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Testing reticulate versus coalescent origins of *Erica lusitanica* using a species phylogeny of the northern heathers (Ericeae, Ericaceae) ☆

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ABSTRACT

Whilst most of the immense species richness of heathers (*Calluna*, *Daboecia* and *Erica*: Ericeae; Ericaceae) is endemic to Africa, particularly the Cape Floristic Region, the oldest lineages are found in the Northern Hemisphere. We present phylogenetic hypotheses for the major clades of Ericeae represented by multiple accessions of all northern *Erica* species and placeholder taxa for the large nested African/Madagascan clade. We identified consistent, strongly supported conflict between gene trees inferred from ITS and chloroplast DNA sequences with regard to the position of *Erica lusitanica*. We used coalescent simulations to test whether this conflict could be explained by coalescent stochasticity, as opposed to reticulation (e.g. hybridisation), given estimates of clade ages, generation time and effective population sizes (Ne). A standard approach, comparing overall differences between real and simulated trees, could not clearly reject coalescence. However, additional simulations showed that at the (higher) Ne necessary to explain conflict in *E. lusitanica*, further topological conflict would also be expected. Ancient hybridisation between ancestors of northern species is therefore a plausible scenario to explain the origin of *E. lusitanica*, and its morphological similarities to *E. arborea*. Assuming either process influences the results of species tree and further evolutionary inference. The coalescence scenario is equivocal with regard the standing hypothesis of stepping stone dispersal of *Erica* from Europe into Africa; whereas reticulate evolution in *E. lusitanica* would imply that the colonisation of Tropical East Africa by *E. arborea* instead occurred independently of dispersals within the rest of the African/Madagascan clade.

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1. Introduction

Erica L. is among the largest genera of flowering plants (Frodin, 2004) with 830–840 species (Oliver and Oliver, 2003; Oliver and Forshaw, 2012). Most of the immense richness of *Erica* is endemic to the Cape Floristic Region (CFR) of South Africa, but species of *Erica* and closely related *Calluna* Salisb. and *Daboecia* D.Don (Ericaceae; Ericeae, commonly referred to as 'heaths' or 'heathers') are archetypal elements of open landscapes of Europe and surrounding areas, in both the Temperate and Mediterranean biomes. These northern heathers (Nelson, 2012) have been the subject of various empirical studies addressing evolutionary and ecological questions including the environmental factors dictating species distributions (Gil-López et al., 2014; Ojeda et al., 1998), and patterns of dispersal and genetic diversity (investigated for individual species by e.g. Beatty and Provan, 2012; Désamoré et al., 2010, 2012). The tree heather, *Erica arborea*, in particular, has been investigated to infer dispersal patterns between Europe and Tropical East Africa (TEA) (Désamoré et al., 2010) and within TEA (Gizaw et al., 2013).

The geographically widespread *E. arborea* is very similar in gross morphology to another species found exclusively in Europe: *E. lusitanica*. They share a tall habit and white corollas, and have been grouped in different formal and informal classifications (e.g. Bentham, 1839; 'Tree heathers', Nelson, 2012). However, the interpretation of morphological variation in *Erica* is far from

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Abbreviations: ITS, Internal Transcribed Spacer regions of nuclear ribosomal DNA; TEA, Tropical East Africa; CFR, Cape Floristic Region.

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straightforward: there is evidence for extensive homoplasy of morphological characters (Oliver, 2000; Pirie et al., 2011). Floral characters may evolve rapidly, probably as adaptations to changing (pollinator) environments (Pirie et al., 2011; Rebelo et al., 1985; Van der Niet et al., 2014), and vegetative characters such as adaptations to recurrent fires (Ojeda et al., 2005) may have undergone similar shifts. As a result, the classic generic classification (Bentham, 1839; Hansen, 1950), based on such characters, has long been considered artificial (Hansen, 1950; Oliver, 2000). In *E. lusitanica*, micromorphological characters such as indumentum and seed coat sculpture appear more similar to some other northern species than they are to *E. arborea* (Fagúndez et al., 2010; Fagúndez and Izco, 2010; Nelson, 2012).

The obvious means to assess this kind of morphological complexity is the molecular phylogeny. However, *E. lusitanica* has not previously been included in phylogenetic analyses, nor have several other European species, and current knowledge of the relationship of species of *Erica* L. in general is limited. Evidence available to date suggests that the heathers (tribe Ericeae; Ericaceae, including *Erica*, *Calluna* and *Daboecia*) comprise a basal grade of ‘northern’, largely European, species (McGuire and Kron, 2005; Pirie et al., 2011) subtending a single, much larger, ‘southern’ clade (‘African/Malagasy *Erica*’; Pirie et al., 2011). Data that we collected in the course of ongoing work on the phylogeny of Ericeae confirmed this general pattern. The results that we report here showed much improved resolution between lineages of the northern grade, revealing strong conflict between phylogenetic trees based on plastid data and independent nuclear ITS with regard to the position of *E. lusitanica*.

This gene tree conflict raises an alternative hypothesis to explain homoplasy of morphological characters in *E. lusitanica*: instead of indicating parallel evolution of traits, it could be the result of hybridisation between morphologically dissimilar species (de Villiers et al., 2013). However, gene tree conflict can instead represent incomplete lineage sorting, being the result of coalescent stochasticity given a linear (rather than reticulate) species tree (Nichols, 2001).

As is the case in many empirical examples of gene tree conflict (Blanco-Pastor et al., 2012; de Villiers et al., 2013; Maureira-Butler et al., 2008; Pirie et al., 2009), both coalescent and reticulate scenarios are in principle plausible for *E. lusitanica*: The possibility of ancient hybridisation events cannot be ruled out since hybridisation between extant *Erica* species is documented: wild hybrids between European species include *Erica* × *stuartii* (MacFarl.) Mast. (*E. tetralix* L. × *E. mackayana* Bab.); *Erica* × *veitchii* Bean (*E. arborea* L. × *E. lusitanica* Rudolphi.); *Erica* × *watsonii* Benth. (*E. ciliaris* L. × *E. tetralix*); *Erica* × *williamsii* Druce. (*E. vagans* L. × *E. tetralix*); and *Erica* × *nelsonii* Fagúndez (*E. tetralix* × *E. cinerea* L.) (Fagúndez, 2006; Fagúndez, 2012; Nelson, 2012; Rose, 2007), and various further crosses have been achieved in cultivation (Nelson, 2012). Moreover, there are no obvious karyological barriers to homoploid hybridisation: polyploidy has not been reported in *Erica* and chromosome counts are constant at $n = 12$ for all studied species with the exception of the European species *E. spiculifolia* that is $n = 18$ (Nelson and Oliver, 2005). Coalescent stochasticity on the other hand can generally be assumed to result in greater or lesser differences between inferred gene trees depending in particular on effective population sizes through time (Nichols, 2001).

In this paper, we attempt to discern whether gene tree conflict in the northern Ericeae with regards to *E. lusitanica* is the result of reticulate evolution or coalescent processes. We present two independent gene trees from DNA sequences (1) of multiple plastid markers and (2) of nuclear ribosomal ITS; from samples representing multiple accessions of all northern species of *Erica*, *Calluna vulgaris* (L.) Hull and *Daboecia cantabrica* (Huds.) K.Koch and exemplar sampling of the large African/Madagascan *Erica* clade. We use coalescent simulations and ancestral state reconstructions of selected morphological characters, and use data concatenation and coalescence based approaches in order to infer and test reticulate and linear species trees under each assumption separately. Finally, we use these trees to reassess the hypotheses concerning colonisation of Tropical East Africa by the putatively closely related tree heather, *Erica arborea*.

2. Materials and methods

2.1. Taxon sampling

We sampled multiple populations from across the geographic distributions of all 21 non-hybrid species and two subspecies of *Erica* recognised by Nelson (2012) within the northern area (including for the first time *E. umbellata* and *E. maderensis*, as well as *E. platycodon* and *E. azorica* not previously included in phylogenetic analyses of the genus), plus multiple accessions of *Calluna vulgaris* and *Daboecia cantabrica* (123 accessions in total). In addition, we sampled one naturally occurring northern *Erica* hybrid (*E.* × *stuartii*); seven *Erica* species from sub-Saharan Africa and Madagascar, representing the African/Madagascan clade (Pirie et al., 2011); and *Empetrum nigrum* L. (Ericoideae) as outgroup. A map illustrating the distribution of the northern samples is presented in Fig. 1, and full accessions details and authors of taxa (following the nomenclature of Nelson, 2012; Oliver and Forshaw, 2012) in Appendix A. Most samples and sequences were obtained newly for this study. Some ITS sequences were taken from Pirie et al. (2011) and further sequences were taken from van der Niet et al. (2014). Samples were obtained largely from field collections, but some were provided by the Bundesgarten-Belvedere Vienna in Austria, Botanic Gardens of the Rheinische Friedrich Wilhelms Universität Bonn in Germany, Gartenbauzentrum Straelen in Germany, and from private collections of known wild origin. Plant material was dried in silica gel and voucher specimens were deposited in herbaria (Appendix A).

2.2. Laboratory protocols

Two different lab protocols were employed: (1) Direct amplification (without DNA isolation) was performed using the method of Bellstedt et al. (2010); and (2) DNA isolation, (followed by separate PCR) was performed using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). In both cases leaf material was ground using a Qiagen Tissuelyser (Retsch GmbH & co., Haan, Germany), respectively: (1) at room temperature in grinding buffer; and (2) dry, having previously been frozen at −80 °C for 24 h. PCR was performed using standard protocols (Appendix B). Plastid regions *atpI-atpH* spacer, *trnK-matK* intron and *matK* gene, *psbM-trnH* spacer, *rbcL* gene, *rpl16* intron, *trnL-rpl32* spacer, *trnT-trnL-trnF-ndhJ* (including genes and intervening intron and spacer regions) and nuclear ribosomal ITS regions (including partial flanking 18S and 26S genes) were amplified and sequenced with primers listed in Appendix B. A targeted supermatrix strategy was employed (Pirie et al., 2008; Wiens, 2006), whereby more variable ITS and *trnL-trnF* spacer sequences were obtained for most samples, and the other, mostly less variable chloroplast markers were added for selected taxa in order to improve resolution of deeper nodes in the chloroplast tree (Appendix A). Sequences in general, and particularly ITS, were inspected for polymorphisms and apparent loss of function (e.g. indels in coding regions) that might be evidence of paralogy. In the absence of these phenomena, and in view of the minimal intra-individual ITS polymorphism across *Erica* and the largely consistent results obtained from cloning polymorphic amplicons reported in Pirie et al. (2011), we did not attempt to

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