using polyethylene glycol (PEG) precipitation (Kusakawa *et al.*, 1990). Sequences were generated using dye terminator cycle sequencing with ABI PRISM BigDye Primer Cycle Sequencing Ready Reaction kits (Applied Biosystems, Foster City, CA, USA), and visualized using an ABI 3730 DNA Analyzer (Applied Biosystems).

Phylogenetic analysis

Contiguous sequences were assembled using SEQUENCHER 4.7 (Gene Codes Corp., Ann Arbor, MI), and aligned datasets were generated using MUSCLE 3.8 (Edgar, 2004) and adjusted manually in MACCLADE 4.06 (Maddison & Maddison, 2000). The aligned matrix is available in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S11839) and upon request from the first author. Models of molecular evolution were evaluated for each marker using Akaike's information criterion (AIC) scores in MODELTEST 3.7 (Posada & Crandall, 1998) and used to inform a mixed-model partitioned phylogenetic analysis. Bayesian inference (BI) analyses were executed on a concatenated sequence alignment of six molecular markers, with the chloroplast (cpDNA) and nuclear ribosomal (nrDNA) datasets organized into two partitions, and the mutation rate, gamma and state frequencies allowed to vary between the two partitions. BI analyses were performed using MRBAYES 3.1.2 (Ronquist & Huelsenbeck, 2003), and two simultaneous runs were initiated starting from random trees. Posterior probabilities of trees were approximated using the Metropolis-coupled Markov chain Monte Carlo (MCMC) algorithm with four incrementally heated chains [Temperature (T) = 0.2] for 20 million generations, and trees were sampled every 2000 generations. Convergence and sampling intensity were evaluated using the potential scale reduction factor (PRSF) and estimated sample size (ESS). To estimate burn-ins, posterior parameter distributions were viewed using TRACER 1.4 (Rambaut & Drummond, 2007). Maximum-likelihood (ML) analyses were conducted using RAxML 7.0.3 (Stamatakis *et al.*, 2008). Tree searches were executed starting from a random stepwise-addition maximum-parsimony (MP) tree and employed the GTRGAMMA (general time-reversible with rate heterogeneity accommodated by a gamma distribution) nucleotide substitution model. RAxML estimated all free model parameters, with GAMMA model parameters estimated up to an accuracy of 0.1 log-likelihood units. Nonparametric bootstrapping under ML was also carried out with RAxML, using 1000 bootstrap replicates. All RAxML analyses were undertaken using the Cyberinfrastructure for Phylogenetic Research (CIPRES) portal (http://www.phylo.org/portal2).

Divergence time estimation

Two fossil calibrations were used within the closely related clade Valerianaceae (Donoghue *et al.*, 2001). To accommodate palaeontological uncertainty, relatively broad constraints on fossil age were chosen. Fossil fruits assigned to stem group *Patrinia* have been documented from the Miocene to Pliocene of Poland and Russia (Lańcucka-Środoniowa, 1967), as well as from the Miocene of Japan (Ozaki, 1980). In a previous study of divergence times in Dipsacales (Bell & Donoghue, 2005), an age of *c.* 45–60 million years (Myr) was estimated for crown group Valerianaceae. Accordingly, the crown group of Valerianaceae was constrained to a lognormal distribution with an upper bound of 60 million years ago (Ma) and a lower bound of 45 Ma (see also Moore & Donoghue, 2007). *Valeriana* is known on the basis of fossil fruits from the Miocene and Pliocene of Europe (Bell & Donoghue, 2005), and the crown group was constrained to a lognormal distribution with an upper bound of 25 Ma and a lower bound of 15 Ma (Moore & Donoghue, 2007).

To estimate divergence times, the Bayesian divergence time method implemented in BEAST 1.5.4 (Drummond & Rambaut, 2007) was employed. This method allows uncertainty in divergence time estimates resulting from topological and fossil uncertainty. The uncorrelated lognormal (UCLN; Drummond *et al.*, 2006) model of rate evolution was chosen, which does not require rates to be heritable and, therefore, allows lineage-specific rate heterogeneity. The BEAST analyses were conducted specifying prior distributions for the fossil nodes discussed above, and the data were partitioned into cpDNA and nrDNA. Two BEAST analyses were run for 50 million generations, sampling every 5000. Convergence to the same posterior distributions of divergence times and parameter estimates were examined in TRACER, and the burn-in was also determined based on the traces. A maximum-credibility tree, representing the maximum a posteriori topology, was calculated after removing burn-ins with TREEANNOTATOR 1.5.4.

Biogeographical reconstructions

Three biogeographical regions (Fig. 1) were used in the analysis: (1) Europe (including the Mediterranean Basin), (2) Africa (south of the Sahara) and (3) eastern Asia. Each *Scabiosa* species was assigned to one or more of these areas based on descriptions of species distributions in the literature (Verlaque, 1986). The biogeographical history of *Scabiosa* was inferred using a ML-based method, LAGRANGE 2.0.1 (Ree *et al.*, 2005; Ree & Smith, 2008), using the maximum clade credibility tree inferred from BEAST. This approach allows for the modelling of geographical areas to estimate the relative probabilities of ancestral lineages according to the phylogeny, and estimates dispersal and extinction parameters as part of the dispersal–extinction–cladogenesis (DEC) model (Ree & Smith, 2008). Two DEC models (A and B) were used that differed in dispersal probabilities between different biogeographical regions. In model A, dispersal probabilities were equal between all biogeographical areas, with no constraints between regions. In model B, dispersal parameters were allowed to vary, reflecting changes in dispersal opportunities through time, beginning from the age of the root node from the BEAST analysis (the details of model B are described in Appendix S2). All possible area combinations with a maximum of three simultaneous areas were permitted, and dispersal between areas was permitted bidirectionally.

RESULTS

Phylogenetic analysis

Bayesian and ML analyses of the combined cpDNA and nrDNA sequences were performed with 24 accessions of *Scabiosa* and five outgroups from the major clades of Dipsacaceae. The fully aligned data matrix was 4081 bp in length, of which 3003 bp was cpDNA and 1077 bp was nrDNA. The topologies of the trees generated for the cpDNA and nrDNA partitions were generally congruent, although support values were relatively low (data not shown). Combining the cpDNA and nrDNA datasets resulted in a well-supported hypothesis of *Scabiosa* phylogeny (Fig. 2). *Scabiosa* was recovered as monophyletic, with *Sixalix* resolved as its sister group, as in previous phylogenetic studies (Caputo *et al.*, 2004; Avino *et al.*, 2009; Carlson *et al.*, 2009). The phylogenetic analyses resolved two major clades in *Scabiosa*: clade 1 and clade 2. Clade 1 consisted of members of Asian section *Prismakena* (Bobrov, 1957) and a European clade of *S. vestina* Facchini, sister to *S. silenifolia* Waldst. & Kit. + *S. canescens* Waldst. & Kit. Support for the monophyly of section *Prismakena* was low (< 0.80 BI posterior probability support, < 70% ML bootstrap support), although *S. comosa* Fisch. ex Roem. & Schult. and *S. mansenensis* Nakai formed a well-supported clade. Clade 2 consisted of the annual species *S. tenuis* Spruner, sister to a large clade ('clade 2, core group') containing members of *S. columbaria* s.l., *S. ochroleuca* s.l. and a clade of all sampled African species. *Scabiosa ochroleuca* s.l. was supported as monophyletic in the BI analysis, with the Balkan endemics *S. triniifolia* Friv. and *S. webbiana* D. Don resolved as sister taxa. Relationships within the large *S. columbaria* species complex were not resolved with the six markers used. Phylogenetic structure was discernable within the African group, with *S. angustiloba* (Sond.) Burtt ex Hutch., *S. beukiana* Eckl. & Zeyh. and *S. tysonii* L. Bolus forming a clade that was sister to *S. africana* L. and *S. transvaalensis* S. Moore + *S. drakenbergensis* Burtt (although support for this clade was low in the ML analysis; ML bootstrap = 66%).

Divergence time and biogeographical analyses

The phylogeny calculated from the Bayesian divergence time analysis resulted in the same topology as that estimated by the

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BI and ML analyses, with higher support (> 0.80 BI posterior probability) for the *Prismakena* clade and for the clade containing the *S. columbaria* and *S. ochroleuca* species complexes (> 0.95 BI posterior probability; Fig. 3). ML reconstructions of geographical ranges for the major nodes of *Scabiosa* are presented in Fig. 4. Analyses using model A and model B yielded similar results with similar log-likelihood scores (model A: $\ln L = -36.98$; model B: $\ln L = -36.03$). Biogeographical and molecular dating analyses inferred that *Scabiosa* diverged from its sister group, *Sixalix*, in Europe sometime in the Miocene between 6.7 and 15.9 Ma. The split between the two main lineages – clade 1 and clade 2 – occurred between 5.3 and 12.7 Ma. Movement to Asia was reconstructed in the lineage leading to clade 1, with the split between the Asian and European clades estimated to have occurred sometime between the late Miocene and early Pleistocene (2.3–6.6 Ma), followed by diversification of both clades during the Pliocene/Pleistocene (Asian clade: 1.5–5.2 Ma; European clade: 1.1–4.6 Ma). In clade 2, the core group originated sometime between the late Miocene and early Pleistocene (2.0–6.8 Ma),

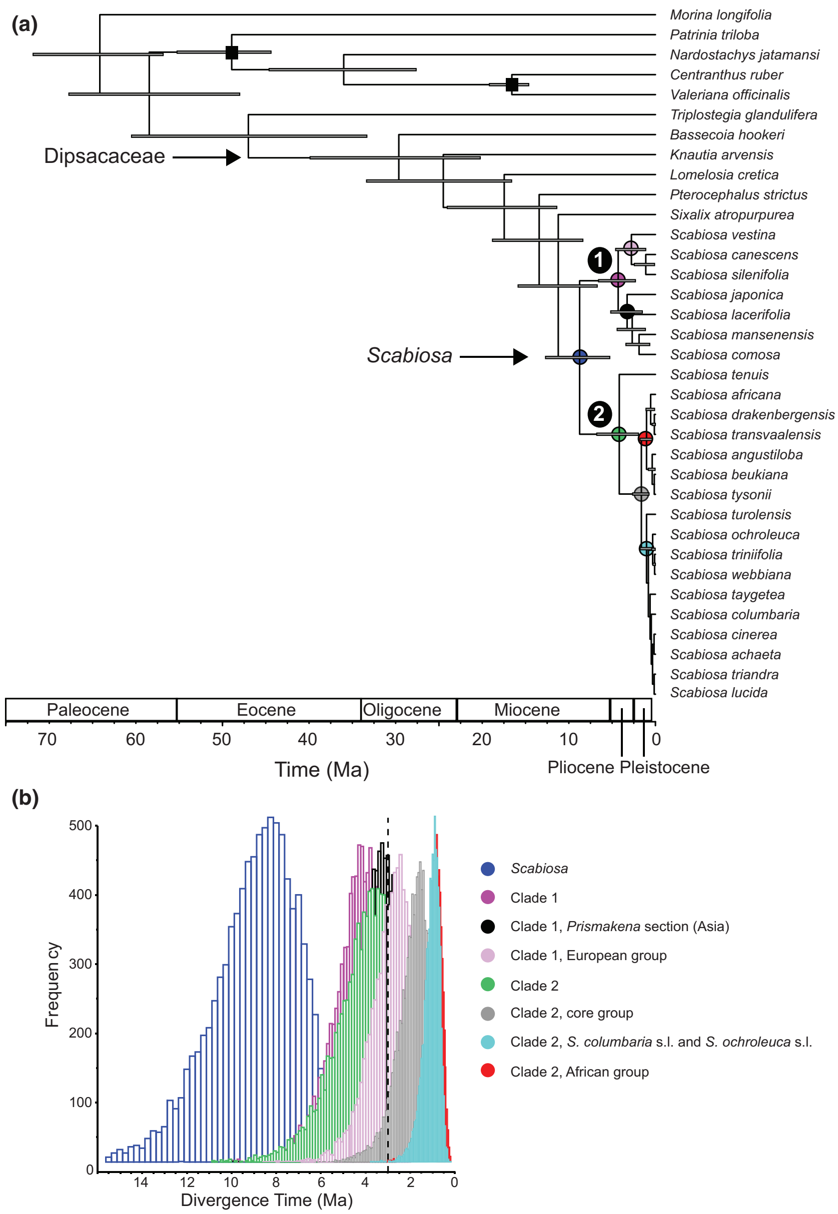

Figure 3 (a) Chronogram of *Scabiosa* produced from the BEAST analysis. Maximum clade credibility tree with mean nodal ages and 95% highest posterior density (HPD) intervals indicated by bars (shaded bars indicate nodes with > 0.80 posterior probability support). The time-scale in Ma (million years ago) and geological time periods are shown at the bottom. Clades 1 and 2 are numbered, black squares represent fossil calibrations, and coloured circles mark clades that correspond to the histograms shown in (b). Histograms display variance in the inferred divergence time estimations for the major clades. The dashed line marks the Pliocene onset of the mediterranean climate in Europe.

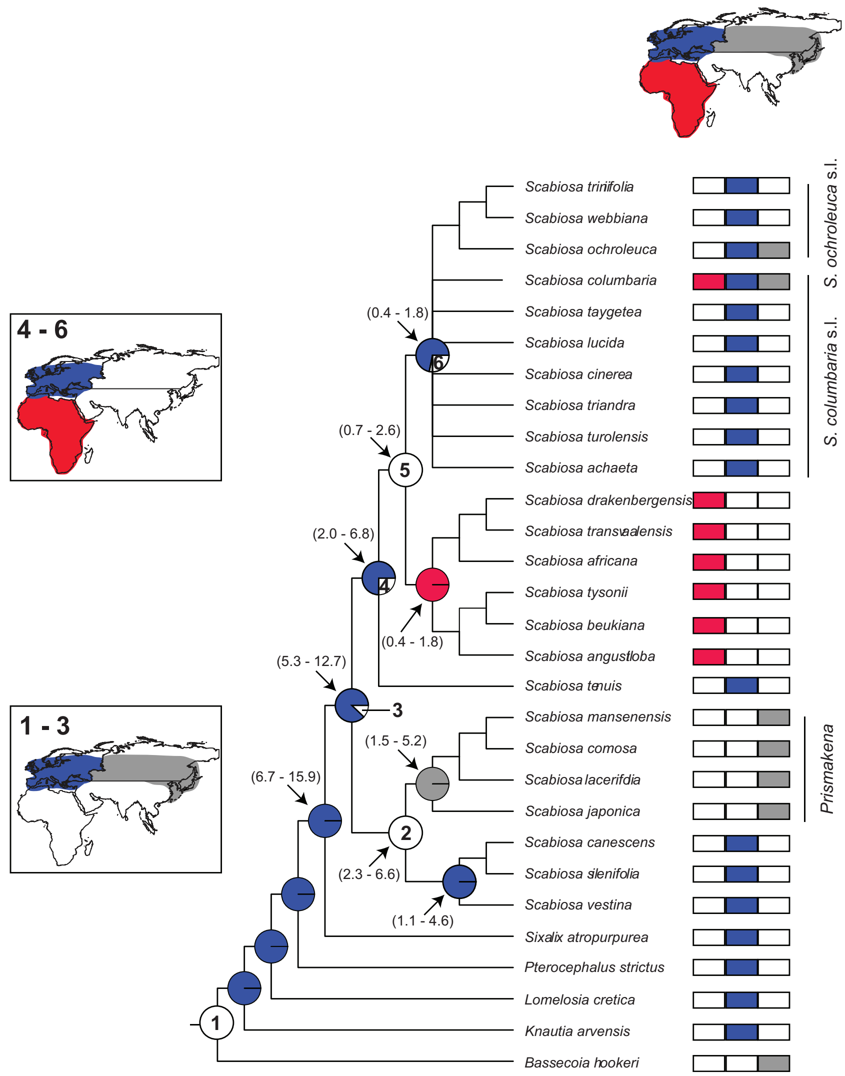

Figure 4 Biogeographical reconstruction of ancestral ranges in *Scabiosa*. Coloured boxes to the right of the species names show current geographical distributions and correspond to the distribution map. Pie charts at nodes represent the probabilities of the most likely ancestral ranges. Ancestral ranges of pie charts labelled with a number (1–6) are shown in the inset maps (e.g. Europe and Asia or Europe and Africa). Numbers in parentheses show inferred divergence time estimates for each node.

and the split between the African clade and the clade containing the *S. columbaria* and *S. ochroleuca* species complexes occurred sometime in the Pleistocene (0.7–2.6 Ma). These clades then radiated in Africa and Europe at a similar time during the Pleistocene (0.4–1.8 Ma for both lineages).

DISCUSSION

Scabiosa phylogeny and character evolution

The phylogenetic results presented here are the most comprehensive for *Scabiosa* to date. Previous studies of Dipsacaceae included relatively few members of *Scabiosa*, and placed the Asian species *S. japonica* Miq. as sister to European and African taxa (Avino *et al.*, 2009; Carlson *et al.*, 2009). Our study shows that there are two major lineages in *Scabiosa* (clade 1 and clade 2), with one clade of European species (containing *S. canescens*, *S. silenifolia*, *S. vestina*) linked with the Asian species in clade 1 (*Prismakena*), and the remaining European species aligned with the African species in clade 2. Members of clade 1 are generally characterized by pleisiomorphic morphological and anatomical features (Verlaque, 1986; Mayer & Ehrendorfer, 1999). In particular, the epicalyx is generally less differentiated than in members of clade 2. The apical part of the epicalyx – the corona – forms a small wing in *Scabiosa*, and in clade 1 the corona is less wing-like and tends to be more irregularly shaped and vertically oriented, with fewer corona nerves (Verlaque, 1986). Furthermore, members of *Prismakena* have a quadrilateral epicalyx that lacks deep grooves in the epicalyx tube, and all examined members of *Prismakena* lacked sclerenchyma – thick cells that are considered an adaptation to arid conditions (Bobrov, 1957; Mayer, 1995; Mayer & Ehrendorfer, 1999). In contrast, members of clade 2 are characterized by an epicalyx with eight prominent grooves, sclerenchyma and a horizontal and more wing-like corona. These epicalyx features are probably related to dispersal and colonization and may have allowed members of clade 2 to successfully colonize regions such as the Mediterranean Basin. The sclerified epicalyx in particular would have given members of clade 2 an advantage in colonizing regions that experience summer drought (Mayer, 1995). Lastly, the European members of clade 1 are united by the presence of entire leaves in the rosette, and our study supports the previously proposed association of *S. canescens* and *S. vestina* based on similar morphology of the cauline leaves (Jasiewicz, 1976).

The major phylogenetic relationships within clade 2 are relatively well resolved, although support for the monophyly of the two species complexes is relatively low in the phylogenetic analysis (but high in the BEAST analysis). *Scabiosa tenuis* is one of only two annuals in *Scabiosa*, and it appears as sister to the remaining perennial species. The African species tend to have mauve flowers (Verlaque, 1986) and are divided into two subclades that differ in elevation and geography. *Scabiosa africana* is the only species in the African clade that occurs in the Mediterranean Cape region (*S. columbaria* s.s. also occurs in this region) and is sister to *S. drakensbergensis* and *S. beukiana* (but with low ML support), which occur at high elevations in the Drakensberg range, which forms the eastern escarpment of the southern African central plateau. Members of the other African subclade (i.e. *S. tysonii*, *S. beukiana* and *S. angustiloba*) occur at lower elevations in the Drakensberg range.

Scabiosa columbaria and *S. ochroleuca* species complexes have long posed a difficult taxonomic problem. Hybridization is common and, as a result, the number of reported species (and subspecies) has varied widely (e.g. Bobrov, 1957; Matthews, 1972; Grossman, 1975; Jasiewicz, 1976). A revision of the species limits in *S. columbaria* s.l. and *S. ochroleuca* s.l. is much needed. In the meantime, our study suggests that several of the proposed species in these complexes do indeed belong to the same evolutionary lineages. Like all species in clade 2, members of the two complexes are morphologically similar, but differ in corolla colour: reddish purple to lilac blue in *S. columbaria* s.l. and white to pale yellow in *S. ochroleuca* s.l. Our finding that members of *S. ochroleuca* s.l. are monophyletic lends support to this taxonomic interpretation and to the utility of corolla colour as a synapomorphy for *S. ochroleuca* s.l.

Origin of *Scabiosa* and Old World disjunctions

The results presented in this study suggest that *Scabiosa* originated sometime in the middle–late Miocene with an initial area of diversification in Europe, which was followed by movement into Asia and Africa. A less specific hypothesis could be that the area of origin cannot be resolved, and is located somewhere within the current distribution areas (‘primitive cosmopolitanism’). However, the more detailed hypothesis of a European area of initial diversification obtains significantly better support than primitive cosmopolitanism or an initial area of diversification in either Africa or Asia (Fig. 4), indicating that this more detailed hypothesis can be preferred using an events-based ML framework. A Miocene origin for *Scabiosa* is consistent with the study of Bell & Donoghue (2005), which showed the major lineages of Dipsacaceae to have originated during this time. During the middle Miocene, global temperatures cooled (Zachos *et al.*, 2001), causing subtropical and warm-temperate elements to retreat from Europe, which opened up niches for herbaceous lineages (Pons *et al.*, 1995). Aridification is also thought to have occurred during this time, caused by changing sea currents owing to the closure of the connection between the Mediterranean Sea and Indian Ocean, which fragmented the Tethys (Krijgsman, 2002). By the late Miocene, palaeo-Mediterranean species began to develop, as the subtropical elements were lost (Thompson, 2005). The origin of *Scabiosa* generally coincides with this shift away from warm-temperate and subtropical elements during the Miocene. The initial diversification of *Scabiosa* may also have been influenced by the Messinian salinity crisis at the end of the Miocene (5.96–5.33 Ma), a geological phenomenon caused by the closing of Mediterranean–Atlantic gateways that resulted in the drying of the Mediterranean Sea and increased salinity (Krijgsman *et al.*, 1999; Krijgsman, 2002). This event allowed the formation of ephemeral corridors that connected land masses throughout the Mediterranean. The impact of the Messinian salinity crisis on plant biogeography is thought to have been driven primarily by differentiation via vicariance (e.g. Sanmartín, 2003; Thompson, 2005; Rodríguez-Sánchez *et al.*, 2008); however, adaptation to saline soils may have also promoted diversification in early diverging *Scabiosa* lineages (Kruckeberg, 1986; Rajakaruna, 2004).

Like all major groups within the Dipsacales, Dipsacaceae may have originated in Asia and subsequently moved west (Bell & Donoghue, 2005; Moore & Donoghue, 2007; Carlson *et al.*, 2009). Our study suggests that movement back into Asia also occurred, as evidenced by the inferred movement of *Scabiosa* into Asia at least once in clade 1. Most work on plant disjunctions in the Northern Hemisphere has focused on plants with an eastern Asia–eastern North America disjunct distribution (e.g. Wen, 1999; Donoghue & Smith, 2004; Winkworth & Donoghue, 2005; Smith & Donoghue, 2009), or with a Eurasia–western North America distribution (i.e. the Madrean–Tethyan disjunction; e.g. Fritsch, 1996; Hileman *et al.*, 2001; Coleman *et al.*, 2003; Smith & Donoghue, 2009; Wen & Ickert-Bond, 2009). There has been less work on disjunctions between eastern Asia and Europe (but see Sun, 2002; Sun & Li, 2003; Wu, 2004), and, unlike other Dipsacales, *Scabiosa* evidently never moved to the New World except as introduced weeds. Northern Hemisphere disjunctions are thought to sometimes have resulted from an earlier, once widespread Cenozoic relict flora that later fragmented (Wen, 1999; Tiffney & Manchester, 2001; Milne & Abbott, 2002). The disjunction between eastern Asia and Europe was the result of extinction owing to the uplift of the

Tibetan Plateau, mainly in the Miocene (Harrison *et al.*, 1992; Axelrod *et al.*, 1998; Sun *et al.*, 2001; Sun, 2002; Zhang *et al.*, 2006; Qiao *et al.*, 2007). In the case of *Scabiosa*, we infer the split between Europe and Asia to be 2.3–6.6 Ma, so we cannot reject the vicariance hypothesis. However, Pliocene climate fluctuations may be a more likely explanation for this disjunction, as is consistent with studies on other plant groups exhibiting more recent disjunctions between Europe and Asia (e.g. Fiz-Palacios *et al.*, 2010; Tu *et al.*, 2010).

Our dating analysis indicates that there were probably no significant barriers to dispersal for the ancestor(s) of Asian *Scabiosa* species north of the Tibetan Plateau. In the Palaeogene, the Turgai Strait created a barrier from the Arctic Ocean to the Tethys Seaway and separated the European and Asian floras until the early Oligocene (Legendre & Hartenberger, 1992), well before the origin of *Scabiosa*. With the demise of the Turgai Strait, a dry and more seasonal continental climate spread through central Asia and is thought to have facilitated exchange between Asia and Europe (Tiffney & Manchester, 2001). While migration is considered to have occurred primarily in an east–west direction (Tiffney & Manchester, 2001), our results with *Scabiosa* provide an example of movement from west to east. The European and Asian members of clade 1 tend to occur in steppe or montane habitats (Bobrov, 1957; Jasiewicz, 1976; Hong *et al.*, 2011), suggesting that perhaps members of *Scabiosa* were 'pre-adapted' to survive in similar environments in Asia and made use of existing corridors (Ackerly, 2004; Donoghue, 2008; Crisp *et al.*, 2009). The wide distributions of *S. columbaria* s.s. and *S. ochroleuca* s.s., which extend through central Asia (Fig. 1), demonstrate the feasibility of migration through Europe and central Asia. The current absence of *Scabiosa* clade 1 between Western Europe and the Altai may be a result of extinction during the glacial climates in the regions in the rain shadows of the Himalaya and Caucasus.

The African *Scabiosa* radiation is also unique in the context of Dipsacaceae biogeography, wherein most lineages occur mostly around the Mediterranean Basin. Plant disjunctions between the Mediterranean Basin and southern Africa are an increasingly well-studied phenomenon, and while many of these disjunctions are associated with xeric conditions in Africa and south-west Asia, others occur in temperate habitats in Africa (e.g. Cape and Afromontane regions) and Eurasia (Hilliard & Burtt, 1971; Linder *et al.*, 1992). Dispersal from a southern African origin is the most common explanation (Caujapé-Castells *et al.*, 2001; Goldblatt *et al.*, 2002; Coleman *et al.*, 2003; Calviño *et al.*, 2006; : Galley *et al.*, 2007; del Hoyo *et al.*, 2009; Sanmartín *et al.*, 2010; Désamoré *et al.*, 2011); however, our study suggests that dispersal to southern Africa from Europe occurred in *Scabiosa*. Although less commonly documented, other African–European disjunct groups also show a European origin, such as *Erica* (McGuire & Kron, 2005) and *Dianthus* (Valente *et al.*, 2010). Our findings support a Pliocene/Pleistocene migration into Africa, perhaps via the East African Rift mountains, which were formed in the late Miocene–Pliocene (Grove, 1983). The current distribution of *S. columbaria* s.s. over the length of east Africa and into southern Africa (Fig. 1) demonstrates the suitability of this track as a corridor for *Scabiosa*.

All members of the African clade are located in the greater Drakensberg range in eastern South Africa, except for *S. africana*, which occurs in the Mediterranean Cape region. Many southern African groups occur in both the Cape and the Drakensberg, but, unlike *Scabiosa*, tend to be more species-poor in the latter (Hilliard & Burtt, 1987; Linder, 2005). This is thought to result from, among other factors (see Linder, 2005), a more stable Pleistocene climate in the Cape, which resulted in less extinction and allowed the range-restricted species characteristic of the fynbos to persist (Galley *et al.*, 2009). The Drakensberg range, on the other hand, is dominated by grasslands, is not characterized by a winter rainfall regime, and experienced greater climatic fluctuations during the Last Glacial Maximum (Harper, 1969). Although the eastern escarpment of southern Africa dates to the Jurassic fragmentation of Gondwana, the current elevation of these mountains may be largely a result of Pliocene uplift (Partridge, 1998). This uplift has been suggested to have triggered diversification of eastern South African plant lineages (Goldblatt *et al.*, 2002; Linder *et al.*, 2006). However, our results suggest that *Scabiosa* radiated in the Drakensberg range after this occurred. The asymmetric distribution of *Scabiosa* species in the Drakensberg range versus the Cape could result from the relatively young age of the African clade, which may have migrated first to the Drakensberg range. The Drakensberg range plays an important role as a 'stepping stone' for plants between the Cape and Afrotemperate regions further north, and migration from the Cape is thought to have occurred predominately through the Drakensberg (Galley *et al.*, 2007; Sanmartín *et al.*, 2010). Because *Scabiosa* has a European origin, migration may have occurred in the opposite direction, with *Scabiosa* arriving first in the Drakensberg before moving into the Cape in the lineage leading to *S. africana*. Like several other Asian and European *Scabiosa* species, species in the Drakensberg range occur in montane habitats, suggesting that this lineage may have filtered into regions to which it was already well adapted. The two clades separate into a Drakensberg foothills clade (*S. tysonii–buekiana–angustiloba*) and summits clade (*S. drakensbergensis–transvaalensis*), and could be radiations out of an original elevational separation. Such an elevational diversification has been documented for the orchids in this region as well (Linder, 1980, 1981). The Cape *S. africana* is related to the high-elevation clade, consistent with the presence of a Cape element at high elevation in the Drakensberg (Weimarck, 1941; Carbutt & Edwards, 2002).

Although we consider it less likely in view of the existence of suitable migration corridors, consideration also needs to be given to the possibility of long-distance dispersal by birds as an explanation for disjunctions in *Scabiosa*. The stiff calyx bristles characteristic of *Scabiosa* diaspores form a pappus that suggests epizoochory (van der Pijl, 1982). Long-distance bird dispersal has been invoked to explain disjunctions in other Mediterranean plant clades such as *Senecio* (Coleman *et al.*, 2003) and

Hordeum (Blattner, 2006), and is regarded as a more common occurrence than previously recognized. Moreover, *Scabiosa nitens*, a species not included in this study but a presumed member of the *S. columbaria* group (Jasiewicz, 1976), occurs on the Azores – oceanic islands that were never connected to continental land masses – which indicates that long-distance dispersal is possible in *Scabiosa*.

Mediterranean diversification

The remarkable species diversity of Mediterranean regions makes the factors underlying diversification of particular interest. The relative importance of the initiation of the mediterranean climate of hot, dry summers and cool, wet winters remains unclear. The origin and diversification of Mediterranean lineages such as *Antirrhinum* (Vargas *et al.*, 2009) and *Senecio* sect. *Senecio* (Coleman *et al.*, 2003) are dated to the Pliocene, suggesting a climatic link. However, the origins of several other lineages pre-date the mediterranean climate, with diversification spanning the Oligocene, Miocene, Pliocene and Pleistocene [e.g. *Androcymbium* (Caujapé-Castells *et al.*, 2001; del Hoyo *et al.*, 2009); *Anthemis* (Lo Presti & Oberprieler, 2009); *Cyclamen* (Yesson *et al.*, 2009); *Ruta* (Salvo *et al.*, 2010)]. Similarly, the origin of *Scabiosa* pre-dates the Pliocene, but the diversification of major subclades within *Scabiosa* may be associated with the Pliocene increase in summer drought. For example, the divergence time estimates of the crown ages of clade 1, clade 2 and the European group of clade 1 encompass the timeframe during which the mediterranean climate was formed, *c.* 3 Ma (Suc, 1984). While the confidence intervals preclude more precise dating of these clades, a correlation between the mediterranean climate and diversification of some of the major *Scabiosa* lineages cannot be ruled out.

The Pleistocene radiation of the clade containing the *S. columbaria* and *S. ochroleuca* complexes has clearly played a prominent role in the evolution of *Scabiosa* in Europe. As with other recent radiations, this clade forms a large polytomy (except for the *S. ochroleuca* group), and further analysis using additional markers is required to discern relationships within this group. Other European radiations, such as *Cistus* (Guzmán *et al.*, 2009) and *Dianthus* (Valente *et al.*, 2010), also diversified primarily in the Mediterranean Basin and are dated to the Pleistocene, suggesting a prominent role for the climatic fluctuations that characterized this time in the evolution of the contemporary Mediterranean flora. Despite climatic instability during the Pleistocene, numerous refugia that allowed long-term species persistence existed in the Mediterranean Basin (Taberlet *et al.*, 1998), where several members of the two *Scabiosa* species complexes occur. These refugia probably provided the source material for the recolonization of previously glaciated areas by members of *Scabiosa* in central Europe (von Hagen *et al.*, 2008).

A further increase in summer drought during the Pleistocene (Mai, 1989; Svenning, 2003; Rodríguez-Sánchez & Arroyo, 2009) may also be associated with the radiation of the *Scabiosa* species complexes. Sclerenchyma in the epicalyx tube, characteristic of all members of clade 2, may have enabled persistence and adaptation to drought conditions (Mayer, 1995). In addition, members of the two groups are differentiated by leaf shape and pubescence traits that are associated with resistance to drought stress and solar irradiance (Lambers *et al.*, 1998). For example, taxa that occur in semi-arid Mediterranean regions (e.g. *S. turolensis*, *S. taygetea*) have leaves that are covered in woolly, dense hairs (i.e. they are 'lanate'), and species in the dry, stony meadows of the Balkan peninsula (e.g. *S. webbiana*, *S. triniifolia*) have leaves covered in a soft mat of short, erect hairs. In contrast, species that occur in more humid, formerly glaciated regions in Central and Eastern Europe, often in the mountains (e.g. *S. lucida*), have glabrous leaves. A similar pattern is reported for *Cistus*, where the diverse microclimatic conditions of the Mediterranean Basin are correlated with the evolution of divergent leaf traits (Guzmán *et al.*, 2009).

Summary and concluding thoughts

The unusual triple disjunction of *Scabiosa* in the Old World provides the opportunity to evaluate the origin and timing of intercontinental disjunctions involving eastern Asia, Europe and southern Africa. Separate movements into Asia and, later, Africa from Europe offer a less commonly documented example of west–east and north–south migration in the Old World. The timing of disjunctions in *Scabiosa* tends not to support the hypothesis of vicariance of a once widespread Cenozoic flora (although this cannot be ruled out in the Europe–Asia disjunction), but rather to point to the importance of Pliocene/Pleistocene climate fluctuations and/or long-distance dispersal. Migration corridors such as the East African Rift mountains may have been of great importance, as many members of *Scabiosa* occur in montane habitats and could have migrated through areas to which they were already well adapted. The immense ranges of *S. columbaria* s.s. and *S. ochroleuca* s.s. demonstrate the current suitability of migration through these corridors, and perhaps provide insight into how the triple disjunction of *Scabiosa* was achieved in the past. That is, the current distributions of *S. columbaria* s.s. and *S. ochroleuca* s.s. may represent 'history repeating itself', with the expectation that these widespread ranges will fragment in the future as populations undergo local adaptation in different parts of the range.

The initiation of the mediterranean climate has been invoked to explain the high species diversity associated with the Mediterranean Basin. The origin of *Scabiosa*, however, does not appear to coincide with this event. Rather, the retreat of subtropical floras in response to increasing aridity in Europe during the Miocene may have been more important. Nevertheless, it appears that the mediterranean climate may have played an important role in *Scabiosa* diversification. Divergence time estimates for several of the major subclades encompass the Pliocene origin of the mediterranean climate, and most taxa within the *S. columbaria* s.l. and *S. ochroleuca* s.l. radiation occur in typical Mediterranean habitats. To successfully colonize this region, these species presumably adjusted their phenology to seasonal rainfall patterns (i.e. severe summer drought, with the majority of rainfall in the winter). Because members of *Scabiosa* were already successful in colonizing dry habitats such as rocky mountain meadows and steppes, perhaps they were pre-adapted to survive in Mediterranean regions (Ackerly, 2004). In other words, their physiological ecology may have been 'half way there', but adaptations to the seasonal drought and rainfall of Mediterranean regions (e.g. leaf shape and pubescence) were more recent.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article:

Appendix S1 Species sampled, voucher information, and GenBank accessions of DNA sequences analysed in this study.
Appendix S2 Description of biogeographical models used in the Lagrange analysis.

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