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Preliminary Isozyme Evidence on the Hybrid Origin and Diploid Progenitors of Bromus pectinatus (Poaceae)

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PRELIMINARY ISOZYME EVIDENCE ON THE HYBRID ORIGIN AND DIPLOID PROGENITORS OF *BROMUS PECTINATUS* (POACEAE)

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ABSTRACT

Isozyme electrophoresis was used to study the origin of *Bromus pectinatus* (Poaceae, Pooideae). The morphological characteristics of *B. pectinatus* are intermediate between *B. japonicus* of sect. *Bromus* and *B. tectorum* of sect. *Genea.* Previous authors have suggested that sects. *Bromus* and *Genea* may be linked through *B. pectinatus,* a species that has been classified in sect. *Bromus,* though this placement has been questioned. Isozyme data support the allotetraploid nature of *B. pectinatus* and its position between sects. *Bromus* and *Genea.* Several heterozymes are consistent with the view that *B. pectinatus* may have arisen through polyploidization of a hybrid between *B. japonicus* of sect. *Bromus* and *B. tectorum* of sect. *Genea.*

Key words: allopolyploids, *Bromus pectinatus,* diploid progenitors, isozymes.

INTRODUCTION

Section *Genea* Dum. of the genus *Bromus* L. (Poaceae, Pooideae) comprises weedy, annual grasses widely distributed in Mediterranean countries, southwest Asia, and extending to northern Europe (Stebbins 1981; Sales 1993). Stebbins (1981) hypothesized that the genus originated in western Eurasia. Many species of sect. *Genea,* except the diploid *B. fasciculatus* C. Presl, are widely introduced in North and South America, Australia, and New Zealand (Mack 1981; Upadhyaya et al. 1986). Some species, e.g., *B. diandrus* Roth*, B. rigidus* Roth, and *B. tectorum* L., have spread rapidly in new areas and become problematic weeds (Kon and Blacklow 1988; DiTomaso 2000; Novak and Mack 2001).

Among the characters provided by Smith (1985) in the description of sect. *Genea* are spikelets cuneiform at maturity, broader at the apex. Sales (1994) regarded the section as ''the most highly evolved in the genus'' and still in the process of further specialization with incomplete species differentiation.

Species of sect. *Genea* often display remarkable inter- and intraspecific variation in morphology. The section includes the following species: diploids ($2n = 14$) *B. fasciculatus, B. sterilis* L., and *B. tectorum*; tetraploids ($2n = 28$) *B. madritensis* L. and *B. rubens* L.; hexa- and octoploids *B. diandrus* and *B. rigidus,* each species with two ploidy cytotypes ($2n = 42, 56$) (Sánchez Anta et al. 1988; Devesa et al. 1990). Polyploidy has thus played a significant role in the evolution of sect. *Genea,* as well as in other sections of *Bromus* (Stebbins 1981). Isozymes have been used successfully (Oja and Jaaska 1996; Oja 2002*b*) to show the allopolyploid nature of the sect. *Genea* polyploids, and to identify some of their putative diploid progenitors. All three diploid species of sect. *Genea* have diverged from each other at a number of isozyme loci (Oja and Jaaska 1996). Only single-banded electrophoretic variants attributed to allozymes have been observed in all diploids examined, indicating that autogamy and self-fertilization are characteristic of them (Oja and Jaaska 1996; Oja 2002*b*).

Each sect. *Genea* polyploid has revealed different fixed heterozygous phenotypes at several heterozyme loci, indicating their independent alloploid origin from different diploid progenitors (Oja and Jaaska 1996; Oja 2002*a*; Oja and Laarmann 2002). Relationships among the *Genea* diploids and tetraploids as evidenced by patterns of isozyme variation (Oja 2002*b*) are summarized in Fig. 1. Tetraploid *B. rubens* may be derived from a hybrid of *B. fasciculatus* and *B. tectorum,* and tetraploid *B. madritensis* from a hybrid of *B. fasciculatus* and *B. sterilis.* The origin of the *B. diandrus*/*rigidus* complex has been a matter of prolonged discussion. Many botanists have recognized the taxonomic problems in this complex (Ovadiahu-Yavin 1969; Tzvelev 1976; Böcker et al. 1990; Sales 1993). Previous isozyme investigations (Oja and Jaaska 1996; Oja and Laarmann 2002) support the view of Esnault and Huon (1987) and Sales (1993), that of a single species with different cytotypes. Cugnac (1933, 1937) suggested that *B. diandrus* is a hybrid between *B. rigidus* and *B. sterilis.* Isozymes indicate that of the three diploids only *B. sterilis* fits well as a genome donor for the *B. diandrus*/*rigidus* complex (Oja 2002*b*). The isozyme data also provide no evidence for the participation of tetraploids *B. madritensis* and *B. rubens* in the hybrid origin of the complex (Oja 2002*b*). The progenitor genotype of the complex may have been missed because of inadequate sampling of *B. fasciculatus* or, alternatively, because of an unknown or even extinct progenitor.

Scholz (1981) and Sales (1993) stated that sects. *Bromus* and *Genea* may be less distinct than previously recognized. Supporting this hypothesis, Oja and Jaaska (1998) performed a cladistic analysis of isozyme variation, which resulted in two sect. *Genea* diploids, *B. sterilis* and *B. tectorum,* being nested among sect. *Bromus* diploids. Scholz (1981) and Sales (1993) suggested that sects. *Bromus* and *Genea* may be linked through *B. pectinatus* Thunb., a species classified

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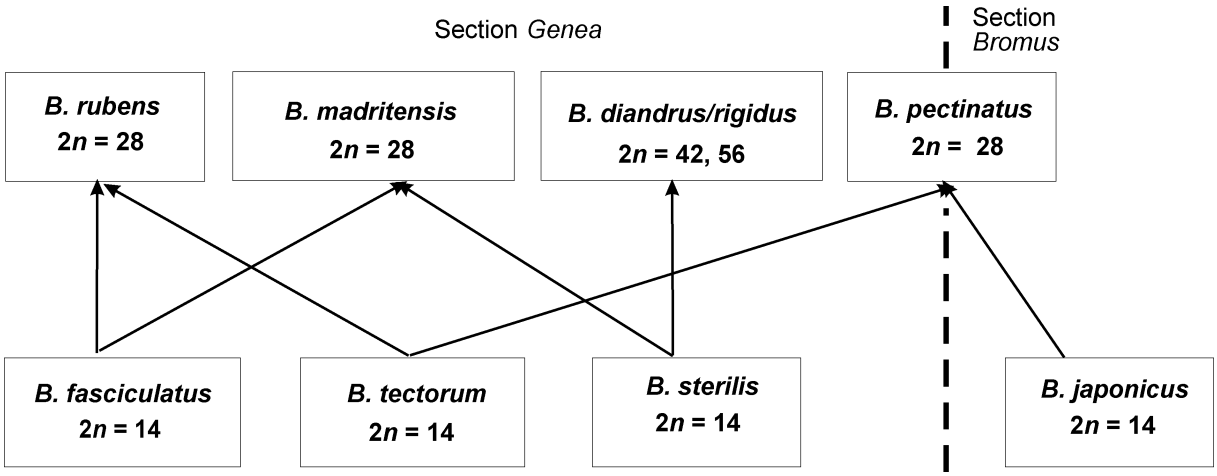

Fig. 1.—The putative phylogenetic position of *Bromus pectinatus* as evidenced from the isozyme data.

in sect. *Bromus* (Smith 1970). Sales (1993) further suggested that *B. pectinatus* is closely related to members of sect. *Genea,* and its placement in sect. *Bromus* is questionable owing to its cuneiform spikelets, characteristic of sect. *Genea.* In his study of the *B. pectinatus* complex, Scholz (1981) suggested a hybrid origin for the complex. The morphological characteristics of *B. pectinatus* are intermediate between *B. japonicus* Thunb. of sect. *Bromus* and *B. tectorum* of sect. *Genea. Bromus japonicus* is a morphologically highly variable diploid ($2n = 14$) species distributed in central Asia and the Mediterranean region, and is introduced to America and Australia (Smith 1985). The similarity of *B. pectinatus* to *B. japonicus* is reflected in a nomenclatural combination, *B. japonicus* var. *pectinatus* (Thunb.) Asch. & Graebn. As well, where the taxa are treated as conspecific. *Bromus pectinatus* resembles *B. tectorum* in general morphology and may have taken part in species formation in sect. *Genea.* Descriptions of *B. pectinatus* (Scholz 1981; Sales 1993; Phillips 1995) mention the vegetative vigor of the species, which may be due to its tetraploid nature ($2n = 28$; Mehra et al. 1968; Smith 1972). *Bromus pectinatus* is a weed species distributed throughout Asia, and introduced in South Africa and Australia (Smith 1972).

This paper summarizes the results of my six previous papers regarding sect. *Genea* allopolyploids and their putative diploid progenitors (Oja and Jaaska 1996; Oja 1999, 2002*a, b*; Oja and Laarmann 2002; Oja et al. 2003). In addition, new isozyme evidence concerning the allopolyploid *B. pectinatus* and its potential diploid progenitors is included.

Table 1. Seed accessions of *Bromus pectinatus* analyzed in this study from the USDA Western Regional Plant Introduction Station at Washington State University (Pullman, Washington, USA). Accession PI 442454 was misdetermined as *B. japonicus*. Detailed locality data for the accessions are unknown. Seeds of each accession were grown to maturity (anthesis) for vouchers and to verify identifications. Vouchers are deposited in TAA, Herbarium of the Institute of Agricultural and Environmental Sciences (Tartu, Estonia).

Accession number	Origin	Voucher
PI 202531	Belgium	*Oja 475/00*
PI 442453	Belgium	*Oja 444/00*
PI 442454	Belgium	*Oja 515/00*

MATERIALS AND METHODS

Plant Material

It proved difficult to obtain seed samples of *B. pectinatus.* No botanical gardens provide this species, and even personal requests to researchers in South Africa were not fruitful. I was able to find only three seed accessions, all originating from Belgium, at the USDA Western Regional Plant Introduction Station at Washington State University (Pullman, Washington, USA). Information on the accessions and vouchers is given in Table 1.

Isozyme Analysis

Enzyme extracts were prepared from the shoots (primary leaf plus coleoptile) of 5–10-day-old etiolated seedlings, subjected to electrophoresis in vertical polyacrylamide gel slabs as described in Oja (1999) and Jaaska and Jaaska (1986, 1990), and stained for the following enzymes: malate dehydrogenase (MDH, EC 1.1.1.37), shikimate dehydrogenase (SKD, EC 1.1.1.25), 6-phosphogluconate dehydrogenase (PGD, EC 1.1.1.44), aspartate aminotransferase (AAT, EC 2.6.1.1.), superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (PRX, EC 1.11.1.7), and phosphoglucoisomerase (PGI, EC 5.3.1.9). Preliminary analyses of seed progeny (up to ten seedlings per accession) revealed no allozyme variation within accessions, reflecting a selfing breeding system (Oja and Jaaska 1996; Oja et al. 2003). Therefore, 2–4 seedlings per accession were analyzed.

Isozyme results are described at the level of isozyme phenotypes, which correspond to respective genotypes. Therefore, isozyme symbols were used, e.g., AAT-B and AAT-C, instead of respective gene symbols in italics, e.g., *Aat-2, Aat-3.* Heterozygous phenotypes of dimeric enzymes were distinguished on the gels as symmetrical triplets with codominant isozyme variants as flanking bands, whereas monomeric enzymes showed two-banded heterozygous phenotypes (Wendel and Weeden 1989).

The isozyme nomenclature used, which distinguishes ge-

Table 2. A survey of diagnostic isozyme phenotypes of malate (MDH), shikimate (SKD), 6-phosphogluconate dehydrogenase (PGD), aspartate aminotransferase (AAT), superoxide dismutase (SOD), peroxidase (PRX), and phosphoglucoisomerase (PGI) isozymes in the diploids *Bromus tectorum* and *B. japonicus* (Oja 1999; Oja et al. 2003) and tetraploid *B. pectinatus*. Fixed heterozygous phenotypes of the tetraploid are given as a fraction of isozymes. The species-specific allozymes of the diploids are designated by subscripts: J = *B. japonicus*, T = *B. tectorum*.

Species	MDH-B	SKD-A	PGD-A	AAT-B	AAT-C	SOD-A	PRX-F	PGI-A
B. tectorum	2, 3_T, 1_T	2, 3, 1_T	2	5_T	1_T	4	5_T	6
B. japonicus	2, 3	2	2/3, 1/3	4_J	3_J	4	6_J	4_J, 3
B. pectinatus	2	2	2	$4_J/5_T$	$1_T/3_J$	4	$5_T/6_J$	4

netically heterologous, homoeologous, and homologous isozymes (abbreviated to heterozymes, homoeozymes, and allozymes, respectively), follows Jaaska and Jaaska (1984). The use of different terms is needed to define more precisely the isozymes encoded by different types of genes in allopolyploids. These terms were used in my previous publications (Oja and Jaaska 1996; Oja 1998, 2002*a*; Oja and Laarmann 2002) describing isozymes in diploid and allopolyploid brome species.

RESULTS AND DISCUSSION

Isozyme variation in *B. pectinatus* and the related diploids *B. tectorum* of sect. *Genea* and *B. japonicus* of sect. *Bromus* is summarized in Table 2. All three species share the same allozyme of MDH-B, SKD-A, and PGD-A, reflecting close genetic affinities among them and between sects. *Bromus* and *Genea. Bromus pectinatus* revealed fixed heterozygosities at three isozyme loci, AAT-B, AAT-C, and PRX-F, suggesting an allopolyploid origin. Fixed heterozygosity means that the same heterozygous isozyme phenotype is found in all plants without genetic segregation into homozygous allozyme phenotypes. Fixed heterozygosity of isozymes in alloploids has been frequently used as a criterion to distinguish them from autoploids and to identify their diploid progenitors (e.g., Jaaska 1969, 1976; Barber 1970; Jaaska and Jaaska 1970, 1984, 1986; Roose and Gottlieb 1976; Watson et al. 1991). Fixed heterozygosities of allopolyploids combine additively electrophoretically divergent and codominantly expressed isozymes of diploid parents, allowing their identification. Allozymes of a heterozyme that differ electrophoretically in diploid parents become two codominant homoeozymes encoded by homoeologous loci (of composite genomes) of an allotetraploid (Jaaska and Jaaska 1984). Thus, the PRX-F5/6, AAT-B4/5, and AAT-C1/3 heterozygosities indicate that *B. tectorum* of sect. *Genea* with species-specific PRX-F5, AAT-B5, and AAT-C1, and *B. japonicus* of sect. *Bromus,* with PRX-F6, AAT-B4, and AAT-C3, may be the diploid progenitors of *B. pectinatus.* However, taking into account the small number of accessions of *B. pectinatus* analyzed in this study, this is only a preliminary hypothesis.

Three chromosome count reports point to two ploidy levels in *B. pectinatus,* $2n = 28$ (Mehra et al. 1968; Spies et al. 1999) and $2n = 14$ (Moinuddin et al. 1994). All three accessions included in this study are allotetraploids based on the fixed heterozygous isozyme phenotypes.

Isozyme data thus evidence the allotetraploid nature of *B. pectinatus* and its intermediate position between sects. *Bromus* and *Genea,* confirming the suggestion of Scholz (1981) and Sales (1993) that the sections may be linked through *B. pectinatus.* Fixed heterozygosities of AAT-B, AAT-C, and PRX-F are consistent with the view that *B. pectinatus* may be the result of the hybridization between *B. japonicus* and *B. tectorum,* followed by the polyploidization of the hybrid. However, variation in allozymes PGD-A and PGI-A is not consistent with this hypothesis because PGD-A3 of *B. japonicus* and PGI-A6 of *B. tectorum* were not found in *B. pectinatus.* There are several possible explanations for this result. First, it is possible that the parental diploids that contributed genomes have evolved since the polyploid formation with the appearance and distribution of new allozymes. Second, homozygous phenotypes of PGD-A and PGI-A of *B. pectinatus* may have arisen through a genome-specific silencing of PGD-A3 and PGI-A6 due to mutation to null-alleles during or shortly after the formation of the initial allopolyploid. Alternatively, PGD-A3 and PGI-A6 might remain undetected in *B. pectinatus* due to insufficient sampling. The putative phylogenetic position of *B. pectinatus* as evidenced from the isozyme data is summarized in Fig. 1.

A wider isozyme survey of *B. pectinatus* is necessary for better understanding the origin of the species and the relationship between the two sections, including whether the sections are even valid, monophyletic sections.

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LITERATURE CITED

BARBER, H. 1970. Hybridization and the evolution of plants. *Taxon* **19**: 157–160.

BÖCKER, R., P. ERZBERGER, AND H. SCHOLZ. 1990. *Bromus diandrus*—Berliner Populationen. *Verh. Berliner Bot. Vereins* **8**: 93–96.

CUGNAC, A. 1933. Hybrides nouveaux du genre *Bromus. Bull. Soc. Bot. France* **80**: 38–39.

———. 1937. Sur quelques Bromes et leurs hybrides. VII. *B. sterilis* var. *velutinus* Volkart obtenu par synthèse expérimentale à partir du croisement de *B. madritensis* L. par *B. sterilis* L. *Bull. Soc. Bot. France* **84**: 711–713.

DEVESA, J., T. LUGUE, AND P. GOMEZ. 1990. Numeros cromosomaticos de plantas occidentales, 591–601. *Anales Jard. Bot. Madrid* **47**: 411–417.

DITOMASO, J. 2000. Invasive weeds in rangelands: species, impacts, and management. *Weed Science* **48**: 255–265.

ESNAULT, M.-A., AND A. HUON. 1987. Etudes morphologiques et caryologiques de *Bromus rigidus* et *Bromus diandrus* Roth: rela-

tions taxonomiques. *Bull. Soc. Bot. France, Lett. Bot.* **134**: 299–304.

JAASKA, V. 1969. Electrophoretic studies of seedling phosphatases, esterases and peroxidases in the genus *Triticum* L. *Proc. Acad. Sci. Estonian SSR, Biology* **18**: 170–183.

———. 1976. Aspartate aminotransferase isozymes in the polyploid wheats and their diploid relatives. *Biochem. Physiol. Pflanzen* **170**: 159–171.

———, AND V. JAASKA. 1970. Biochemical data on the origin of Transcaucasian endemic wheats. *Proc. Acad. Sci. Estonian SSR, Biology* **19**: 344–354.

———, AND ———. 1984. Isoenzymes of aromatic alcohol dehydrogenase in rye and triticale. *Biochem. Physiol. Pflanzen* **179**: 21–30.

———, AND ———. 1986. Isoenzyme variation in the barley genus *Hordeum* L. 1. Alcohol dehydrogenase and superoxide dismutase. *Biochem. Physiol. Pflanzen* **181**: 301–320.

———, AND ———. 1990. Isoenzyme variation in Asian beans. *Bot. Acta* **103**: 281–290.

KON, K., AND W. BLACKLOW. 1988. Identification and population variability of great brome (*Bromus diandrus* Roth) and rigid brome (*Bromus rigidus* Roth). *Austral. J. Agric. Res.* **39**: 1039–1050.

MACK, R. 1981. Invasion of *Bromus tectorum* L. into western North America: an ecological chronicle. *Agro-Ecosystems* **7**: 145–165.

MEHRA, P., P. KHOSLA, B. KOHLI, AND J. KOONAR. 1968. Cytological studies in the North Indian grasses (Part 1). *Res. Bull. Punjab Univ. New Ser., Sci.* **19**: 157–230.

MOINUDDIN, M., A. VAHIDY, AND S. ALI. 1994. Chromosome counts in Arundinoideae, Chloridoideae, and Pooideae (Poaceae) from Pakistan. *Ann. Missouri Bot. Gard.* **81**: 784–791.

NOVAK, S., AND R. MACK. 2001. Tracing plant introduction and spread: genetic evidence from *Bromus tectorum* (Cheatgrass). *BioScience* **51**: 114–122.

OJA, T. 1998. Isoenzyme diversity and phylogenetic affinities in the section *Bromus* of the grass genus *Bromus* (Poaceae). *Biochem. Syst. & Ecol.* **26**: 403–413.

———. 1999. Allozyme diversity and interspecific differentiation of the two diploid bromegrass species, *Bromus tectorum* L. and *B. sterilis* L. (Poaceae). *Pl. Biol. (Stuttgart)* **1**: 679–686.

———. 2002*a*. Genetic divergence and interspecific differentiation in the *Bromus madritensis* complex (Poaceae) based on isozyme data. *Biochem. Syst. & Ecol.* **30**: 433–449.

———. 2002*b*. *Bromus fasciculatus* Presl—a third diploid progenitor of *Bromus* section *Genea* allopolyploids (Poaceae). *Hereditas* **137**: 113–118.

———, AND V. JAASKA. 1996. Isoenzyme data on the genetic divergence and allopolyploidy in the section *Genea* of the grass genus *Bromus* (Poaceae). *Hereditas* **125**: 249–255.

———, AND ———. 1998. Allozyme diversity and phylogenetic relationships among diploid annual bromes (*Bromus,* Poaceae). *Ann. Bot. Fennici* **35**: 123–130.

———, ———, AND V. VISLAP. 2003. Breeding system, evolution and taxonomy of *Bromus arvensis, B. japonicus* and *B. squarrosus* (Poaceae). *Pl. Syst. Evol.* **242**: 101–117.

———, AND H. LAARMANN. 2002. Comparative study of the ploidy series *Bromus sterilis, B. diandrus* and *B. rigidus* (Poaceae) based on chromosome numbers, morphology and isozymes. *Pl. Biol. (Stuttgart)* **4**: 484–491.

OVADIAHU-YAVIN, Z. 1969. Cytotaxonomy of genus *Bromus* of Palestine. *Israel J. Bot.* **18**: 195–216.

PHILLIPS, S. 1995. *Bromus* L., pp. 54–55. *In* I. Hedberg and S. Edwards [eds.], Flora of Ethiopia and Eritrea, Vol. 7. Addis Ababa University, Ethiopia, and Uppsala University, Sweden.

ROOSE, M., AND L. GOTTLIEB. 1976. Genetic and biochemical consequences of polyploidy in *Tragopogon. Evolution* **30**: 818–830.

SALES, F. 1993. Taxonomy and nomenclature of *Bromus* sect. *Genea. Edinburgh J. Bot.* **50**: 1–31.

———. 1994. Evolutionary tendencies in some annual species of *Bromus* (*Bromus* L. sect. *Genea* Dum. (Poaceae)). *Bot. J. Linn. Soc.* **115**: 197–210.

SÁNCHEZ ANTA, M., M. GALLEGO, AND A. NAVARRO. 1988. Aspectos anatomicos de la epidermis de algunas especies de *Bromus* L. y su cariologia. *Acta Botanica Barcinon* **37**: 335–344.

SCHOLZ, H. 1981. Der *Bromus-pectinatus*-Komplex (Gramineae) im Nahen und Mittleren Osten. *Bot. Jahrb. Syst.* **102**: 471–495.

SMITH, P. 1970. Taxonomy and nomenclature of the brome-grasses (*Bromus* L. s.l.). *Notes Roy. Bot. Gard. Edinburgh* **30**: 361–375.

———. 1972. Serology and species relationships in annual bromes (*Bromus* L. sect. *Bromus*). *Ann. Bot.* **36**: 1–30.

———. 1985. *Bromus* L., pp. 272–301. *In* P. Davis [ed.], Flora of Turkey and the east Aegean Islands, Vol. 9. University Press, Edinburgh, UK.

SPIES, J., T. BURGER, AND M. VAN WYK. 1999. Chromosome studies on African plants. 12. The tribes of subfamily Pooideae. *Bothalia* **29**: 335–341.

STEBBINS, G. 1981. Chromosomes and evolution in the genus *Bromus* (Gramineae). *Bot. Jahrb. Syst.* **102**: 359–379.

TZVELEV, N. 1976. Zlaki SSSR (Poaceae USSR). Nauka, Leningrad, USSR. 787 p.

UPADHYAYA, M., R. TURKINGTON, AND D. MCILVRIDE. 1986. The biology of Canadian weeds, 75: *Bromus tectorum* L. *Canad. J. Pl. Sci.* **66**: 689–709.

WATSON, L., W. J. ELISENS, AND J. R. ESTES. 1991. Electrophoretic and cytogenetic evidence for allopolyploid origin of *Marshallia mohrii* (Asteraceae). *Amer. J. Bot.* **78**: 408–416.

WENDEL, J., AND N. WEEDEN. 1989. Visualization and interpretation of plant isozymes, pp. 5–45. *In* D. Soltis and P. Soltis [eds.], Isozymes in plant biology. Dioscorides Press, Portland, Oregon, USA.