Isozyme evidence on the genetic diversity, mating system and evolution of *Bromus intermedius* (Poaceae)

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Abstract. Genetic diversity and differentiation among the *B. intermedius* accessions of different geographic origin has been studied using isozyme analysis. The mating system was evaluated on the basis of allozyme polymorphism. Outcrossing rate \((t)\) in *B. intermedius* was mostly 0, except one population with \(t = 0.16\), indicating nearly complete autogamy in this species. Given that *B. arvensis* and *B. intermedius* had common allozymes of all isozymes studied, it is suggested that *B. intermedius* may be a direct autogamous derivative of the outcrosser *B. arvensis*. Contrary to expectations, the allozyme diversity in *B. intermedius* was higher than in *B. arvensis*, 23 and 16 allozymes, respectively. Geographic pattern was found among the accessions of *B. intermedius*.

Key words: *Bromus*, isozymes, genetic diversity, breeding system.

Introduction

The evaluation of a distribution of genetic variation within and among related species is important for understanding their evolution and differentiation. It has been shown that closely related plant species often demonstrate considerable variation in mating modes (Barrett and Eckert 1990, Kohn et al. 1996). The widespread occurrence of selfing species in the plant kingdom indicates that the transition from outcrossing to self-pollination is one of the most common trends in the evolutionary history of plants (Stebbins 1974, Brown 1979, Gottlieb 1981, Hamrick and Godt 1989). It is interesting to compare the allozyme variability between related species with different breeding systems for testing whether genetic variability is reduced as expected for autogamy (Charlesworth and Charlesworth 1995), e.g. predominantly selfing species *Diploptaxis muralis* was “nearly devoid of genetic variation” in comparison with strictly outbreeding *D. tenuifolia* (Eschmann-Grupe et al. 2004).

*Bromus intermedius* Guss. is an annual diploid \((2n = 14)\) species of section *Bromus*. *Bromus intermedius* has mostly Mediterranean distribution: South of Europe, North of African and Southwest of Asia. In the Iberian Peninsula it is only found in the southern part of Spain (Acedo and Llamas 1999).

A detailed general description of *B. intermedius* is given in Acedo and Llamas monograph about *Bromus* in the genus Iberian Peninsula (1999). The authors observed some variation in relation to the organization of the...
inflorescence: there are specimens with simple inflorescences and with more or less ramified inflorescence, although B. intermedius is generally quite uniform morphologically. Acedo and Llamas also mentioned that B. intermedius has often been confused with B. hordeaceus and B. lanceolatus, although there are clear differences between them. My personal field experience shows that B. intermedius may be easily confused with B. japonicus var. villosus Koch, which has hairy spikelets, especially when the latter grows in a poor environment. Taxonomists usually admitted Bromus japonicus as a greatly variable taxon with two subspecies and two varieties (Tzvelev 1976; Smith 1980, 1985). The main difference between B. japonicus and B. intermedius, according to a recent very accurate key to Bromeeae in the Mediterranean climatic zones (Spalton 2004) is the dry lemma texture which is papery, usually with protruding veins in B. intermedius and leathery, usually without protruding veins in B. japonicus. Inflorescence structure, panicle branches length and spikelet measurements are greatly influenced by environment conditions during the vegetation period (Smith and Sales 1993, Smith 1985) and are of limited value to distinguish the two species. Unfortunately, pubescence can hide protruding veins and frequently in B. japonicus leathery lemmas can be quite thin and thus with protruding veins. Spalton emphasized this fact in the notes of his paper (Spalton 2004).

Morphological diversity in the genus Bromus and great variation within its species is well known and has caused substantial difficulties in taxonomic delimitations for various species. Section Bromus contains several species complexes with taxonomically problematic species which are closely related and difficult to delimit, e.g. the B. mollis or B. secalinus complexes. Bromus japonicus, B. squarrosus and B. arvensis form a similar cluster of closely related species (Oja et al. 2003), and it seems that B. intermedius also belongs to this complex. Among them, B. arvensis has a more wide distribution, extending from the Mediterranean to South and Central Europe and B. intermedius has a more restricted range, being more confined to Mediterranean. Ribosomal DNA ITS sequences data (Ainouche and Bayer 1997) also showed that the diploid species B. japonicus, B. squarrosus, B. arvensis and B. intermedius are weakly divergent from each other and form a group within the section Bromus. Isozyme results (Oja 1998, Oja and Jaaska 1998) revealed the same closely related species couples: B. japonicus – B. squarrosus and B. intermedius – B. arvensis. Ainouche et al. (1995) suggested that self-fertilizing diploid species B. intermedius and B. squarrosus may have substantial amounts of allogamy. In our study based on a limited number of seed accessions (Oja 1998, Oja and Jaaska 1998), no heterozygous phenotypes of any heterozyme, except duplicated PGD-A, were observed in B. intermedius, suggesting high rates of selfing in this taxon. In our late study (Oja et al. 2003) we found that B. japonicus is extreme selfer with outcrossing rate $t = 0.00$ and B. arvensis is a nearly complete allogamous species.

Present paper describes allozyme diversity in B. intermedius more thoroughly on the basis of larger seed material collected from natural populations from different parts of the distribution area. The major goal of the present study is to characterize the breeding system of B. intermedius quantitatively and to compare the allozyme variation and outcrossing rates between the three closely related species in section Bromus in order to make inferences about their phylogenetic relationships.

Material and methods

Plant material. A total of 35 accessions of B. intermedius were studied. Accessions from Turkey and Greece were collected in nature by the author, accessions from Crete were collected by K. Loolaid and one accession from Italy (denoted below by *), was received from botanical garden. Each accession is a bulk seed sample collected from 2-3 neighbour plants in a local population. Some of them were grown in a garden to have vouchers to verify identifications. Vouchers will be deposited in TAA, Herbarium of the Institute of Zoology and Botany.