Rapid diversification of Tragopogon and ecological associates in Eurasia

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Rapid diversification of *Tragopogon* and ecological associates in Eurasia


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Abstract

*Tragopogon* comprises approximately 150 described species distributed throughout Eurasia from Ireland and the UK to India and China with a few species in North Africa. Most of the species diversity is found in Eastern Europe to Western Asia. Previous phylogenetic analyses identified several major clades, generally corresponding to recognized taxonomic sections, although relationships both among these clades and among species within clades remain largely unresolved. These patterns are consistent with rapid diversification following the origin of *Tragopogon*, and this study addresses the timing and rate of diversification in *Tragopogon*. Using BEAST to simultaneously estimate a phylogeny and divergence times, we estimate the age of a major split and subsequent rapid divergence within *Tragopogon* to be ~2.6 Ma (and 1.7–5.4 Ma using various clock estimates). Based on the age estimates obtained with BEAST (HPD 1.7–5.4 Ma) for the origin of crown group *Tragopogon* and 200 estimated species (to accommodate a large number of cryptic species), the diversification rate of *Tragopogon* is approximately 0.84–2.71 species/Myr for the crown group, assuming low levels of extinction. This estimate is comparable in rate to a rapid Eurasian radiation in *Dianthus* (0.66–3.89 species/Myr), which occurs in the same or similar habitats. Using available data, we show that subclades of various plant taxa that occur in the same semi-arid habitats of Eurasia also represent rapid radiations occurring during roughly the same window of time (1.7–5.4 Ma), suggesting similar causal events. However, not all species-rich plant genera from the same habitats diverged at the same time, or at the same tempo. Radiations of several other clades in this same habitat (e.g. *Campanula*, *Knautia*, *Scabiosa*) occurred at earlier dates (45–4.28 Ma). Existing phylogenetic data and diversification estimates therefore indicate that, although some elements of these semi-arid communities radiated during the Plio-Pleistocene period, other clades sharing the same habitat appear to have diversified earlier.

Introduction

The most rapid plant radiations have been shown to have occurred in well-known diversification hot spots, including the Andes, Canary Islands, Hawaiian Islands, South Africa and certain regions with Mediterranean climates (Paun et al., 2005; Linder, 2008; Sauquet et al., 2009). Valente et al. (2010) recently documented an exceptionally rapid rate of diversification in *Dianthus* (carnation; Caryophyllaceae) in temperate Eurasia; *Dianthus* comprises over 300 species, with over 100 species involved in the radiation examined. With this exception, rapid, recent radiations have not been well
documented in the Mediterranean or other north-temperate Eurasian floras. In addition, the rapid diversification of Dianthus appears to coincide with an increase in aridity and seasonality in the region, mostly during the Plio-Pleistocene [1.2–7.0 million years ago (Ma)], marking the onset of the typical Mediterranean climate (Suc, 1984).

Species of Dianthus commonly occur in semi-arid, steppe-like communities in frequent association with species of Tragopogon (Asteraceae) (Carni, 1997; Fekete et al., 2002; Safronova, 2008). Based on several published treatments, Tragopogon comprises up to 150 species (reviewed in Mavrodiev et al., 2004, 2005); however, this number may well be an underestimate due to the proposed presence and recent detection of many cryptic species (see also Mavrodiev et al., 2007, 2008a,b). Tragopogon occurs throughout Eurasia from Ireland and the UK to India and China with a few species in North Africa. Most species occur in Iran, Iraq, Afghanistan and neighbouring areas of the Caucasus, Turkey, Eastern Europe and Central Asia. Although many species of Tragopogon occur in and near the Mediterranean, the distribution of Tragopogon corresponds more accurately to the Paratethys region, an ancient basin that covered 3 million km² and that is represented today by the Mediterranean, Black, Caspian and Aral Seas (reviewed in Sprovi et al., 2003), and the adjacent mountainous areas (Tzvelev, 1985). Thus, although the large number of Mediterranean species of Tragopogon may initially suggest a diversification in response to the onset of Mediterranean climate with wet, mildly cool winters and long, dry summers, the diversification may have actually occurred in semi-arid steppe vegetation further east and north-east, where winters are colder and precipitation is more evenly distributed throughout the year. These different climates naturally harbour quite different vegetation with sclerophyllous shrublands and low-growing forests occurring in the Mediterranean and grasslands with interspersed forest islands farther east.

Despite extensive sampling of genes and species, the phylogeny of Tragopogon is not yet fully resolved (Mavrodiev et al., 2005, 2008a,b). Poorly resolved phylogenies in many groups of organisms, including diverse insect lineages, birds, turtles, mammals and angiosperms, have been interpreted as the result of rapid radiations (reviewed in Whitfield & Lockhart, 2007). We therefore hypothesize that the species-rich Tragopogon may similarly represent a rapid radiation that perhaps occurred in response to the same drying trends that are proposed to have prompted rapid diversification in Dianthus. Indeed, we postulate that other floristic elements of these same communities may have similarly diversified during this same time period, potentially brought on by similar climatic, ecological and/or geological factors. To test this hypothesis, we estimated divergence times to compute absolute rates of diversification for Tragopogon. In addition, we investigated the variability in diversification rate through time using a variety of methods and simulations. Results for Tragopogon are congruent with those for Dianthus. Thus, we propose that rapid diversification may be characteristic of many lineages from semi-arid grassland communities in Eurasia. We therefore searched the literature for further possible examples of rapid diversification in this habitat. We tried to control for the place of diversification to distinguish between hypotheses that the Mediterranean climate is responsible for the diversification versus diversification in response to environmental changes farther east.

Materials and methods

Sampling strategy

Tragopogon is part of Scorzoneraeae (Cichorieae, Asteraeae), a well-supported clade that also comprises Epilasia, Podospermum, Koelpinia, Lasiospora, Pterachaea, Tournesia, Geropogen, Takhtajananthia and the polyphyletic Scorzonera (Mavrodiev et al., 2004). We used the intergeneric transcribed spacer (ITS) data set for Scorzoneraeae from Mavrodiev et al. (2004) and supplemented it with data for additional species of Tragopogon from subsequent work (Mavrodiev et al., 2005, 2007, 2008a,b). We included nearly all presumed diploid species listed in all recent comprehensive taxonomic treatments of Tragopogon (summarized in Nikitin, 1937; Richardson, 1976; Mavrodiev et al., 2005, 2008a). However, for 13 species listed in these treatments, we were unable to obtain material: T. alicus, T. albomarginatus, T. bjelouricus, T. erosris, T. gorskiwanus, T. heteropappus, T. idae, T. lassithicus, T. lithuanicus, T. pichleri, T. karjagini, T. savranicus and T. verrucosobracteatus. Lactua sp. was included as an outgroup (see Mavrodiev et al., 2004). ITS sequences were aligned using MAFFT ver. 6.0 (Katoh & Toh, 2008) with default settings for gap opening penalty and offset value.

Phylogenetic and divergence time analyses

To determine adequate models of molecular evolution for our likelihood and Bayesian analyses, we used the program MrModelTest ver. 2.2 (Nylander, 2004), in conjunction with PAUP* (Swofford, 2003). We selected models based on the Akaike Information Criterion (AIC). For these data, a GTR+I model was selected as the most appropriate.

We performed a likelihood ratio test (Felsenstein, 1981) to determine whether our sequence data were evolving in a clock-like fashion. Because rate constancy among lineages was rejected (P < 0.001), we estimated divergence times using a Bayesian uncorrelated lognormal model as implemented in BEAST 1.7.1 (Drummond & Rambaut, 2007). In our analysis, we set the
underlying model of molecular evolution to be GTR+Γ and simultaneously estimated both tree topology and divergence times. BEAST also allows for uncertainty in the age of calibrations to be represented as prior distributions rather than as strict/fixe calibration points. For each analysis, we initiated four independent Markov chain Monte Carlo (MCMC) analyses from starting trees with branch lengths that satisfied the priors on divergence times. A starting tree with branch lengths satisfying the fossil prior constraint was generated by r8s v.1.7 using nonparametric rate smoothing (NPRS; Sanderson, 2003). For each MCMC analysis, we ran six independent chains for 100 million generations and assessed convergence and stationarity of each chain to the posterior distribution using Tracer v.1.5 (Rambaut & Drummond, 2007) and by plotting time series of the log posterior probability of sampled parameter values. After stationarity was achieved, we sampled each chain every 1000 steps until an effective sample size (ESS) of >200 samples was obtained. If convergence between the independent chains was evident, we combined the samples from each run using LogCombiner v.1.6.1 (part of the BEAST package).

Tremetsberger et al. (2005; reviewed in Kilian et al., 2009) estimated the age of Cichorieae and its subtribes based on both fossil evidence and DNA sequences. Subtribe Scorzonerinæ originated 17.4–21.2 Ma, and the minimum age of pollen of Scorzonera hispanica based on the fossil record is 3.4 Ma. Using 17.4 and 21.2 Ma (Tremetsberger et al., 2005) as minimum age constraints, the ages of Tragopogon as well as of other subclades of Scorzonerinæ were obtained using the Bayesian uncorrelated lognormal method as implemented in BEAST.

### Estimation of diversification rates in Tragopogon

To test the null hypothesis of constant diversification rates through time, we used both the birth–death likelihood (BDL) and pure-birth likelihood (PBL) method of Rabosky (2006) and compared them to three models that relax the assumption of rate constancy in diversification. Under these models, the method provides the most likely break points for models with two or more rates. We used the AIC to evaluate the five different diversification models. We selected the best-fitting model by comparing the difference in AIC values (AAIC) between the best rate-constant model and the AIC values of the rate-variable models. Type I error was evaluated by creating a null distribution of expected differences in AIC values for the best-fit rate-constant (\(\text{AIC}_{\text{RC}}\)) and rate-variable (\(\text{AIC}_{\text{RV}}\)) models. We simulated 10 000 pure-birth trees with taxon sampling in Phylogen version 1.1 (Rambaut, 2002). We also tested for departures from a constant diversification rate using the constant rates (CR) test of Pybus & Harvey (2000). For this test, a positive \(\gamma\) (gamma) value suggests a history of an increase in diversification rate through time, whereas a negative value indicates a decrease in diversification rate towards the tips of the clade. To assess type I error due to incomplete sampling, we used the Monte Carlo CR (MCCR) test of Pybus & Harvey (2000). This test calculates a critical value for \(x = 0.05\) for a given data set by simulating a distribution of pure-birth phylogenies with a specified proportion of incomplete sampling (see Supporting Information). For this test, we simulated 10 000 incomplete sampled trees using Phylogen. To evaluate whether differential rates of diversification resulted from an excess of expected species in Tragopogon or from an excess within a subclade of Tragopogon, we performed analyses of BDL and PBL models, the CR test and the MCCR on both the whole tree with outgroups (i.e. the Scorzonerinæ) and a tree that included only species of Tragopogon.

We further explored the effect of missing taxa on estimates of differential diversification rates using methods described in Day et al. (2008) and Valente et al. (2010). We followed their protocol to add all missing species of Tragopogon randomly to the maximum clade credibility (MCC) tree from the BEAST analysis. We repeated this process on each of 10 000 sample BEAST trees to create a distribution of branching times that incorporates topological uncertainty, branch-length variation, as well as uncertainty in the placement of the missing taxa. Current standing diversity of Tragopogon was estimated by taking names from the International Plant Names Index (IPNI) and the Euro-Med database (Greuter, 2006–2009), as well as floras and taxonomic treatments of Tragopogon (summarized in Mavrodiev et al., 2004, 2005, 2007, 2008a,b; see Table S1). We integrated information from our own phylogenetic analyses with species names and interpretations from the literature. Lineage-through-time (LTT) plots were then constructed using the R package APE (Paradis et al., 2004). All analyses assumed an extant species diversity of at least 300 species for Scorzonerinæ and 200 species for Tragopogon (see Accounting for missing taxa section below).

We estimated the absolute rate of diversification in Tragopogon for five different models. Net diversification rates were estimated using the whole-clade method of Magallón & Sanderson (2001) that does not assume complete taxon sampling. We estimated rates for both stem and crown group Tragopogon for two extremes: one, where we assumed no extinction (\(e = 0\)), and another, where we assumed that the extinction rate was high (\(e = 0.9\)). All calculations were made using GEIGER (Harmon et al., 2008).

For the broader comparison with other genera co-occurring with Tragopogon and Dianthus, we investigated the available data on the composition of Eurasian plant communities that include species of Tragopogon (Carni, 1997; Fekete et al., 2002; Safronova, 2008). Rates for
these taxa were calculated as described previously for *Tragopogon*. When ranges in the number of species were given, we used the maximum value to calculate rate values.

**Accounting for missing taxa**

As described previously, we explored the effect of missing taxa on our estimates of differential diversification rate estimates using methods described in Day et al. (2008) and Valente et al. (2010). We conducted two separate sampling strategies. First, we added all missing species of *Tragopogon* randomly to the MCC tree generated by the BEAST analysis. Second, we assigned each missing taxon to group with a putative relative (see below, Table S1). For each, we repeated this process on each of 10 000 sample BEAST trees to create a distribution of branching times that incorporates uncertainty in topology, branch-length variation, as well as the placement of the missing taxa. Current standing diversity of *Tragopogon* was estimated by taking names from the IPNI (www.ipni.org) and the Euro-Med database, as well as floras and taxonomic treatments of *Tragopogon* (Table S1). We integrated information from our own phylogenetic analyses with species names and interpretations from the literature. Species labelled ‘not clear’ in Table S1 were randomly assigned to fall anywhere within the crown group. For the remaining lineages, we assigned missing numbers of taxa based on values in Table S2. Because *Scorzonerinae* is clearly polyphyletic in our analysis (Fig. S1), we assigned equal numbers of missing taxa (i.e. ~48) to each of the four positions for *Scorzonerinae* in our tree.

The distribution of branches throughout the tree was evaluated across all completely sampled trees using the gamma statistic (Pybus & Harvey, 2000). Positive values for this statistic suggest that there has been an increase in diversification towards the tips of the tree (i.e. more shorter branches towards the tips of the tree), whereas negative values suggest a deceleration in diversification towards the tips of the tree.

**Results**

**Phylogenetic analyses**

Results from the simultaneous estimation of topology and divergence times using BEAST are presented in Fig. 1. *Lactuca* was specified as the outgroup (see Mavrodiev et al., 2004), and the tree is rooted between *Lactuca* and *Scorzonerinae*. The major clades recovered here are similar to those reported by Mavrodiev et al. (2004) and clearly demonstrate the polyphyly of *Scorzonerinae* and the validity of the taxa segregated from it. This Bayesian tree, however, differs slightly from both the maximum parsimony (MP) and maximum likelihood (ML) trees presented in Mavrodiev et al. (2004), but only in those regions of the MP and ML trees that received low bootstrap support. For example, *Gereopogon* is sister to *Tragopogon* in the BEAST tree reported here, but is sister to *Podospermum* in both the MP (< 50% bootstrap support) and ML (63% bootstrap support).

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**Fig. 1** Timing and diversification of *Tragopogon*. (a) Chronogram of maximum clade credibility (MCC) tree inferred with BEAST. Red clade represents *Tragopogon*, whereas black branches are for other lineages of *Scorzonerinae* in addition to one species of *Lactuca*. Horizontal axis represents time, in millions of years before the present. (b) Lineage-through-time (LTT) plots of 10 000 resampled trees with missing taxa added using the method of Day et al. (2008). The black line represents the MCC tree from the BEAST analysis. Horizontal axis represents time, in millions of years before the present. The vertical axis represents number of lineages present. See Supporting information for more details.
support) trees. The sampled species of *Tragopogon* form a well-supported (PP = 1.0) clade. However, posterior probability values for relationships among species of *Tragopogon* were typically low (PP < 0.70). For more details, see Supporting Information.

**Divergence times**

The BEAST analyses estimated the age of the most recent common ancestor (mrca) of extant *Tragopogon* (i.e. crown group age) as 2.6 Ma (1.7–5.4 highest posterior density (HPD); Fig. 1). *Tragopogon* was subsequently split into two major clades (see also Mavrodiev et al., 2005), followed by further diversification. The age of stem group *Tragopogon* was estimated at 7.4 Ma (3.7–11.6 HPD). The age of the entire clade (*Lactuca + Scorzonerinae*) was estimated at 24.6 Ma (17.2–35.4 HPD).

**Estimated species richness and differential diversification of *Tragopogon***

In our previous analyses (Mavrodiev et al., 2004, 2005, 2007, 2008a,b) we recognized 122 diploid species of *Tragopogon*. Our survey of the literature has revealed an additional 53 species that appear to be diploid, are legally published and either do not appear to be synonyms of other species or were treated as synonyms without a comprehensive investigation. These species have not been included in any phylogenetic analyses, and their placements can only be inferred from taxonomies and suggested relationships proposed in the literature (Table S1). These 53 species were placed in those clades in the BEAST topology that possessed their highest support. The sampled species of *Tragopogon* was recently named (Sua rez-Santiago et al., 2011). Hence, the total number of species (summarized in Mavrodiev et al., 2004, 2007, 2008a,b) is likely an underestimate. Based on the number of named species, we estimate the diversification rate of *Tragopogon* to be approximately 0.84–2.71 species/Myr for the crown group, assuming no extinction, and 0.55–1.76 species/Myr, assuming a high proportion of extinction (c = 0.9). Estimates based on different values of current species diversity, as well as stem-group-based diversification estimates, are presented in Table 1.

Based on the BDL analysis, we strongly rejected the hypothesis of a constant diversification rate in Scorzonerinae. Likewise, the calculated $\gamma$ statistic (11.9) suggested strong departure from the null hypothesis of a constant diversification rate for the clade ($P < 0.0001$, based on MCCR test) and an increase in diversification rate through time. We estimated the best-fit model to be the rate-variable, yule2rate model ($P < 0.001$). This model assumes a pure-birth process, followed by a shift in diversification rate (either up or down) at a specific break point. With the sampling used, our analysis suggests that Scorzonerinae diversified at a constant rate (0.12 ± 0.06 species/Myr) until roughly 2.5 Ma, when there was a >10-fold increase to 2.3 ± 0.18 species/Myr. This increase in diversification rate is illustrated in a lineage-through-time plot (Fig. 1) that shows a strong and sudden increase in diversification before the present. These results were consistent across all 10 000 trees that had missing taxa randomly added. We stress, however, that this analysis of Scorzonerinae should be considered an initial assessment. Although our sampling of most genera of Scorzonerinae was nearly comprehensive, we are still lacking several species of *Tragopogon*, and the large, polyphyletic *Scorzoneravara* requires improved representation, with only ten species (of 170–200) was being sampled for this analysis.

For the *Tragopogon* clade, we calculated a $\gamma$ of 2.51 ($P < 0.0001$, based on MCCR test). Our analysis, based on the best-fit yule2rate model ($P < 0.01$, based on simulations), suggests that *Tragopogon* diversified at a constant rate (1.83 ± 0.16 species/Myr) until roughly 0.48 Ma, when there was a decrease in rate to 0.33 ± 0.08 species/Myr (see Fig. 1).

**Accounting for missing taxa**

Each subsampling strategy showed very similar results as assessed by the gamma statistic, with all subsampling routines having overlapping values across the 10 000

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**Table 1** Diversification rates in *Tragopogon*. Calculated net diversification rates (birth–death) based on method of Magallón & Sanderson (2001)

<table>
<thead>
<tr>
<th>Clade</th>
<th>n = 150</th>
<th>n = 200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crown Group, $c = 0$</td>
<td>1.26 (0.79–2.44)</td>
<td>1.34 (0.84–2.71)</td>
</tr>
<tr>
<td>Crown Group, $c = 0.9$</td>
<td>0.79 (0.50–1.53)</td>
<td>0.87 (0.55–1.69)</td>
</tr>
<tr>
<td>Stem Group, $c = 0$</td>
<td>0.68 (0.43–1.38)</td>
<td>0.72 (0.46–1.44)</td>
</tr>
<tr>
<td>Stem Group, $c = 0.9$</td>
<td>0.37 (0.24–0.75)</td>
<td>0.41 (0.26–0.82)</td>
</tr>
</tbody>
</table>
trees (range = −148.87 to −211.89). The overall similarity in values suggests little effect of subsampling, or choice of placement of the missing taxa. The negative values across all sampling strategies suggest a deceleration in diversification rates towards the tips of the trees.

**Discussion**

The estimated rate of diversification for *Tragopogon* is 0.84–2.71 species/Myr (assuming 200 extant species and little to no extinction during the age of the clade), which overlaps with the rate estimated for *Dianthus* (0.66–3.89 species/Myr; Valente et al., 2010). Therefore, *Tragopogon* appears to provide a second clear example of a rapid Eurasian radiation during the Plio-Pleistocene.

Significantly, the rate of *Tragopogon* radiation is also close to many other examples of rapid plant radiations from around the world (reviewed in Valente et al., 2010). For example, *Tragopogon* radiated at rates comparable with Mediterranean *Cistus* (Guzmán & Vargas, 2009; speciation rate of 1.46–2.44 species/Myr), alpine *Soldanella* (Kadereit et al., 2004: 1.64–2.55 species/Myr), South American *Lupinus* (Hughes & Eastwood, 2006: 1.30–3.78 species/Myr), South American *Valeriana* (Bell & Donoghue, 2005: 1.71–3.2 species/Myr) and Neotropical *Gentianella* (von Hagen & Kadereit, 2001: 1.64–2.55 species/Myr).

In Eurasia, different species of *Tragopogon* occur in various types of open habitats, often with *Dianthus* species (Vicherek, 1972; Karpov et al., 1987; Golub & Saveljeva, 1991; Golub, 1994; Carni, 1997; Tyschenko, 1998; Umanets & Solomakha, 1998; Fekete et al., 2002; Golub et al., 2002, 2009; Chytřý & Rafajová, 2003; Dubina et al., 2003, 2004; Voityuk, 2005; Safronova, 2008), as well as with species of other clades thought to represent rapid radiations. Such communities commonly include species of *Artemisia*, *Astragalus*, *Campanula*, *Centaurea*, *Dianthus*, *Knautia*, *Scabiosa*, *Scorzoneria*, *Silene* and *Veronica*, in addition to *Tragopogon* (Carni, 1997; Fekete et al., 2002; Noroozi et al., 2008; Safronova, 2008). Thus, these genera may represent other cases of rapid diversification. Our analyses of diversification rates for some of these genera (Table 2) demonstrate that *Dianthus* and *Tragopogon* may be extreme cases of diversification in this region.

The diversification rates for *Tragopogon*, *Dianthus* and other examples from the Mediterranean coincide with

### Table 2 Comparison of diversification rates among Mediterranean plant clades. Rates were calculated using methods described by Magallón & Sanderson (2001). When ranges in the number of species were given, we used the maximum value to calculate rate values. In addition, we assumed the proportion of extinction parameter (e) to be equal to zero in all cases. Ages of clades were taken from reference supplied in last column.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Age of crown group (Myr)</th>
<th>Diversification rate (crown group, sp/Myr)</th>
<th>Number of species</th>
<th>Distribution</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td><em>Anthemis</em></td>
<td>8.83</td>
<td>0.50</td>
<td>170</td>
<td>E Mediterranean</td>
<td>Lo Presti &amp; Oberprieler (2009)</td>
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<tr>
<td><em>Antirrhinum</em></td>
<td>4.1</td>
<td>0.62</td>
<td>25</td>
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<td>Vargas et al. (2009)</td>
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<td><em>Arum sect. Dioscorides</em></td>
<td>3.9</td>
<td>0.61</td>
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<td>Linz et al. (2010)</td>
</tr>
<tr>
<td><em>Campanula</em></td>
<td>45–80</td>
<td>0.07–0.12</td>
<td>500</td>
<td>Mediterranean</td>
<td>Cellinese et al. (2009)</td>
</tr>
<tr>
<td><em>Centaurea (behen + involucrata Group)</em></td>
<td>7.68</td>
<td>0.55</td>
<td>150</td>
<td>Mediterranean</td>
<td>Barres et al., unpub. data</td>
</tr>
<tr>
<td><em>Centaurea (depressa + ñingulata Group)</em></td>
<td>6.03</td>
<td>0.47</td>
<td>30</td>
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<td>Barres et al., unpub. data</td>
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<td><em>Centaurea sect. Acrocentron</em></td>
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<td>1.95</td>
<td>100</td>
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<td>4.25</td>
<td>0.55</td>
<td>21</td>
<td>W Mediterranean</td>
<td>Fernández-Mazuecos &amp; Vargas (2010)</td>
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<tr>
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<td>5.2</td>
<td>0.54</td>
<td>33</td>
<td>Europe</td>
<td>Guzmán &amp; Vargas (2009)</td>
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<tr>
<td><em>Cousinia – cousioïd clade</em></td>
<td>3.4–16.9</td>
<td>0.34–1.68</td>
<td>200–600</td>
<td>SW Asia</td>
<td>López-Vinyalloneta et al. (2009)</td>
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<td>0.66–3.84</td>
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<tr>
<td>‘eu-Scorzonera’</td>
<td>3.8</td>
<td>0.89</td>
<td>50–60</td>
<td>Mediterranean and Irano-Turanian</td>
<td>This study</td>
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<td>21</td>
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<td>This study</td>
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</tbody>
</table>
marked climatic and topographic changes in this area. The inferred diversification time follows the Messinian Salinity Crisis (MSC), one of the most dramatic geological events that occurred in this area in the late Miocene, between 5.3 and 5.96 Ma (Hsu et al., 1977; Krijgsman et al., 1999). During this time, the Mediterranean Sea became temporarily isolated from the Atlantic Ocean, resulting in the gradual drying of the basin. The cause of the closure of the Strait of Gibraltar is not completely clear, and a large body of literature documents that the MSC was driven by a complex combination of tectonic and glacio-eustatic processes that progressively isolated the Mediterranean Sea from the open ocean (Clauzon et al., 1996; Krijgsman et al., 1999; Duggen et al., 2003; Fauquette et al., 2006; Jolivet et al., 2006; Rouchy et al., 2006; Gargani & Rigollet, 2007). Following the MSC, the warm, humid climate of the Miocene further deteriorated and shifted to clear seasonality with summer droughts and cold, humid winters (Valente et al., 2010). These Pliocene changes may not have happened simultaneously but seem to have started in the eastern Mediterranean (Thompson, 2005). Glaciations in the north led to a sharper climatic differentiation between the northern and southern sides of the Mediterranean (Pons et al., 1995). Furthermore, significant tectonic uplift from Turkey to the Iberian Peninsula (Güldali, 1979; Meulenkamp & Sissingh, 2003) led to an increased altitudinal differentiation in the vegetation (Combournieu-Nebout, 1993; Valente et al., 2010).

Although many species of *Tragopogon* (and *Dianthus* and other co-occurring floristic elements) occur in the Mediterranean region, diversification may in fact have been greater in the areas bordering the current Mediterranean and corresponding to the ancient Paratethys. The latter was a series of inland seaways, brackish lakes and wetlands that developed from the Tethys Ocean during the Oligocene–Neogene. The Paratethys had ongoing connections with the Mediterranean Sea both before and after the MSC (Clauzon et al., 2005; Popov et al., 2006; Krijgsman et al., 2010). Climatic interactions across the broad Paratethys basin had long-term geographical and biological consequences (Sprovieri et al., 2003), with much of this basin now corresponding to meadow steppe, steppe, dry steppe and semi-desert regions. These areas – beyond the bounds of the current Mediterranean basin – were formed post-MSC (Krijgsman et al., 2010) at a time that seems to coincide with the origin of *Tragopogon* and other key elements of the steppe vegetation in this region.

Only an in-depth biogeographic analysis requiring a comprehensively sampled, well-supported and well-resolved phylogeny is actually able to discern between a Mediterranean centre of diversification and a Paratethyan centre. However, because approximately one-third of all species of *Dianthus* are endemic to the Mediterranean region but only 5–10% of the species of *Tragopogon* are, different centres of diversification for the two genera are possible. Furthermore, such biogeographic analyses of plants from this region are scarce (Lo Presti & Oberprieler, 2009; Mansion et al., 2009; Roquet et al., 2009), and we can therefore only provide preliminary insights from studies on other species-rich Eurasian lineages occurring in the same dry steppe habitats as *Dianthus* and *Tragopogon*. It is also noteworthy that the pattern reported here even extends to insects from these habitats (Esseghir et al., 2000).

A few lineages within Eurasian *Campanula* may have diversified before the onset of drier climates, but a large number of species appear to be the product of more recent diversification events that may have occurred during the Plio-Pleistocene (Cellinese et al., 2009; Roquet et al., 2009). However, as with *Tragopogon* and *Dianthus*, species-rich lineages may be susceptible to bias due to poor taxon sampling, clearly the case so far with *Campanula* and related taxa.

*Centauraea* sect. *Acrocentron* (100 species) may have diversified c. 5 Ma (Font et al., 2009) with a crown group in the western Mediterranean that was dated at c. 2 Ma (L. Barres, I. Sanmartín, C.-L. Anderson, A. Susanna, S. Buerki, M. Galbany-Casals, & R. Villatersana, unpubl. data); this latter group is similar in age to *Tragopogon* and *Dianthus* (Table 2). One well-supported subclade (‘*eu-Scorzonera’*) of the former genus *Scorzonera* may be another example of a rapid radiation during the same general time frame, although it may have originated a little earlier. We estimate that this radiation occurred about 3.8 Ma (Table 2) and generated at least 50–60 species (Lipschiz, 1964; Kamelin & Tagaev, 1986; reviewed in Mavrodiev et al., 2004), mostly in Western Asia. Note that based on Mavrodiev et al. (2004), *Scorzonera* is not monophyletic (see also Fig. S1) and remains in need of more comprehensive study and revision. Therefore, the actual number of species in this well-supported subset of *Scorzonera* is unclear (Kilian et al., 2009), but may be at least twice as high as the estimate used previously.

Allopolyploidy is a common mode of speciation in *Tragopogon*; at least 12 Eurasian species are of polyploid origin or are reported to comprise diploid and polyploid cytotypes (Mavrodiev et al., 2008b). Through the union of divergent parental genomes, allopolyploids exhibit novel genetic combinations and may display novel phenotypes. Such phenotypic novelty may be manifested in adaptation to new habitats, such as those resulting from the climatic and topographic changes that followed the MSC. At least some of the diversification in *Tragopogon* during the past 2 + Ma can be attributed to polyploid speciation rather than to cladogenic events.

Other species-rich clades occurring in the same basic habitats with *Tragopogon* apparently radiated somewhat earlier (45–4.28 Ma) than *Tragopogon* (e.g. *Campanula, Scabiosa, Knautia, Cousinia* subgenus *Cousinia*), a few
subgroups within *Centaurea*, and notably the major meadow grasses of the area; Table 2). Such temporal differences are not surprising, not just for methodological reasons, but also because diversification may have occurred not only at different places and at different times, but also with different causes. Given that the MSC coincided with a time of increasing aridity and was followed by a time of increasing seasonality, it is possible that plant taxa already present in the Mediterranean diversified first in response to aridity and later to increased seasonality, as demonstrated in *Anthemis* (Lo Presti & Oberprieler, 2009). Moreover, diversification may already have happened earlier in response to increased salinity during the MSC. Studies of pollen records suggest no significant climatic changes during this period and show that plant migration and regional extinction were driven exclusively by the increased salinity caused by the desiccation of the Mediterranean basin (Fauquette et al., 2006).

Taxa already adapted to aridity before the late Miocene, such as those in the mountains of Central Asia, may show a different pattern. The closest relatives found in Central Asia may not have undergone radiation events and habitat change before the late Miocene; rather, they may have radiated subsequently in the Mediterranean during the late Pliocene in response to increased seasonality and the development of dry meadow communities. For example, this pattern of Mediterranean species with closest relatives in mountainous areas is found in *Veronica* (Albach et al., 2004). However, not all taxa native to Asia necessarily underwent habitat shifts before migrating to Europe, as illustrated by *Aquilegia*, which evolved from forest species in Asia (e.g. Bastida et al., 2010) and exhibits only a late Pliocene radiation in the meadows of Western Europe. The east-to-west migration was favoured by the earlier onset of the climatic events in the east. Consequently, plant radiations in the western Mediterranean seem to have started later than those in the east (e.g. Vargas et al., 2009).

Thus, our comparisons reveal three potential cases of ‘pseudocongruence’ – that is, common spatial patterns that originated at different times through different causes (Cunningham & Collins, 1994) – in these clades that radiated in the same habitat. First, the place of diversification may have differed as semi-arid grasslands formed in parallel over a large area from Western Europe to Central Asia. This parallel formation subsequently allowed migration across the region, blurring patterns of initial diversification. Second, different factors, such as aridity, changes in seasonality or increasing salinity, responsible for the formation of semi-arid grasslands may have initiated diversification in different taxa. Third, differences in the timing of diversification may be reflected in analyses of diversification patterns in taxa from these habitats.

The late Pliocene has been inferred as the time frame for a major east–west vicariance event in *Microcnemum* (Kadereit & Yaprak, 2008) and *Campylanthus* (Thiv et al., 2010). Superficially, it may therefore seem as if the subclades of such taxa radiated in the late Pliocene, but in fact, they are more likely remnants of larger extinctions. This emphasizes that the same climatic and tectonic events can lead to rapid radiations in some groups but to extinction in others.

In summary, our comparison of diversification rates and patterns in *Tragopogon* with those of other clades from dry meadow communities of the Mediterranean and surrounding regions indicates that these plant communities did not evolve all at once but represent more malleable assemblages, with different components originating and diversifying at different times. These results also demonstrate the fairly rapid evolution of modern plant communities themselves. Detailed studies so far suggest that these speciation events occurred in allopatry and as a consequence of habitat shifts (Lo Presti & Oberprieler, 2009; Bastida et al., 2010) rather than in response to pollinator shifts. Thus, our data for *Tragopogon* and results summarized in Table 2 support the recent hypothesis that nonadaptive allopatric diversification can proceed remarkably rapidly (Kozak & Wiens, 2006; Valentie et al., 2010). However, we also stress that other modes of speciation, such as hybridization and polyploidy, may have already generated additional species of *Tragopogon* (reviewed in Mavrodiev et al., 2004, 2007, 2008a,b), *Veronica* (summarized in Albach et al., 2008) and other clades represented in these meadow communities.

Many other clades may have similarly radiated rapidly throughout this region, but have not yet been investigated in detail. Examples include *Acantholimon* (Plumbaginaceae, 120 species), *Silene* (Caryophyllaceae, 194 species), *Acanthophyllum* (Caryophyllaceae, 70 species) and perhaps *Eremurus* (Asphodelaceae, 50 species). Hence, future studies should focus on these and other species-rich Eurasian lineages.

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**References**

Rapid diversification of Tragopogon and ecological associates in Eurasia


Supporting information

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

Table S1 Additional putative diploid species of Tragopogon placed into the BEAST tree and their putative closest relatives, based on selected taxonomic treatments.

Table S2 Scorzonerinae diversity.

Figure S1 Maximum Clade Credibility tree inferred using BEAST. Numbers above the branches equal posterior probability values (> 0.50) multiplied by 100.

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