Quaternary range dynamics and polyploid evolution in an arid brushland plant species (*Melampodium cinereum*, Asteraceae)

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ARTICLE INFO

ABSTRACT

Pleistocene climatic fluctuations played a principal role for range formation and population history of many biota, including regions not directly affected by glaciations, such as the arid habitats of the southwestern United States and adjacent Mexico. Specifically, drought-adapted species are expected to have persisted during cooler and wetter periods in one or more refugia, resulting in lineage differentiation, from where they reached their current distribution after range expansion in the course of Holocene aridification. Here, we test this hypothesis using *Melampodium cinereum* (Asteraceae), a morphologically and cytologically variable species of dry brushlands of Texas and adjacent Mexico. In line with the hypothesized presence of several refugia, AFLP data provide strong evidence for the presence of geographically distinct genetic lineages, which, however, only partly agree with current intraspecific taxonomy. Despite multiple origins, tetraploids form a genetically cohesive group. The exclusive occurrence of tetraploids in a range parapatric to that of the diploids likely results from former geographic isolation of cytotypes, lending further support for the presence of Pleistocene refugia. Whereas plastid sequence data show a clear signal for the expected Holocene range and population expansion, they show little geographic structure and high levels of intrapopulational diversity. This may be due to lineage sorting during periods of population separation and/or substantial gene flow among populations via seeds, which has not been sufficient to erode the overall pattern of genetic divergence resulting from geographic isolation.

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1. Introduction

Phylogeography infers past population processes in a geographical context and determines the processes underlying the origin, distribution and maintenance of biodiversity (Beheregaray, 2008). Its success over the past two decades has been spurred by conceptual, methodological and computational advances allowing testing of explicit models (statistical phylogeography: Knowles and Maddison, 2002; Knowles, 2004) with increasingly sophisticated tools (e.g., Bayesian skyline plot and Bayesian skyride: Drummond et al., 2005; Minin et al., 2008).

Numerous phylogeographic studies have shown the important role of Pleistocene climatic fluctuations for range formation and population history of many biota. This is true not only for regions that have been directly affected by glaciation, such as mountain ranges both in Europe and North America or the Arctic (e.g., Brunsfeld et al., 2001; Hewitt, 2001; Abbott and Brochmann, 2003; Dobeš et al., 2004; Schönswetter et al., 2005; Guo et al., 2008), but also for other regions, such as eastern North America (Soltis et al., 2006 and references therein). Pleistocene climatic oscillations also affected the arid habitats of the southwestern United States and adjacent Mexico, which reached their current extent only after the last glacial maximum in a phase of large-scale aridification (McClaran and van Devender, 1995; Bousman, 1998; Metcalfe et al., 2000; Musgrove et al., 2001; Holmgren et al., 2007). Prior to this aridification, drought-adapted species persisted in one or more distinct refugia, from where they reached their current distribution after range expansion within the last 12,000 years (van Devender, 1990; Holmgren et al., 2007). Hence, genetic patterns in those species are expected to reflect both phylogeographic structure due to isolation in refugia and population expansion and secondary contact due to Holocene range expansions (Hewitt, 2001, 2004). In contrast to animals (Taulman and Robbins, 1996;

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Pook et al., 2000; Riddle and Hafner, 2006 and references therein; Castoe et al., 2007; Waltari et al., 2007), these predictions have been rarely tested in plants. The few available studies do show evidence for Pleistocene refugia and population expansion (Nason et al., 2002; Clark-Tapia and Molina-Freaner, 2003; Fehlberg and Ranker, 2009; Garrick et al., 2009; Sosa et al., 2009), but they also highlight that each species has its idiosyncratic phylogeographic history. For instance, in the Sonoran Desert *Lophocereus schottii* (Cactaceae) expanded northwards from a single refugium in the southern Baja California peninsula region and shows strong signal of vicariance between peninsular and mainland populations (Nason et al., 2002), whereas *Euphorbia lomelii* expanded southwards and the mainland populations likely originated after long distance dispersal across the Sea of Cortez from the Baja California peninsula (Garrick et al., 2009).

A major force in plant evolution and diversification is polyploidy (Ramsey and Schemske, 1998, 2002; Wendel, 2000). It is estimated that at least 70% (but probably all higher plants, with the possible exception of *Amborella*) have undergone at least one round of polyploidization in their history (Leitch and Bennett, 1997, 2004; Leitch and Leitch, 2008; Soltis et al., 2009). Polyploidy is recognized as an important mode of diversification by, for instance, promoting adaptation to new ecological niches or conferring reproductive isolation, which may eventually lead to speciation (Otto and Whitton, 2000). While allopolyploids may differ conspicuously from their diploid progenitors in morphology and physiology, autopolyploids are often more difficult to distinguish on the basis of morphology alone (Levin, 1983, 2002). Autopolyploids often co-exist within diploid parental populations (Husband, 2004; Suda et al., 2007), and despite several studies on rates and mechanisms of polyploid origin and their establishment relative to their progenitors (Jackson and Hauber, 1982; Felber, 1991; Husband and Schemske, 2000; Baack, 2005; Ramsey et al., 2008), the factors involved in new cytotype formation and particularly in establishment remain insufficiently understood (Baack and Stanton, 2005). In a phylogeographic context, polyploidy can provide information on the direction of the evolution of populations because the evolution of polyploids from lower ploidy levels is largely unidirectional (Meyers and Levin, 2006).

A good system to investigate range dynamics in conjunction with polyploid evolution in xeric habitats is series *Leucantha* of the genus *Melampodium* (Asteraceae). This phylogenetically distinct complex (Blöch et al., 2009) includes three species, with a collective distributional area in the southwestern USA and northern Mexico (ranging from the Sonoran via the Chihuahuan Desert to the Southern and Tamaulipan Plains; Stuessy, 1971a). Here, we focus on *M. cinereum*, which is restricted to the Tamaulipan mezquital ecoregion (Olson et al., 2001), characterized by grass- and thorny scrublands, and the eastern margins of the Chihuahuan Desert (Stuessy, 1971a, 1972). Within its range, *M. cinereum* shows extensive geographically structured morphological and cytological variation (Stuessy, 1971a, 1972). Stuessy et al. (2004) hypothesized that this pronounced geographical structure might be connected to major climatic changes taking place in this region during the Pleistocene, especially during the last glacial maximum (Holmgren et al., 2007).

Our general aim is to elucidate the phylogeographical history of *M. cinereum*, also in the context of polyploid evolution. To this end, we generated amplified fragment length polymorphism (AFLP) and plastid DNA sequence data from 25 populations covering the entire distribution of the species, determined the ploidy level of most individuals with flow-cytometry, and analyzed those data using, among others, a coalescent-based Bayesian approach for hypothesis testing and molecular dating. Specifically, we want to test: (1) whether the current grouping based on morphology is congruent with the genetic population structure likely determined by isola-

tion in different refugia; (2) whether and how this xerophilous species responded to the massive aridification after the last glacial maximum (Holmgren et al., 2007); and (3) whether polyploids originated once, as suggested by their narrow distribution parapatric to diploids, or recurrently.

2. Materials and methods

2.1. Study species

Melampodium cinereum is a drought-tolerant, low-growing, perennial subshrub with white-rayed flowering heads distributed in xeric habitats of southern Texas and adjacent Mexico (Fig. 1). It is morphologically most easily distinguishable from its closest relatives *M. leucanthum* and *M. argophyllum* by the outer phyllaries being connate only one-fourth of their length (Stuessy, 1972). Based on morphological variation concerning size of flower heads, shape of leaves, and indumentum of leaves and stems (length and density of hairs), three varieties *cinereum*, *hirtellum* and *ramosissimum* have been distinguished. Although largely separated geographically (Fig. 1), the ranges of the three varieties overlap near the Rio Grande valley in southern Texas, where the occurrence of morphological intermediates has been hypothesized to be the result of hybridization (Stuessy, 1972). Flowering time spans several months and, despite some differences, flowering times of the varieties overlap at least five months (Stuessy, 1972). Based on the chromosome base number $x = 10$ (Weiss-Schneeweiss et al., 2009), the species comprises morphologically indistinguishable diploid and tetraploid cytotypes (with occasional tri-, penta- or hexaploids; Stuessy et al., 2004; Obermayer et al., unpublished data), especially in var. *cinereum*, where tetraploids are frequent and occupy a distinct distributional area in eastern Texas to the exclusion of diploids (Stuessy et al., 2004).

2.2. Plant material

Plant material from 113 individuals was collected in 25 populations of *M. cinereum* covering the whole range of the species (Fig. 1): 17 of var. *cinereum* (pops. 1–17), six of var. *hirtellum* (pops. 18–23) and two of var. *ramosissimum* (pops. 24 and 25; Table 1). Samples were dried and stored in silica gel until DNA isolation. Vouchers are deposited in the herbarium of the University of Vienna (WU).

2.3. Ploidy level determination

DNA ploidy levels (Suda et al., 2006) were determined on a Partec PAII flow cytometer equipped with a mercury lamp (Partec, Münster, Germany), using DAPI stained DNA of silica-dried leaf material following the method of Suda and Trávníček (2006) except for using only half of the amount of Otto II buffer. Buffer composition was further optimized by adding ascorbic acid and polyvinylpyrrolidone (PVP40) to final concentrations of 0.586 mg/ml and 16.666 mg/ml, respectively (Bharathan et al., 1994). As internal reference size standard, fresh leaves of *Glycine max* 'Merlin' (1C = 1.134 pg; Greilhuber and Obermayer, 1997) were co-analyzed with the test material. A few individuals (7%) could not be analyzed due to the poor quality of the leaf material. The correct interpretation of DNA ploidy levels was confirmed by chromosome numbers determined for selected individuals using standard Feulgen staining as described by Weiss-Schneeweiss et al. (2007).