Cytological Studies on Seven Species of Korean *Carex* (Cyperaceae)

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Summary Chromosome numbers of seven *Carex* taxa collected from the Korean peninsula are reported. This study is the first observations of meiotic chromosomes for *Carex* species from Korean populations. A univalent is observed in *C. ischnostachya* Steud. var. *fastigiata* T. Koyama ($n=26\text{II}+\text{I}$), and two different chromosome numbers are observed in an individual of *C. leucochlora* Bunge ($n=27\text{II}$, 29II). For the first time, the chromosome number for *C. polyschoena* H. Lév. & Vaniot ($n=26\text{II}$) is reported here. The results of diverse chromosome numbers suggest that polyploidy and aneuploidy including agmatoploidy and symploidy (increases and decreases in chromosome number due to fission or fusion, respectively) have played an important role for *Carex* species diversity in Korea.

Key words Agmatoploidy, *Carex*, Chromosome, Cyperaceae, Korea.

Genus *Carex* (Cyperaceae) is the largest angiosperm genus in the temperate zone, comprised of more than 2000 species worldwide (Reznicek 1990, The Global *Carex* group 2015). The genus exhibits high variance in chromosome numbers ranging from $n=6$ to $n=66$ with every haploid number between $n=6$ and $n=48$ (Tanaka 1949, Davies 1956, Roalson 2008, Hipp *et al.* 2009). The cytogenetic variance is hypothesized to be due to the non-localized centromeres (holocentric chromosomes), which facilitate chromosome number increases (agmatoploidy) and/or decreases (symploidy) by fission and fusion, respectively (Hoshino 1981, Nishikawa *et al.* 1984, Luceño and Guerra 1996, Chung *et al.* 2011, Hipp *et al.* 2013). Cytological studies have been very informative on taxonomy and phylogeny of *Carex* (Rothrock *et al.* 2009, Yano *et al.* 2010) and high chromosome number variations have been hypothesized to play an important role on rapid speciation in the genus (Hipp 2007, Hipp *et al.* 2009, Hipp *et al.* 2010, Chung *et al.* 2012, Escudero *et al.* 2012).

About 157 taxa of *Carex* are distributed in Korea, with Cyperaceae one of the most important components in the Korean flora as the second largest family following Asteraceae (Oh 2007, Kim *et al.* 2008). Although chromosome number variations are well-known features in *Carex* speciation, only a few chromosome studies have been conducted on Korean *Carex* populations.

Kim (2006) and Lee and Kim (2008) reported somatic chromosome numbers of three species: *C. blepharicarpa* var. *stenocarpa* Ohwi ($2n=20$), *C. siderosticta* Hance ($2n=12$), and *C. okamotoi* Ohwi ($2n=12$). Recently, somatic chromosome numbers of *Carex* sect. *Siderostictae* were counted from multiple populations of Korea. The study confirmed the chromosome evolution from large chromosomes (about $2\text{--}4\mu\text{m}$ long) with small numbers ($2n=12$) to smaller chromosomes (less than $1\mu\text{m}$ long) with high numbers (Chung *et al.* 2013). The deficiency of cytological information on Korean *Carex* species has resulted in difficulty in understanding Korean species diversity and cytological character evolution in *Carex* as a whole.

In this paper, we report chromosome numbers of seven *Carex* species from Korean populations and discuss the significance of their taxonomic and cytological characteristics.

Materials and methods

The chromosome numbers of seven species in *Carex* from Korean populations were analyzed. To determine meiotic chromosomes, live plants were collected from natural populations and then cultivated at the Jungwon University (Province Chungcheongbuk-do, Korea) and/or Korea National Arboretum (Province Gyeonggi-do, Korea). The methods for chromosome observation mainly followed Rothrock and Reznicek (1996), fixing immature spikes in a mixture of methanol, chloroform, and

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Fig. 1. Photomicrographs of *Carex* meiotic metaphase chromosomes. A. *C. ischnostachya* var. *fastigiata* ($n=26\text{II}+\text{I}$, Chung *et al.* 14), the arrow indicates a univalent. B. *C. leucochlora* ($n=27\text{II}$, Chung *et al.* 30); C. *C. leucochlora* ($n=29\text{II}$, Chung *et al.* s.n.). D. *C. neurocarpa* ($n=54\text{II}$, Chung *et al.* s.n.). E. *C. polyschoena* ($n=26\text{II}$, Hoshino *et al.* s.n.). F. *C. sabynensis* ($n=27\text{II}$, Yang *et al.* s.n.). G. *C. siderosticta* ($n=6$, Hoshino *et al.* s.n.). H. *C. tristachya* ($n=21\text{II}$, Chung *et al.* 10). Scale bar= $5\mu\text{m}$.

Table 1. Species, localities, voucher specimens, and chromosome numbers of *Carex* collected from Korea.

Specie, locality, and voucher information	Chromosome number counted in this study ($2n$)	Previous report ($2n$)
<i>Carex ischnostachya</i> Steud. var. <i>fastigiata</i> T. Koyama Mt. Ilseongsan, Hwawon-myeon, Haenam-gun, Jellanam-do, South Korea (Chung <i>et al.</i> 14, April 28 2012, KH)	53 ($n=26\text{II}+\text{I}$)	62 (Ohkawa and Yokota 1998)
<i>C. leucochlora</i> Bunge Mt. Jujaksan, Bugil-myeon, Haenam-gun, Jeollanam-do, South Korea (Chung <i>et al.</i> 30, April 28 2012, KH)	54 ($n=27\text{II}$) 58 ($n=29\text{II}$)	64, 68 (Tanaka 1939) 72 (Okuno 1939) 68 (Hoshino 1981, Ohkawa and Yokota 1998)
<i>C. neurocarpa</i> Maxim. Mochung-dong, Seowongu, Cheongju-si, Chungcheongbuk-do, South Korea (Chung <i>et al.</i> s.n., May 11 2011, KH)	108 ($n=54\text{II}$)	108 (Tanaka 1937)
<i>C. polyschoena</i> H. Lév. & Vaniot Korea National Arboretum, Socheur-eup, Pocheon-si, Gyeonggi-do, South Korea N 37.73919, E 127.17045 (Hoshino <i>et al.</i> s.n. Mar. 15 2015, KH)	52 ($n=26\text{II}$)	
<i>C. sabynensis</i> Less. ex Kunth Korea National Arboretum, Socheur-eup, Pocheon-si, Gyeonggi-do, South Korea (Yang <i>et al.</i> s.n., April 12 2012, KH)	54 ($n=27\text{II}$)	40 (Krogulevich 1971) 60 (Yurtsev and Zhukova 1982)
<i>C. siderosticta</i> Hance Korea National Arboretum, Socheur-eup, Pocheon-si, Gyeonggi-do, South Korea N 37.74615, E 127.17094 (Hoshino <i>et al.</i> s.n. Mar. 15 2015, KH)	12 ($n=6\text{II}$)	12, 24 (Hoshino and Tanaka 1977, Hoshino 1981) 12 (Chung <i>et al.</i> 2013) 24 (Tang and Xiang 1989, Starodubtsev 1989, Hoshino <i>et al.</i> 1993, Probatova <i>et al.</i> 1998, Probatova 2000)
<i>C. tristachya</i> Thunb. Mt. Ilseongsan, Hwawon-myeon, Haenam-gun, Jellanam-do, South Korea (Chung <i>et al.</i> 10, April 28 2012, KH), Seosan-si, Chungcheongnam-do, South Korea (Yang <i>et al.</i> s.n. Feb. 9 2015, KH)	42 ($n=21\text{II}$)	40, 41, 64 (Tanaka 1948)

propionic acid (6:3:2). Fixed anthers were squashed in 2% acetic-orcein and observed at $1000\times$ magnification and photographed. At least three meristematic cells per sample were analyzed to determine meiotic chromosome

numbers. All voucher specimens are housed at the Korea National Arboretum Herbarium (KH) (Table 1).

Results and discussion

Meiotic chromosome numbers are summarized with previous records in Table 1. Bivalents and a univalent are observed. None of the species exhibit distinct primary constriction, and all chromosomes are less than $1\ \mu\text{m}$ long except for *Carex siderosticta* (Fig. 1).

Carex ischnostachya Steud. var. *fastigiata* T. Koyama ($n=26\text{II}+\text{I}$, Fig. 1A)—*Sect.* Ischnostachyae

The chromosome number for *C. ischnostachya* var. *fastigiata* from a Korean population is $n=26\text{II}+\text{I}$, which is a new number for the taxa. The observation of a univalent is common in *Carex* due to holocentric chromosome structures (Hoshino 1981, Luceño and Guerra 1996, Chung *et al.* 2011). This number differs from previous reported somatic chromosome number $2n=62$ collected from Japan (Ohkawa and Yokota 1998, Hoshino *et al.* 2011). Hoshino (1981) confirmed the somatic chromosome number of *C. ischnostachya* var. *ischnostachya* $2n=62$, which was collected from Hiroshima, Japan. Our new number for the taxon is the first report from meiotic chromosomes of a Korean population. The species occurs only in Korea and Japan, and chromosome numbers from the two geographical regions are incongruent. The chromosome number variations among populations require further investigation with geographic and morphological features together.

C. leucochlora Bunge ($n=27\text{II}$, 29II , Fig. 1B, C)—*Sect.* Mitratae

In an individual of *C. leucochlora*, two meiotic chromosome numbers $n=27\text{II}$, 29II are observed, which is the first count from a Korean population. Previous chromosome counts for the species vary from $2n=64$ to $2n=72$ (Table 1, Tanaka 1939, Okuno 1939, Hoshino 1981, Ohkawa and Yokota 1998). *C. leucochlora* is widely distributed in East Asia including Korea, Japan, northeast China, Taiwan, and the Himalayas (Hoshino *et al.* 2011). Various chromosome numbers for the species might be related to broad geographic distribution and recent speciation events (Chung *et al.* 2011). To understand the chromosome variation, taxonomic and phylogenetic studies with cytological research on more population samples should be conducted.

C. neurocarpa Maxim. ($n=54\text{II}$; Fig. 1D)—*Sect.* Phleoidae

The meiotic chromosome of *C. neurocarpa* has $n=54\text{II}$, which is congruent from the previous reports from Japanese populations (Tanaka 1937). Our count is the first report from Korean populations. *C. neurocarpa* is widely distributed in East Asia. Further cytological research covering broad distribution areas is required to understand chromosome number variation in the taxon. *C. neurocarpa* is well distinguished from closely related

taxa by leaf-like bracts and winged perigynia (Oh 2007, Dai *et al.* 2010, Hoshino *et al.* 2011).

C. polyschoena H. Lév. & Vaniot ($n=26\text{II}$, Fig. 1E)—*Sect.* Mitratae

The meiotic chromosome number for the species is $n=26\text{II}$, which is the first count for the taxa. The species occurs in forests of Japan, Korea, and China (Hoshino *et al.* 2011). In Japan, *C. polyschoena* is found only in Tsushima Island of Nagasaki Prefecture. However, in Korea, the species occurs throughout the country (Oh 2007, Yang *et al.* 2015). Dai *et al.* (2010) treated the species as a variant of *C. pisiformis* Boott. Further taxonomic and phylogenetic studies are needed to clarify conflicting species delimitations and understand species evolution.

C. sabynensis Less. ex Kunth ($n=27\text{II}$, Fig. 1F)—*Sect.* Mitratae

We observed meiotic chromosomes of $n=27\text{II}$ from *C. sabynensis*, which is incongruent with previous reports $2n=60$ (Yurtsev and Zhukova 1982) and $2n=40$ (Kroglevich 1971). The species is distributed in broad areas in East Asia, growing in open meadows or wet places in Japan, Korea, eastern Siberia, and northeast China (Hoshino *et al.* 2011). The broad geographic distribution associated with morphological character variations might result in the incongruent cytological characters.

C. siderosticta Hance ($n=6$, Fig. 1G)—*Sect.* Siderostictae

Six chromosomes are observed at pollen mitotic metaphase. Previous counts from Korea are diploids with chromosome numbers of $2n=12$ (Chung *et al.* 2013). In China, Japan, and Russia, both diploids, tetraploids, or either have been reported: $2n=12$, 24 from Japan (Hoshino and Tanaka 1977, Hoshino 1981, Hoshino *et al.* 1993), $2n=24$ from China and Russia (Tang and Xiang 1989, Starodubtsev 1989, Probatova *et al.* 1998, Probatova 2000). Although aneuploidy is known to play an important role in *Carex* speciation, in *Carex* sect. *Siderostictae*, polyploidy is an important speciation mechanism.

C. tristachya Thunb. ($n=21\text{II}$, Fig. 1H)—*Sect.* Mitratae

The chromosome number counts from two Korean populations are reported as $n=21\text{II}$, which disagrees with previous reports ($2n=40$, 41 , 64 ; Tanaka 1948). The species is found on the sunny sides of grassland and forest edges in Korea, Japan, and China (Dai *et al.* 2010). Although it is common to have chromosome number variation within a species, among populations, and even in individuals in *Carex* (Roalson 2008, Hipp *et al.* 2011, Chung *et al.* 2011), taxonomic research on the species is desired to resolve obscure species delimitation by studies on morphological variation.

The chromosome number $2n=52$ for *C. polyschoena*

is first reported in this study. Although this species is common in South Korea, it is designated as Endangered II in Japan, and found only on Tsushima Island. *C. polyschoena* is very close to *C. stenostachya* Franch. & Sav., which is common in western Japan. A continuous aneuploid series, $2n=58, 59, 60$, and 61 , is reported (Hoshino *et al.* 1993). Further cytological studies are needed to elucidate the chromosomal differentiation in South Korean and Japanese populations. Although only diploid chromosome numbers are found in *C. siderosticta* from South Korean populations, tetraploids are common in Chinese and Japanese populations. Additional cytological data from South Korea, which is located between China and Japan, is needed. More cytological studies are also needed in order to explain the mechanisms of the aneuploidal diversification of four species, *C. ischnostachya* var. *fastigiata*, *C. leucochlora*, *C. sabynensis*, and *C. tristachya*, for which different chromosome numbers from previous reports are found.

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