Chromosome Karyotypes of *Echinacea angustifolia* var. *angustifolia* and *E. purpurea*

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Abstract. Chromosome karyotypes of the most commonly cultivated and medicinally used *Echinacea* taxa, *E. angustifolia* DC. var. *angustifolia* and *E. purpurea* (L.) Moench., were analyzed. The chromosomes of both taxa are medium in length, ranging from 4.12 to 5.83 μm in *E. angustifolia* var. *angustifolia* and 3.99 to 6.08 μm in *E. purpurea*. No abrupt length changes in the chromosomes were noted. The karyotypes of the two species are generally similar, but a distinguishable feature exists in one pair of chromosomes. The centromere of chromosome pair 10 is subterminally located in *E. purpurea*, but terminally located in *E. angustifolia* var. *angustifolia*, which can be readily recognized in mitotic metaphase cell plates. This finding may provide useful information for *Echinacea* evolutionary, genetic, and breeding studies.

Chromosome size and morphology may help indicate evolutionary relationships among plant species (Clark and Wall, 1996). In plant taxonomy, breeding, and genetic studies, information about chromosome karyotypes can be useful in species identification and analysis of hybrid populations. Native to North America, *Echinacea* species have an important place in herbal medicine. *Echinacea* has long been used by Native Americans to treat many conditions, including venomous bites, rabies, cold, headache, and stomach cramps (Foster, 1991; Kindscher, 1989; Li, 1998). Its non-selective, immune-enhancing properties have promoted the use of and demand for *Echinacea* products in recent years and, consequently, its field production. Cultivation of *Echinacea* has gained international attention and has been conducted in many countries, including the United States, Canada, Germany, Norway, Romania, Finland, Australia, Poland, Russia, New Zealand, Egypt, and China (Dou et al., 2001; El-Gengaihi et al., 1998; Finger et al., 1998; Li, 1998). Of the nine species and two varieties in *Echinacea* recognized by McGregor (1968), *E. angustifolia* var. *angustifolia* and *E. purpurea* are used most commonly (Li, 1998), followed by *E. pallida*.

Chromosome numbers and ploidy levels have been investigated in *Echinacea*. *Echinacea angustifolia* var. *angustifolia* and *E. purpurea* are diploids with a somatic chromosome number of $2n = 22$. *Echinacea pallida* is a tetraploid ($2n = 44$) and was suspected to

be derived from chromosome doubling of a hybrid between *E. simulata* McGregor and *E. sanguinea* Nutt. (McGregor, 1968). Chromosome karyotypes of *Echinacea* species have been reported to be quite similar (McGregor, 1968); however, precise descriptions and visual presentation of karyotypes of these species have not been published. This research was to analyze the karyotypes of *E. angustifolia* var. *angustifolia* and *E. purpurea*.

Materials and Methods

Root tips from germinating seeds and young leaves from field-grown plants were used for chromosome preparation. *Echinacea angustifolia* var. *angustifolia* and *E. purpurea* seeds were provided by the North Central Regional Plant Introduction Station (NCRPIS), U.S. Dept. of Agriculture (USDA) (Ames, Iowa), and purchased from Johnny's Selected Seeds (Albion, Maine), Wind River Seed (Manderson, Wyo.), and Richters (Goodwood, Ont., Canada) (Table 1). Seed germination was conducted in a growth chamber held at 25 °C. Seeds were soaked with deionized (DI) water in beakers for 15 min before they were placed in petri dishes on two layers of Whatman no. 1 filter paper thoroughly wetted with DI water. Germinating seeds with 1- to 5-mm radicles were treated with 0.08% colchicine solution for

≈3.5 h in small vials at room temperature. After colchicine treatment, the seeds were placed in freshly prepared Carnoy's fixative II (1 glacial acetic acid : 3 chloroform : 6 ethanol) (Li and Zhang, 1991) and stored in a refrigerator for at least 24 h before use.

The remaining seeds were also sown in a greenhouse in cell flats in mid-March (the USDA seeds were germinated in the growth chamber and transplanted into cell flats in late April after germination), and plants were transplanted into a field at Gaia Herbs Farm (Brevard, N.C.) in mid- to late May. Vegetative buds that were elongating were excised from the field-grown plants at ≈11:00 AM in early Sept. 2002 and immediately treated as in root tip preparation. Some of the *E. angustifolia* var. *angustifolia* plants and most of the *E. purpurea* plants were blooming at the time of sampling.

Plant morphologies of *E. angustifolia* var. *angustifolia* and *E. purpurea* are readily distinguishable. Only one *E. angustifolia* var. *angustifolia* was noted in ≈4000 plants grown from the commercial *E. purpurea* seed lots and vice versa, and no off-types were found in the USDA seeds. The morphological characteristics of the field-grown plants matched well with the published species descriptions (McGregor, 1968). Identities were also confirmed by plant taxonomist William Hess (Morton Arboretum, Lisle, Ill.). We have labeled the field-grown plants sampled for chromosome preparations, and voucher specimens from these plants will be made and deposited at Gaia Herbs, Inc.

For chromosome preparation, the fixed samples were placed in DI water for ≈30 min at room temperature, and then root tips of ≈1 mm or young leaf tips were excised and hydrolyzed in 1 N HCl for ≈6.5 min at 60 °C. The tips were then transferred to DI water and held for ≈30 min at room temperature. Chromosome slides were prepared by macerating the tips on slides in carbol fuchsin stain (Li and Zhang, 1991) and squashing under cover glass. Chromosome examination and photography were conducted by using a microscope (Zeiss IM35) under 1000× magnification.

For each seed source, chromosome evaluation was conducted from at least five root tips (five seeds) and young leaves from two plants. Chromosome measurements were made on the enlarged prints and converted to micrometers by relating measurements made in the microscope with a micrometer. Karyotype analysis was based on at least seven high-quality metaphase cell plates.

Table 1. Seed sources and origins of *E. angustifolia* var. *angustifolia* and *E. purpurea* used in chromosome evaluation.

Species	Accession no.	Seed origin	Provider
<i>E. angustifolia</i>	Ames 23930	Iowa	NCRPIS ² , USDA
<i>E. angustifolia</i>	Ames 24060	Kansas	NCRPIS, USDA
<i>E. angustifolia</i>	13370	Wyoming	Wind River Seed
<i>E. angustifolia</i>	16420	Minnesota	Johnny's Selected Seeds
<i>E. purpurea</i>	Ames 23967	North Carolina	NCRPIS, USDA
<i>E. purpurea</i>	Ames 25104	Louisiana	NCRPIS, USDA
<i>E. purpurea</i>	S2280	Oregon	Richters
<i>E. purpurea</i>	12448	Colorado	Wind River Seed

²North Central Regional Plant Introduction Station.

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Fig. 1. *Echinacea* metaphase chromosomes prepared from root tips of germinating seeds: (a) early metaphase stage and (b) metaphase stage of *E. angustifolia* var. *angustifolia*; and (c) early metaphase stage and (d) metaphase stage of *E. purpurea*. Arrows indicate the chromosomes with a terminal centromere region. Bar = 10 μ m.

Fig. 2. Chromosome karyogram of: (a) *E. angustifolia* var. *angustifolia* prepared from Fig. 1b; and (b) *E. purpurea* prepared from Fig. 1c. Bar = 10 μ m.

Results and Discussion

Typical mitotic metaphase cell plates are shown in Figure 1. As previously reported (McGregor, 1968), chromosome numbers of *E. angustifolia* var. *angustifolia* and *E. purpurea* are $2n = 22$. Results of the karyotype analysis are presented in Figure 2 and Table 2. In general, the chromosomes of both species are medium in length, ranging from 4.12 to 5.83 μ m in *E. angustifolia* var. *angustifolia* and from 3.99 to 6.08 μ m in *E. purpurea*. No abrupt length changes in the chromosomes were noted. Both species have four pairs of chromosomes with median-region centromeres and two pairs with submedian-region centromeres. The remaining five pairs have subterminal-region centromeres in *E. purpurea*. In *E. angustifolia* var. *angustifolia*, four of the five remaining pairs have subterminal-region centromeres, but chromosome pair 10 has its centromeres in the terminal region. Pair 10 can be readily recognized in *E. angustifolia* when compared with those of *E. purpurea*. The other pairs of chromosomes of the two species cannot be readily distinguished without detailed measurements. Within-species karyotypic variation was not observed in this study.

The readily distinguishable chromosome pair 10 in *E. angustifolia* might be used in hybrid identification in crosses of these two species. Although some quantitative differences in chromosome length and arm ratios exist in other pairs of the variety and the species, the overall similarity of the chromosomes and their subtle differences may make them unsuitable in species identification. Further research using chromosome banding (Tuna et al., 2001) or fluorescent dye staining (Schwarzacher et al., 1992) may help discriminate these pairs in more detail.

The plant materials used in this research represent four different growth locations (four states in the United States) for both taxa (Table 1), and no within-species karyotypic variation was observed. This may indicate that karyotypic differentiation, which is particularly prominent due to the presence of the pair of terminal-region centromere chromosomes in *E. angustifolia* var. *angustifolia*, is broadly true in the two taxa. It may be interesting to examine whether chromosome karyotype differences exist among other species in *Echinacea*.

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Table 2. The chromosome (Chr.) karyotypes of *E. angustifolia* var. *angustifolia* (E.a) and *E. purpurea* (E.p).^z

Chr.	Total length		Long arm		Short arm		Arm ratio ^y		Chr. type ^x	
	E.a	E.p	E.a	E.p	E.a	E.p	E.a	E.p	E.a	E.p
I	5.83 ± 1.03	6.08 ± 1.02	3.36 ± 0.61	3.53 ± 0.59	2.47 ± 0.43	2.55 ± 0.42	1.36 ± 0.06	1.38 ± 0.07	m	m
II	5.61 ± 0.98	5.61 ± 1.08	3.14 ± 0.56	3.27 ± 0.64	2.47 ± 0.42	2.33 ± 0.43	1.27 ± 0.05	1.40 ± 0.07	m	m
III	4.93 ± 1.01	5.16 ± 0.96	3.81 ± 0.79	3.59 ± 0.69	1.12 ± 0.21	1.57 ± 0.29	3.40 ± 0.13	2.29 ± 0.09	st	sm
IV	4.93 ± 0.89	5.15 ± 0.84	2.73 ± 0.69	2.80 ± 0.48	2.20 ± 0.21	2.35 ± 0.39	1.24 ± 0.08	1.19 ± 0.05	m	m
V	4.89 ± 0.76	4.75 ± 0.91	3.62 ± 0.55	3.27 ± 0.61	1.27 ± 0.18	1.48 ± 0.28	2.85 ± 0.07	2.21 ± 0.06	sm	sm
VI	4.84 ± 0.73	4.57 ± 0.82	3.45 ± 0.53	3.79 ± 0.69	1.39 ± 0.21	0.78 ± 0.14	2.48 ± 0.10	4.86 ± 0.08	sm	st
VII	4.82 ± 0.85	4.50 ± 0.89	2.53 ± 0.44	2.67 ± 0.54	2.29 ± 0.42	1.83 ± 0.34	1.10 ± 0.05	1.46 ± 0.10	m	m
VIII	4.53 ± 0.92	4.48 ± 0.76	3.81 ± 0.77	3.81 ± 0.63	0.72 ± 0.14	0.67 ± 0.12	5.31 ± 0.07	5.69 ± 0.06	st	st
IX	4.31 ± 0.83	4.22 ± 1.02	3.32 ± 0.65	3.21 ± 0.74	0.99 ± 0.18	1.01 ± 0.27	3.36 ± 0.04	3.18 ± 0.05	st	st
X	4.30 ± 0.79	4.12 ± 0.73	3.99 ± 0.74	3.25 ± 0.59	0.31 ± 0.06	0.87 ± 0.14	12.87 ± 0.12	3.73 ± 0.13	t	st
XI	4.12 ± 0.81	3.99 ± 0.99	3.36 ± 0.67	3.00 ± 0.73	0.76 ± 0.16	0.99 ± 0.26	4.42 ± 0.08	3.03 ± 0.09	st	st

^zChromosome type nomenclature is based on Levan et al. (1964), and chromosome length is in mm.^yArm ratio = length of the long arm : length of the short arm.^xm = median region centromere; sm = submedian region centromere; st = subterminal region centromere; and t = terminal region centromere.

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