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Bipolar disjunctions in *Carex*: Long-distance dispersal, vicariance, or parallel evolution?

Marcial Escudero [a,*], Virginia Valcárcel [a], Pablo Vargas [b], Modesto Luceño [a]

[a] Department of Molecular Biology and Biochemical Engineering, Pablo de Olavide University. Ctra. Utrera km 1, 41013-Sevilla, Spain
[b] Royal Botanical Garden of Madrid, CSIC. Pza. Murillo no. 2, 28014-Madrid, Spain

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ABSTRACT

The long-standing fascination of naturalists and scientists in the evolutionary and biogeographical causes behind the pattern of distribution of bipolar plants has lead to an intense debate around the three well-known biogeographical hypotheses of vicariance, long-distance dispersal, and parallel evolution. Genus *Carex*, despite lacking any general long-distance dispersal devices, represents six of the 30 plant species with bipolar distribution. We aimed to evaluate the role of the three alternative mechanisms mentioned above in the origin and evolution of five bipolar *Carex* species. Phylogenetic and phylogeographical reconstructions using Bayesian Inference, maximum parsimony, and statistical parsimony were performed with plastid (*rps*16 intron) and nuclear (ITS) DNA sequences. As a result, five cases of long-distance dispersal are proposed (*C. canescens*, *C. macloviana*, *C. magellanica*, *C. maritima*, *C. microglochin* s.str.) with an inferred southward migration from Northern to Southern Hemisphere for three of them. On the other hand, parallel evolution seems to be the most plausible explanation to understand the particular case of the bipolar species *C. microglochin* s.l.

Introduction

The striking fact that many species from different plant families display similar bipolar distributions (Moore and Chater, 1971) has fascinated scientists since the beginning of the nineteenth century (Darwin, 1859; Humboldt, 1817). Moore and Chater (1971) compiled 30 bipolar species considering those present at high latitudes, reaching at least the Strait of Magellan in the southern hemisphere and Alaska or Arctic Europe in the northern and irrespective of their presence at lower latitudes. Six of these 30 species are *Carex* species (*C. canescens* L., *C. capitata* L., *C. macloviana* D' Urv., *C. magellanica* Lam., *C. maritima* Gunn., *C. microglochin* Wahlenb.). More recently, Vollan et al. (2006) considered two additional *Carex* species (*C. echinata* Murray, *C. lachenalii* Schkuhr) as examples of bipolar plants, although these plants do not reach such high latitudes in the southern hemisphere. Many studies have focused on elucidating the origin of such disjunctions (Ball, 1990; Darwin, 1859; Du Rietz, 1940; Heide, 2002; Humboldt, 1817; Moore, 1972; Moore and Chater, 1971; Smith, 1986; Steffen, 1939; Vollan et al., 2006; Wilson, 1986). As a result, three main hypotheses have been proposed to explain the origin of present-day bipolar disjunctions: parallel evolution, vicariance, and long-distance dispersal. Historically, parallel evolution was proposed as the most likely mechanism behind bipolar disjunctions (Humboldt, 1817); however, this hypothesis is nowadays discarded by most authors. Vicariance, which implies the disruption of a previous continuous distribution, has also been proposed as the origin of bipolar disjunctions (Du Rietz, 1940). In particular, this hypothesis placed the origin of disjunctions in the Mesozoic (65–250 Myr B.P.), when transtropical highland bridges disappeared. Finally, the third and most popular hypothesis currently proposed for bipolar species is long-distance dispersal, either direct (Van Steenis, 1962) or over stepping stones (mountain hopping; Moore and Chater, 1971; Vollan et al., 2006).

Morphological and ecological studies have historically prevailed in the investigation of bipolar plants (see authors cited above). Improved molecular techniques during the last three decades have provided the suitable new tools for tackling with some evolutionary questions such as the origin of disjunctions. To our knowledge, only the AFLP fingerprinting technique has been used for elucidating the origin of *Carex* bipolar disjunctions (Vollan et al., 2006). In this paper we sequenced genome regions in an attempt to elucidate the origin of five of the six bipolar *Carex* species. Particularly, we analysed the variability of the nuclear ribosomal ITS region and the plastid *rps*16 intron. The ITS region has a level of variation suitable for evolutionary studies at the species level (review: Nieto Feliner and Roselló, 2007) and has been successfully used in studies of *Carex* with biogeographical conclusions (Escudero et al., 2008a, b; Hipp et al., 2006; Roalson

* Corresponding author. Tel.: +34 954349383; fax: +34 954349813.
E-mail address: amesclir@upo.es (M. Escudero).

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and Friar, 2004). Plastid DNA sequences have been widely used to reconstruct phylogenetic and phylogeographical patterns (Taberlet et al., 1998). In particular, the *rps*16 intron has been proven to be one of the most variable plastid regions in *Carex* (Escudero et al., 2008b) and was used for phylogeographical studies (Schönswetter et al., 2006).

In this study, we analysed nuclear and plastid sequences in order to investigate the origin of disjunctions in five bipolar *Carex* species and evaluate the role of the three main biogeographical hypotheses for these taxa, parallel evolution, vicariance, and long-distance dispersal.

Material and methods

Study species

Five *Carex* species (*C. canescens*, *C. macloviana*, *C. magellanica*, *C. maritima*, *C. microglochin*) were investigated in the northern and southern hemispheres. We did not include the sixth bipolar *Carex* species, *C. capitata*, as we were unable to obtain recent material of this species from the southern hemisphere. Outgroup taxa for each *Carex* species were selected based on previous phylogenetic information (Waterway and Starr, 2007). There are four main clades in the tribe *Cariceae* related to these five studied species (Starr and Ford, 2009; Starr et al., 2008; Waterway and Starr, 2007). Clade 1: most species of the subgenera *Carex* and *Vigneastra* (group 1, including *C. magellanica*). Clade 2: most species of the subgenus *Vignea* (group 2, including *C. canescens*, *C. macloviana*, *C. maritima*). Clades 3 and 4: the unispicate clade (most species of subgenus *Psyllophora*, plus a few atypical *Carex* species such as *C. curvula*, and the genera *Cymophyllus*, *Kobresia*, and *Uncinia*) and the *Schoenoxiphium* clade (genus *Schoenoxiphium* and some species of *Psyllophora*), respectively (group 3, including *C. microglochin*).

Sample for ITS sequencing

A total of 28 samples of the five study species were analysed. Five samples were additionally taken from the GenBank database (http://www.ncbi.nlm.nih.gov/): one sample of *C. magellanica* (Waterway and Starr, 2007), three samples of *C. canescens* (Hipp et al., 2006; Roalson et al., 2001; Waterway and Starr, 2007) and one sample of *C. macloviana* (Hipp et al., 2006). The remaining 23 samples were obtained in the field or from herbarium species and sequenced for this study: five samples of *C. magellanica*, seven samples of *C. microglochin*, four samples of *C. canescens*, three samples of *C. macloviana* and four samples of *C. maritima* (Table 1). In group 1, nine species closely related to *C. magellanica* were included, together with *C. tuckermanii* and *C. trichocarpa* (outgroup). In group 2, four sequences of *C. macloviana*, seven of *C. canescens* and four of *C. maritima* were analysed together because of their close phylogenetic relationship, together with 31 closely related species and *C. pensylvanica*, *C. curvula* and *Cymophyllus fraserianus* as an outgroup. In group 3, sequences of seven samples of *C. microglochin* and 19 samples of 19 closely related species, *C. bromoides* and *C. deweyana* (outgroup) were analysed. Sequences of closely related species and outgroups were obtained from Waterway and Starr (2007).

Sample for plastid rps16 sequencing

A total of 23 samples of five species obtained in this study were analysed: five samples of *C. magellanica*, seven samples of *C. microglochin*, four samples of *C. canescens*, three samples of *C. macloviana*, and four samples of *C. maritima* (Table 1). No *rps*16 sequences of the five species and closely related species were deposited in the GenBank. In group 1, three species of the subgenus *Carex* (two sequences got in the present study, (Table 1); one species from Escudero et al., 2008b) were included together with *C. furva* (outgroup) (Table 1). In group 2, *C. macloviana*, *C. canescens*, and *C. maritima* were analysed together with four subgenus *Vignea* species plus *C. microglochin* and *C. binervis* (outgroup) (Table 1; Escudero et al., 2008b). In group 3, *C. microglochin*, five closely related species (five samples) and *C. canescens* and *C. maritima* (outgroup) were analysed (Table 1).

Molecular analysis

PCR amplification and sequencing

Total DNA was extracted from silica-dried material collected in the field as well as from herbarium specimens (UPOS, SI, TRH; abbreviation according to *Index Herbariorum*) using DNeasy Plant Mini Kit (Qiagen, California). Direct and reversal primers were used for amplifications of the ITS region (ITS-A, ITS-4; Blattner, 1999; White et al., 1990) and *rps*16 intron (rpsF, rps2R2; Oxelman et al., 1997). Amplifications were obtained in a Perkin Elmer PCR-system 9700 (California) under the conditions specified by Escudero et al. (2008b) for the ITS and Schönswetter et al. (2006) for *rps*16. PCR products were cleaned using spin filter columns (PCR Clean-up kit, MoBio Laboratories, California). Cleaned products were sequenced using dye terminators (Big Dye Terminator v. 2.0, Applied Biosystems, California) and run in polyacrylamide electrophoresis gels (7%) using an Applied Biosystems Prism Model 3700 automated sequencer. Sequences were edited using the program Seqed (Applied Biosystems). Limits of the ITS region and *rps*16 intron were determined following Starr et al. (1999) and Schönswetter et al. (2006), respectively.

Sequence analyses

Six matrices were manually aligned, three for the ITS and three for the *rps*16 analyses: group-1 matrices (17 of ITS/nine of *rps*16 sequences); group-2 matrices (49/18 sequences); group-3 matrices (28/14 sequences). In light of the first results, the *rps*16 matrices were rebuilt and reanalysed when length mutations were found in the ingroup. The gaps were coded as a simple base (A/T, presence/absence) in the *rps*16 matrices.

Each of the three ITS matrices was split into three different matrices (ITS-1, ITS-2, 5.8S). The resulting nine ITS and three *rps*16 matrices were additionally analysed to determine the simplest model of sequence evolution that best fits the data, under the Akaike Information Criterion (AIC), as implemented in MrModeltest 1.1b (Nylander, 2002). The selected models were used to (1) perform Bayesian inference (BI), and (2) calculate pairwise distances. When gaps were found in the *rps*16 matrices, the selected model for coded gaps was F81 following the manual of MrBayes v3.0b4 (Ronquist and Huelsenbeck, 2003). The six matrices (three ITS/three *rps*16) were analysed using MrBayes v3.0b4 (Ronquist and Huelsenbeck, 2003). Four Markov chain Monte Carlo runs were performed simultaneously in each BI analysis for 5,000,000 generations with an interval of 100 generations. Burn-in was evaluated over generations. After discarding trees yielded before the Likelihood stationary point was reached, the remaining trees were compiled in a majority rule consensus tree, using posterior probability (pp) as a measure of clade support (Alfaro et al., 2000). ITS-1 and ITS-2 pairwise genetic distances were calculated together with PAUP (Swofford, 2003).

For the six same matrices (three ITS/three *rps*16), maximum parsimony (MP) analyses were conducted under Fitch parsimony, as implemented in TNT (Goloboff et al., 2003) with equal