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Molecular phylogeny and biogeography of the bipolar *Euphrasia* (Orobanchaceae): Recent radiations in an old genus

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ABSTRACT

Euphrasia includes perennial or annual green parasitic plants, and has a disjunct bipolar distribution except for one transtropical connection across the high mountains of Oceania. The disjunction is coupled with strikingly contrasting patterns of morphological diversity between the southern and northern hemispheres, making it an exciting model to study processes of evolutionary diversification which shaped present floras. We inferred the relationships among 51 species representing 14 of the 15 sections of the genus based on nrDNA ITS and cpDNA *trn*L intron, *trn*L-*trn*F and *atp*B-*rbc*L intergenic spacers. Maximum parsimony and Bayesian inference support monophyly of the genus and of several intrageneric groups characterized by morphology, ploidy level, and geographic range. Molecular phylogenetic dating using Bayesian "relaxed" clock methods suggests that the earliest *Euphrasia* radiations occurred minimum 11–8 Mya with bipolarity being achieved 7–5 Mya. Biogeographic analyses using explicit model-based approach inferred Eurasia as an ancestral area for the genus. The most parsimonious reconstruction found by a dispersal-vicariance analysis requires 17 dispersals to account for the current biogeographic pattern and supports Eurasian origin for *Euphrasia*. Both long-distance dispersal and across land vicariance can be invoked to explain the diversification in the genus, which experienced rapid radiations driven by new ecological opportunities of the late Pliocene and Pleistocene but also retained a set of local endemic or relict species of an earlier origin.

1. Introduction

The phenomenon of bipolarity represents the most extreme geographic separation in distribution patterns of organisms. Bipolar distributions have been investigated since the mid-nineteenth century (Crame, 1993), and were described for a wide range of groups, e.g., molluscs, nematods, flowering plants, lichens and mosses (Pearce et al., 2007). Several explanations for disjunct distributions have been proposed: geological/climatic vicariance, migration along the continents or land-bridges, long or short distance dispersal (reviewed in Linder and Crisp, 1995). General acceptance of Wegener's continental drift theory in the late 1960s made vicariance the predominant mechanism offering an explanation of various disjunct distributions (Willey, 1988). Now, almost after half a century of dominance by vicariance, biogeography paradigm is shifting. Recent developments of molecular divergence time estimation provide increasing amount of evidence supporting transoceanic dispersal versus vicariance in order to explain pattern and/or timing of cladogenesis revealed by molecular data (e.g., Givnish and Renner, 2004; Sanmartin and Ronquist, 2004; De Queiroz, 2005).

Euphrasia L. (*Orobanchaceae* Ventenat emend. Young et al., 1999, formerly subfam. Rhinanthoideae Wettst., family *Scrophulariaceae* Juss.) comprises approximately 350 species (Fisher, 2004) of perennial and annual green parasitic plants. The genus is distributed throughout temperate regions of the southern and northern hemispheres, with one transtropical connection across the high mountains of Oceania.

The bipolar distribution pattern of *Euphrasia* has intrigued generations of phytogeographers. According to Croizat (1952), Barker (1982, 1986) and Heads (1994), its bipolar disjunction was created by continental drift and can be compared to that of the Gondwanian groups, such as *Fagus*–*Nothofagus*. Similar views were expressed by Du Rietz (1931a,b, 1940), who proposed that the transtropical connection between the austral and the boreal parts, formed by a series of species on the high mountains of New Guinea, Ceram, Celebes, Borneo, Luzon and Taiwan, reflects the breaking up of a previously continuous distribution. A land connection between South American and Australian *Euphrasia* was possible, according to Du Rietz (1940), through Antarctica in the Cretaceous. An alternative hypothesis (Fig. 1) suggests that much more recent transoceanic dispersal is the main factor responsible

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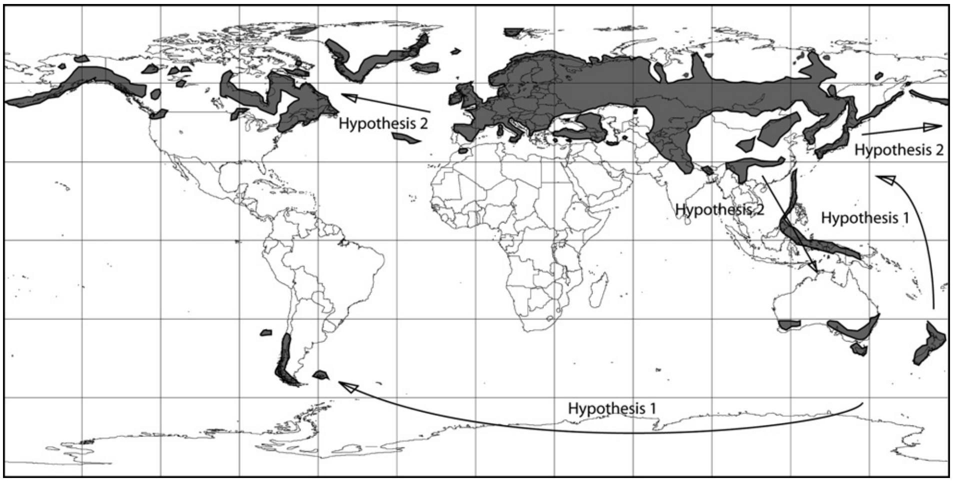

Fig. 1. Distribution map of genus *Euphrasia* with hypothesized dispersal events: Southern Hemisphere origin (Hypothesis 1) versus Eurasian origin (Hypothesis 2).

for the biogeographic pattern in *Euphrasia* (Raven and Axelrod, 1972). Based on dating of the mountain uplift in Malaysia, New Guinea, Australia and New Zealand, Raven (1973) suggested that these mountains provided a series of stepping stones between Asia and Australasia in the late Pliocene and Pleistocene. Moreover, according to paleobotanical data (Muller, 1970, 1981) the major evolutionary radiation of the sympetalous angiosperms occurred in the Tertiary. Molecular phylogenetic dating of the Asterids using a set of reference fossils (Bremer et al., 2004) estimated crown node age of the core lamiids, as Late Cretaceous. *Euphrasia*, along with other groups of sympetalous angiosperms, is a young lineage, and probably attained its world distribution from a northern origin since the Neogene (Raven and Axelrod, 1972) or Late Tertiary (Wolfe et al., 2005).

These competing biogeographic hypotheses can be tested using molecular phylogenetic data, including molecular age estimates. The validity of such testing and its drawbacks are extensively discussed in literature (e.g., Heads, 2005). Shortcomings such as uncertainties in estimations of branch lengths, molecular clock and other model assumptions, and the validity of geological and paleontological calibrations, are widely recognized. At the same time, methodological developments, in particularly implementation of relaxed-clock models (Drummond et al., 2006), as well as accumulation of factual information have made it possible to conclude that approaches based on complementary use of molecular and paleobotanical data offer some of the most powerful techniques now available for inferring the age and, therefore, the possible mechanism by which the change in the range of a lineage occurred (Givnish and Renner, 2004; Sanmartin and Ronquist, 2004). Lately, several molecular studies have strongly supported hypotheses of recent transoceanic dispersal rather than old vicariance in some plants with disjunct distribution patterns, e.g., *Myosotis* and *Hebe* (Wagstaff and Garnock-Jones, 1998; Wagstaff et al., 2002; Winkworth et al., 2002; Cook and Crisp, 2005).

Euphrasia is remarkable among bipolar disjuncts by its strikingly contrasting patterns of morphological diversity in the southern and northern hemispheres. The genus exhibits considerable morphological diversity in the south: eleven of the 15 sections have been described from this region based primarily on a variety of growth forms, predominant perennial or annual life habit, leaf shape and characters of corolla, anthers and capsules. Two extraordinary long-tubed New Zealand species are morphologically so divergent from other *Euphrasia* that they have been suggested to represent separate genera (*Siphonidium* Armstr. and *Anagosperma* (Hook) Wettst.). Later Cheeseman (1925) united *Siphonidium* Armstr. and *Anagosperma* (Hook) Wettst. into one species *Siphonidium longiflorum* Armstr. and Du Rietz (1931a) referred it to the genus *Euphrasia* as more morphologically intermediate New Zealand species were described.

In contrast, the highest number of *Euphrasia* species occur in the northern hemisphere, where the genus is notorious for its taxonomic complexity with numerous—not always well distinguished—taxa and putative hybrids. All the northern hemisphere species are annuals, with the exception of endemic Azorean, Malaysian and Taiwanese species. The northern hemisphere annuals belong to the large section *Euphrasia*. Interspecific hybridization has been considered to be a major factor explaining the complex patterns of variation in this section (Yeo, 1978), in addition to recent origin of species and ongoing radiations driven by new ecological opportunities (Karlsson, 1974, 1986).

Generic delimitations within the tribe *Rhinantheae* Lam. & DC., where *Euphrasia* belongs, have been much debated. Monophyly of *Euphrasia* is supported by its distinct morphological characters (corolla and seed coat morphology, phyllotaxy and leaf shape) and chromosome numbers. All species, diploids and tetraploids, as well as high polyploids (6*x*, 8*x* and 12*x*), appear to share the basic chromosome number of $x = 11$ (Yeo, 1956, 1966, 1978; Vitek, 1986; Vitek and Kiehn, 1996; Barker et al., 1988), although records from outside Europe are still scarce. A molecular phylogeny of the *Orobanchaceae* based on phytochrome A (Bennet and Mathews, 2006) supported monophyly of *Euphrasia* and revealed its sister group relationship with a clade comprised of *Odontites*, *Bartsia* and *Parentucellia*. However, only four species of *Euphrasia* (two from the northern hemisphere sect. *Euphrasia* and two from two Australian sections) were included in that study.

Wettstein's (1896) monograph of *Euphrasia* was the first comprehensive treatment of the genus, and also contained discussions on speciation and phylogeny based on morphology and geographic distributions. Wettstein placed all the South American species in a separate section *Trifidae* based on their tripartite leaves, while the Australian and New Zealand species with their entire to digitate leaves were treated as a subsection of section *Euphrasia*, thus implying a closer relationship with the northern hemisphere taxa. There have been numerous later contributions to *Euphrasia* systematics, which further developed the "narrow species concept" introduced by Wettstein as well as modified his intrageneric classification (Chabert, 1902; Jørgensen, 1919; Pugsley, 1930, 1936; Juzepczuk, 1955; Smejkal, 1963; Yeo, 1970, 1972, 1978; Karlsson (1976); Tzvelev, 1981; Barker, 1982; Vitek, 1985; Silverside, 1991; Gussarova, 2005). The most recent worldwide revision of