Extreme phylogeography in *Carex* (*Cyperaceae*)

T. VILLAVERDE, S. MARTÍN-BRAVO, M. ESCUDERO and M. LUCEÑO

RIASSUNTO - *Filogeografia di estremità di areale in* Carex *(*Cyperaceae*)* - Le disgiunzioni negli areali di distribuzione dei *taxa* hanno suscitato l'interesse dei naturalisti sin dall'inizio del XIX secolo. Sono note solo trenta specie aventi distribuzione bipolare e *Carex* risulta il genere con il maggior numero di specie bipolari. Una disciplina emergente, la filogeografia, dovrebbe essere usata per testare le ipotesi sulle specie bipolari poiché tenta di spiegare le cause e i processi di evoluzione relativi alla distribuzione delle specie e alle loro relazioni genetiche. Sono proposte diverse ipotesi per spiegare le disgiunzioni bipolari: vicarianza, parallelismo, convergenza, dispersione a lunga distanza e spostamento a balzi sui rilievi.

Key words: bipolar disjunctions, *Carex,* long distance dispersal, mountain hopping, parallelism, phylogeography, vicariance

INTRODUCTION

DARWIN committed two chapters of *The Origin of the Species* (1872) to explain the past and present distribution of the species. He stated that patterns of species distribution may often be seen as clear footsteps of their evolution and diversification. Thus, present-day *taxa* distribution may be frequently explained by a combination of historical events (climatic oscillations like aridifications or glaciations; geological movements like plate tectonics or orogenies) and evolutionary processes (extinction, vicariance, dispersal...).

One of the most fascinating distribution patterns is range disjunction. The largest and most challenging disjunction for biologists, the bipolar distribution, already captivated HUMBOLDT (1817) and DARWIN (1859) who realized the existence of common plant species in the flora of remotely distant areas (Tierra del Fuego in the southern hemisphere and Northern Europe/North America in the northern). DARWIN (1872) tried to explain this disjunction as a forced migration of species due to climate change. During glacial periods, the advance of the ice-sheet in the northern hemisphere might have pushed arctic species towards lower latitudes; then, a remigration would have occurred on the returning warmth, since those lowland areas are too warm for their existence. Some species might have found a refugium in mountain summits in southern latitudes in stead of moving northward. DARWIN (1859) then thought that there were few species identically the same in both hemispheres and on the mountains of intermediate tropical regions, whose mutual relations will not have been much disturbed. Admirably, he stated all these hypotheses without even knowing the existence of the genetic material.

Phylogeography is a rising discipline that tries to understand the reasons for the distribution of species and closely related genetic lineages, over time and the evolutionary processes involved (AVISE *et al.*, 1987; AVISE, 2000). This discipline emerged in a multidisciplinary context and it integrates methods and concepts from population genetics (microevolution) and from systematics (macroevolution).

Bipolar biogeography is still the focus of numerous studies after Darwin's first approach based on organism migrations (DU RIETZ, 1940; WILSON, 1986; MOORE, CHATER, 1971; MOORE, 1972; SMITH, 1986; BALL, 1990). Researchers have proposed different hypotheses to unravel the evolutionary history of bipolar *taxa* based on molecular and morphological data (ESCUDERO *et al.*, 2010; POPP *et al.*, 2011). The objective of this work is to briefly review the hypotheses suggested to explain bipolar disjunctions and how to test them with molecular techniques.

BIPOLAR DISTRIBUTIONS IN *CAREX*

MOORE, CHATER (1971) termed bipolar species following a single and simple criterion: such species must reach latitudes as high as the European Arctic or Alaska (c. 55° N. lat.) in the northern hemisphere and the Straight of Magellan (c. 52° S. lat.) in the southern hemisphere, regardless of their occurrences elsewhere. Under this criterion, they pointed 30 species belonging to 12 families (Tab. 1) and 23 genera, 6 of them (20%) corresponding to the genus

Carex L. (*Cyperaceae*). Although *Poaceae* family has 8 bipolar species, they are belonging to three different genera (*Phleum*, *Poa* and *Deschampsia*). Then, *Carex* is the genus with more bipolar species. Why such a striking pattern is so commonly found within *Carex*? This is not only the largest genus within the *Cyperaceae* family, with more than 2.000 species (KÜKENTHAL, 1909) distributed across all floristic regions (REZNICEK, 1990), but also the most diverse angiosperm genus of the northern temperate zone (ESCUDERO *et al.*, 2012). It contains many cold-adapted species that are eligible to have reached extreme latitudes in both hemispheres and, therefore, the bipolar distribution. Amphitropical species of lower latitudes might not be considered as bipolar because their environmental and biological histories are likely to separate them from the arctic-alpine flora when investigating the factors involved in the patterns of their distributions (RAVEN, 1963).

TABLE 1

Number of bipolar species in vascular plants families (MOORE, CHATER, 1971).

Numero delle specie bipolari nell'ambito delle famiglie di piante vascolari (MOORE, CHATER, 1971).

Family	Number
Lycopodiaceae P. Beauv. *ex* Mirb.	1
Plumbaginaceae Juss.	1
Gentianaceae Juss.	1
Scrophulariaceae Juss.	1
Juncaginaceae Rich.	1
Hymenophyllaceae Mart.	2
Polygonaceae Juss.	2
Ranunculaceae Juss.	2
Plantaginaceae Juss.	2
Caryophyllaceae Juss.	3
Cyperaceae Juss.	6
Poaceae Barnhart	8

Carex is divided in four sub-genera based on morphological and molecular data (STARR, FORD, 2009). Three of them have at least one bipolar species: *C. arctogena* H.Sm. and *C. microglochin* Wahlenb. in subgenus *Psyllophora* (Degl.) Peterm.; *C. canescens* L., *C. macloviana* D'Urv. and *C. maritima* Gunn. in subgenus *Vignea* (P. Beauv. ex Lestib.f.) Peterm.; *C. magellanica* Lam. in subgenus *Carex* L. All these species are present in Tierra del Fuego (Patagonia, Argentina) and in arctic-alpine areas of Eurasia and North America. *Carex canescens* is also present in Australia. A detailed taxonomic study of each bipolar species encompassing populations across its range should be mandatory before further steps towards elucidating the origin of such disjunctions. For example, some populations of *C. microglochin* in South America were found to belong to a different species, *C. camptoglochin* (WHEELER, GUAGLIANONE, 2003).

HYPOTHESES TO TEST

Once the taxonomy of the bipolar species has been clearly solved, it is possible to study their biogeography and evolutionary relationships. The geographical separation found in bipolar species might be considered as a major factor disrupting the pattern of gene flow. If genetic isolation allows northern and southern populations to diverge separately, it may lead to the accumulation of molecular and morphological differences to clearly distinguish them. Different degrees of differentiation may account for different hypotheses.

VICARIANCE, PARALLELISM AND CONVERGENCE

Molecular differences between Northern and Southern Hemisphere populations might be due to their dissimilar evolution. Populations might have rested genetically isolated or fragmented, allowing a distinct molecular divergence but not a morphological one.

To reject vicariance hypothesis, estimation of divergence times may help to test the congruence between phylogenetic splits and ecological requirements. The relative age of this disjunction was evaluated through estimation of divergence times and fossil evidences.

Parallelism or convergence hypotheses imply the accumulation of evolutionary steps in the same fashion in northern and southern populations of distantly related lineages, in this case populations would belong to polyphyletic groups. Therefore, to reject these hypotheses, extreme edges populations must be shown as together monophyletic.

DIRECT LONG DISTANCE DISPERSAL

If there are no observed molecular differences between populations, it might be possible to consider a long distance dispersal hypothesis, occurred in recent times. To reject this hypothesis, populations at the extremes of the distribution must belong to ancient monophyletic divergent groups.

ESCUDERO *et al.* (2010) sampled five of the six bipolar *Carex* species (*C. arctogena* was not included) for a molecular approach and suggested long-distant dispersal, sensu lato, as the most plausible cause of bipolar disjunction for *C. canescens, C. macloviana, C. magellanica* and *C. maritima.* Although alternative dispersal hypotheses (e.g. direct long-distant dispersal) could not be tested due to their sampling mrthod, genealogical relationships of chloroplast haplotypes indicated a southwards direction of dispersal for *C. canescens, C. macloviana* and *C. magellanica* (ESCUDERO *et al.*, 2010).

MOUNTAIN HOPPING

The mountain-hopping hypothesis (BALL, 1990) relies on the idea that species have progressively migrated from one mountain chain to the next (as stepping-stones) through the low latitude regions to achieve such distribution (MOORE, CHATER, 1971; VOLLAN *et al.*, 2006). Under this hypothesis, if a broad sampling of the distribution areas is obtained, nearby populations should have higher molecular affinity than the populations at the extreme of the distribution. To reject this hypothesis, molecular dissimilarities must not clearly distinguish northern and southern populations.

MEANS OF DISPERSAL

Although some mechanisms and syndromes for dispersal have been described within the genus *Carex* (ALLESSIO LECK, SCHÜTZ, 2005) it generally lacks of any means for long-distance dispersal (STEENIS VAN, 1962; RAVEN, 1963). Only two bipolar species, *C. macloviana* and *C. microglochin*, display some features for dispersal (winged and hooked utricules, respectively, MASTROGIUSEPPE *et al.,* 2002; SAVILE, 1972). However, there is not enough evidence to explain such wide dissemination simply by morphological dispersal peculiarities (ESCUDERO *et al.*, 2010).

DARWIN (1859) suggested that some plants might also be influenced by occasional vectors of distribution such as sea water (survival in sea water for days in spite of not being adapted for this mean of dispersal) or birds (adhering to foot or kept in bird gizzards). RAVEN (1963) also pointed to birds as important vectors in plant dispersal, suggesting that many of the disjunctions, such as bipolar or amphi-Atlantic, correspond with bird migration routes. An example of a recent long distant dispersal favoured by birds was proposed in *Empretrum* (POPP *et al.*, 2011).

CONCLUSIONS

The fact that bipolar species' taxonomy is often unresolved (e.g. *Armeria maritima* Willd., BAUMBACH, HELWIG, 2007; *Osmorhiza berteroi* DC., WEN *et al.*, 2002) could be a problem when interpreting molecular results. Besides, the exact number of bipolar species might fluctuate when they are the subject of taxonomic revisions (MOORE, CHATER, 1971).

While testing hypotheses for bipolar disjunctions in clear biological entities, nuclear and chloroplast DNA are recommended to be explored. Nuclear DNA (e.g. ITS region, nuclear low copy genes, or AFLPs phenotypes) is commonly used in biogeographical studies and gives enough evidence to elucidate whether there is gene flow between populations. On the other hand, chloroplast DNA (e.g. *matk, rps16, trnL*) is suitable to reconstruct phylogeographical patterns and follow plant dispersion directions, by performing analyses such as haplotype network estimations.

Once the taxonomy of a species group is solved and accurate phylogenies are made, estimation of lineage divergence times could be inferred.

Although fossil evidence in *Carex* is scarce (SMITH *et al.*, 2009), attempts to date speciation events such as that conducted by DRAGON, BARRINGTON (2009) could facilitate the understanding of evolutionary processes in the genus.

Acknowledgements - The authors would like to thank the Società Botanica Italiana for the invitation to present this communication at the Symposium on *Cyperaceae* 2012, and especially E. Martinetto and A. Pistarino for the support given at the meeting.

LITERATURE

ALLESSIO LECK M., SCHÜTZ W., 2005 – *Regeneration of Cyperaceae, whit particular reference to seed ecology and seed banks*. Perspect. Plant Ecol. Evol. Syst. *7*: 95-133.

AVISE J.C., 2000 – *Phylogeography: the history and formation of species*. Harvard University Press, Cambridge, Massachusetts, USA.

AVISE J.C., ARNOLD J., BALL R.M. JR, BERMINGHAM E., LAMB T., NEIGEL J.E., REEB C.A., SAUNDERS N.C., 1987 – *Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics*. Ann. Rev. Ecol. Syst., *18*: 489-522.

BALL P.W., 1990 – *Some aspects of the phytogeography of* Carex. Can. J. Bot., *68*: 1462-1472.

BAUMBACH H., HELLWIG F.H., 2007 – *Genetic differentiation of metallicolous and non-metallicolous* Armeria maritima *(Mill.) Willd. taxa (*Plumbaginaceae*) in central Europe*. Plant Syst. Evol., *269*: 245-258.

DARWIN C., 1859 – *On the Origin of the Species by Means of Natural Selection, or the Perservation if Favoured Raves in the Struggle for Life.* Murray, London.

—, 1872 – *The Origin of the Species.* Murray, London, 6th ed.

DRAGON J.A., BARRINGTON D.S., 2009 – *Systematics of the* Carex aquatilis *and* Carex lenticularis *lineages: Geographically and ecologically divergent sister clades of* Carex *section* Phacocystis *(*Cyperaceae*)*. Am. J. Bot., *96*: 1896-1906.

DU RIETZ G.E., 1940 – *Problems of the bipolar plant distribution*. Acta Phytogeographica Suecica, *13*: 215-282.

ESCUDERO M., HIPP A.L., WATERWAY M.J., VALENTE L.M., 2012 – *Diversification rates and chromosome evolution in the most diverse angiosperm genus of the temperate zone (*Carex*,* Cyperaceae*)*. Mol., Phyl. Evol., *63*(*3*), 650-655.

ESCUDERO M., VARCÁRCEL V., VARGAS P., LUCEÑO M., 2010 – *Bipolar disjunctions in* Carex*: long-distant dispersal, vicariance or parallel evolution?*. Flora, *205*: 118-127.

HUMBOLDT F.W.H., 1817 – *De distributione geographica platarum secundum coeli temperiem et altitudem montium, Prolegomena*. Libraria graeco-latino-germanica, Paris.

KÜKENTHAL G., 1909 – Cyperaceae-Caricoideae. In: ENGLER A. (Ed.), *Das Pflanzenreich*, IV(20): 1-814. Engelmann, Leipzig.

MASTROGIUSEPPE J., ROTHROCK P.E., DIBBLE A.C., REZNICEK A.A., 2002 – Carex *L.* sect. Ovales Kunth. In: FLORA OF NORTH AMERICA EDITORIAL COMMITTEE (Ed.), *Flora of North America North of Mexico*, vol. 23: 332-378. Oxford University Press, New York.

MOORE D.M., 1972 – *Connections between cool temperate floras, with particular refrence to southern South America.* In: VALENTINE D.H. (Ed.), *Taxonomy, Phytogeography and Evolution*: 115-138. Academic Press, London.

MOORE D.M., CHATER A.O., 1971 – *Studies on bipolar species I*. Bot. Not., *124*: 317-334.

POPP M., MIRRÉ V., BROCHMAN C., 2011 – *A single mid-Pleistocene long-distance dispersal by a brid can explain the extreme bipolar disjunction in crowberris (*Empetrum*)*. Proc. Nat. Acad. Sci., *108*: 6520-6525.

RAVEN P.H., 1963 – *Amphitropical relationships in the floras of North and South America.* Quart. Rev. Biol., *38*: 151-177.
REZNICEK A.A., 1990 – *Evolution in sedges (*Carex*,* Cyperaceae*)*. Can. J. Bot., *68*: 1409-1432.
SAVILE D.B.O., 1972 – *Arctic adaptations in plants*. Monograph 6, Research Branch, Canada Department of Agriculture, Ottawa.
SMITH J.M.B., 1986 – *Origins of the Australasian tropicalpine and alpine floras*. In: BARLOW B.A. (Ed.), *Flora and Fauna of Alpine Australia*: 109-128. CSIRO, Melbourne.
SMITH S.Y., COLLINSON M.E., SIMPSON D.A., RUDALL P.J., STAMPANONI F.M., 2009 – *Elucidating the affinities and habitat of ancient, widespread* Cyperaceae*:* Volkeria messelensis *gen. et sp. nov., a fossil mapanoid sedge from the Eocene of Europe*. Am. J. Bot., *96*: 1506-1518.
STARR J.R., FORD B.A., 2009 – *Phylogeny and evolution in* Cariceae *(*Cyperaceae*): current knowledge and future directions.* Bot. Rev., *75*: 110-137.
STEENIS C.G. VAN, 1962 – *The mountain flora of the Malaysian tropics*. Endeavour, *21*: 183-193.
VOLLAN K., HEIDE O., HEUN M., 2006 – *Genetic variation, taxonomy and mountain-hopping of four bipolar* Carex *species (*Cyperaceae*) analysed by AFLP fingerprinting*. Australian J. Bot., *54*: 304-313.
WEN J., PORTER P.P., LOWRY I.I., WALCK J.L., YOO K., 2002 – *Phylogenetic biogeographic diversification in* Osmorhiza *(*Apiaceae*)*. Ann. Missouri Bot. Garden, *89*: 414-428.
WHEELER G.A., GUAGLIANONE E.R., 2003 – *Notes on South American* Carex *(*Cyperaceae*):* C. camptoglochin *and* C. microglochin. Darwiniana, *41*: 193-206.
WILSON J.B., 1986 – *Alpine species of* Cyperaceae *and* Juncaceae. In: BARLOW B.A. (Ed.), *Flora and Fauna of Alpine Australia*: 472-488. CSIRO, Melbourne.

ABSTRACT - Great distribution disjunctions of *taxa* have captivated naturalists since the beginning of the XXth century. Only thirty species are known to have a bipolar distribution, being *Carex* the genus with the largest number of bipolar species. A rising discipline, phylogeography, could be used to test for hypotheses in bipolar species since it tries to understand the reasons and the evolutionary processes involved for the distribution of species and their closely related genetic lineages. Different hypotheses are suggested for bipolar disjunctions: vicariance, parallelism, convergence, long distant dispersal and mountain hopping.

AUTHORS

Tamara Villaverde (tvilhid@upo.es), Santiago Martín-Bravo, Marcial Escudero, Modesto Luceño, Área de Botánica, Departamento de Biología Molecular e Ingeniería Bioquímica, Universidad Pablo de Olavide, Crt. de Utrera s/n, 41008 Sevilla, Spain