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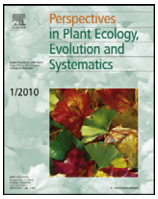

Research article

Evolutionary classification: A case study on the diverse plant genus *Ranunculus* L. (Ranunculaceae)

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ABSTRACT

Evolutionary classification attempts to integrate information on shared ancestry, evolutionary process and phenetic information into the taxonomic concept. Here we exemplify this concept on the monophyletic, species-rich and cosmopolitan plant genus *Ranunculus*. Previous classifications have rendered almost all traditional sections as polyphyletic, and a modern revision based on phylogenetic principles was so far lacking. Maximum parsimony and Bayesian inference analysis of a combined nuclear (ITS of nrDNA) and plastid DNA dataset (*matK*/*trnK*, and *psbJ*–*petA*) provided a phylogenetic framework for the genus with nine well-supported subclades. Neighbor Net analysis revealed a reticulate data structure within two subclades with frequent polyploidy and/or hybridization. Character evolution was studied by McClade reconstructions of morphological data mapped on to the molecular tree topology. Morphological characters show a mosaic-like distribution, but express several shared states congruent to molecular clades. A total evidence approach (TE) based on the combined morphological and molecular dataset suggests a subdivision of *Ranunculus* into a paraphyletic, temperate to arctic group of five subclades (subg. *Auricomus*), and a temperate to subtropical clade with four subclades (subg. *Ranunculus*). Infrageneric classification of two subgenera and 17 sections is based on both monophyly s.l. as evident from TE and a minimum of shared morphological characters. Six subclades have shared morphological or karyological features (sects. *Auricomus*, *Flammula*, *Oreophili*, *Polyanthemos*, *Ranunculus*, *Thora*, and *Trisecti*). One subclade was subdivided into three smaller clades according to morphological data (sects. *Epirotes*, *Leucoranunculus*, *Ranuncella*, *Aconitifolii*). In the case of reticulate evolution and uncertain ancestry we accept well-supported genetic clusters with shared morphological features, as revealed by Neighbor Net analysis (sections *Batrachium*, *Hecatonia*, *Pseudadonis*). Character evolution connected to ecological shifts characterizes the paraphyletic section *Ranunculastrum*, the holophyletic section *Euromontani* (sect. nov.), and the monotypic sect. *Echinella*. The information content of our classification is compared to alternative concepts.

Introduction

Biological classification has been revolutionized by phylogenetic principles, and nowadays there is a broad consensus that shared ancestry is a primary criterion for grouping concepts. However, beside this theoretical foundation, information content and practicability are also important criteria for classification (Mishler, 2009). The combination of these features is the goal of evolutionary classification (e.g., Hörandl, 2007). Unlike traditional descriptive or phenetic concepts, evolutionary classifications are based on phylogenetic principles, i.e., taxonomic grouping is primarily based on shared ancestry. Additionally, evolutionary classification attempts to integrate information about diversification process into the concept (Mayr and Bock, 2002; Hörandl, 2007; Stuessy et al., 2011). Beside cladogenesis, also reticulate evolution and anagenesis are the main processes of diversification. Hybridization potentially can result in speciation both in plants and animals (Rieseberg and Willis, 2007; Mallet, 2007; Mavárez and Linares, 2008). Reticulate evolution may result in conflicting phylogenies that are based on different data sets, which complicates interpretations of phylogenetic reconstruction (McDade, 1992). Polyploidy, as a whole-genome duplication, is often followed by waves of diversification in all major groups of eukaryotes, and is often connected to hybridity (Van de Peer et al., 2009; Soltis et al., 2009).

Evolutionary classification further attempts to integrate information on structure and function into the taxonomic concept

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(Stuessy, 1987; Mayr and Bock, 2002; Hörandl, 2007, 2010; Stuessy and König, 2008). The phenotype contains important information about adaptation, rapid evolutionary change, and ecological features (e.g., Piersma and Van Gils, 2011). Morphological characters include important phylogenetic information, as each of them integrates a phylogenetic signal based on multiple, independent loci (Wiens, 2004; Wortley and Scotland, 2006). However, homoplasious similarity may easily arise as response to similar ecological constraints and is difficult to disentangle from a phenotypic similarity resulting from shared ancestry. Low phylogenetic signal is specifically a problem for groups with low differentiation of organs, structures and tissues (e.g., plants, fungi). Therefore, morphology-based phylogenetic analyses in flowering plants usually remain unresolved (Scotland et al., 2003). Morphological characters rather act in functional combinations (e.g., Stuessy, 2004), while phylogenetic reconstruction relies on isolated synapomorphies. Moreover, morphological characters may be synapomorphic for one clade and be present in the majority of its members, but can disappear or change again in some terminal taxa (Endress, 2010). Because of their restricted information content for reconstruction of shared ancestry, morphological data have lost their prior primary role in phylogenetic classification compared to molecular data, but are still regarded an important data set (Wiens, 2004). Morphological features, after all, are easy to perceive and therefore aid practicability, which is in general an important desirable feature of classifications (Mishler, 2009).

To combine the information content of morphological and molecular data for classification, three main approaches are currently used in the taxonomic literature (see also Wiens, 2004): (1) character states are directly used for phylogenetic reconstruction (mostly in studies including fossils); (2) character evolution is reconstructed by optimizing character states on tree topologies based on molecular data, and synapomorphic characters congruent with molecular clades are used as criterion for delimitation of taxa; (3) total evidence approaches combine molecular and morphological data to present a new phylogenetic hypothesis. All three approaches have methodological limitations if the assumption of a tree-like phylogeny is not correct. Hybridization can cause segregating, mosaic-like morphological patterns in later generations, which makes character distribution uninformative about ancestry. Reticulate evolution is a major cause for homoplasy of morphological traits in phylogenies (e.g., Pirie et al., 2009). The second approach, i.e., mapping of single characters on a molecular tree, neglects functional combinations of characters (unless character states are a priori defined via a combination of features). Moreover, this approach will render rapidly evolving morphological characters as homoplasious if they have differentiated earlier or later than the molecular markers used for tree reconstruction; this phenomenon contributes to the frequently observed scarcity of morphological characteristics for clades in molecular trees (Hörandl, 2010). Total evidence approaches are expected to maximize the phylogenetic signal and explanatory power of the phylogeny (Kluge, 1989) but can be only applied in the case of congruent or weakly conflicting data sets. The combination of morphological and molecular data matrices bears the danger that the latter simply override the phylogenetic signal of morphology because DNA sequences usually have more informative sites (Wortley and Scotland, 2006). However, comparisons of empirical studies suggest that combined molecular and morphological datasets mostly results in improved resolution and higher statistical support (Wortley and Scotland, 2006).

In this study, we intend to present a case study of an explicit application of evolutionary classification based on morphological and molecular data as an alternative model to cladistic principles. Few systematists made explicit efforts to present case studies of evolutionary classifications based on phylogenetic analyses (e.g., Carpenter, 1993; Stuessy et al., 2011). The theoretical debate about pros and cons of cladistic and evolutionary classification has been long and controversial up to present (Stuessy, 1997; Mayr and Bock, 2002; Brummitt, 2002; Hörandl, 2007; Hörandl and Stuessy, 2010; Schmidt-Lebuhn, 2011). But, explicit case studies are needed to validate the concept and practicability of evolutionary classification. The great majority of taxonomists still follow cladistic principles even if taxa are apparently not informative about shared phenotype (e.g., Richter et al., 2009).

Ranunculus L. (buttercups) is a suitable model system for studying various evolutionary processes in flowering plants. The genus comprises about 600 herbaceous species (plus c. 600 agamospecies), with a cosmopolitan distribution (Tamura, 1995; Hörandl et al., 2005; Emadzade et al., 2010). It is the largest genus of Ranunculaceae and ranges among the 50 biggest genera of angiosperms (Frodin, 2004). *Ranunculus* has its greatest diversity in the submeridional to temperate zones of both hemispheres, and in high mountain systems (Emadzade et al., 2011; Hörandl and Emadzade, 2011). The genus occupies both freshwater and terrestrial habitats, ranging from semi-deserts to temperate forests and grassland, arctic-alpine tundra, from mountain rain forests to anthropogenic habitats (Tamura, 1995). Underground parts are at least partly adaptive to ecological conditions (Paun et al., 2005). However, it remained an open question whether ecological shifts and respective characters would correspond to the biggest clades within the genus. The bauplan of flowers is of a simple two-whorled type without synorganization of parts (separated sepals, petals, stamens, and carpels). The perianth shows low variation in color and shape of the parts and forms a more or less wide cup, representing a generalist pollinator syndrome (Steinbach and Gottsberger, 1994). Variation in the shape of the nectary scale on the petals has been thought to characterize major infrageneric taxa (Benson, 1948). Fruit morphology has long been regarded as useful for classification of infrageneric taxa (e.g., Tamura, 1995). However, morphology of diaspores (achenes) could be also adaptive to certain dispersal mechanisms, as suggested by Müller-Schneider (1986). Recent biogeographical studies have shown that long distance dispersal and multiple colonizations of continents have happened frequently in the history of the genus (Emadzade et al., 2011).

In many aspects, *Ranunculus* provides a model for a highly diverse, successful genus with quite a complex evolutionary history. The genus originated about 21 Mya and has a crown group age of c. 18 My; the genus diversified later on during the Cenozoic in several waves. Many high mountain clades originated and diversified during the Quaternary (Emadzade and Hörandl, 2011) under the influence of Pleistocene glaciations. Polyploidy is known for c. 40% of species and plays a major role in the diversification of the genus. However, polyploidy is not equally distributed among the genus, but clustered in some clades; therefore, diversification of the genus cannot be referred to polyploidy alone (Hörandl et al., 2005; Hoffmann et al., 2010). Hybridization has been documented in many groups with or without polyploidy, which means that the assumptions of a strictly tree-like phylogeny are often not valid (Cook, 1966; Huber, 1988; Lockhart et al., 2001; Hörandl et al., 2005, 2009). Anagenetic change can be assumed in some morphologically peculiar species that have been classified as monotypic sections (Tamura, 1995). The variety of evolutionary processes involved, and the high morphological and ecological diversity made classifications notoriously difficult.

Ranunculus s.s. has been confirmed as monophyletic in all previous phylogenetic studies based on DNA sequence data (Ro et al., 1997; Johansson, 1998; Hörandl et al., 2005; Paun et al., 2005; Hoot et al., 2008; Hoffmann et al., 2010; Gehrke and Linder, 2009; Emadzade et al., 2010, 2011). The circumscription of the genus