Cytological Studies of 4 Species of the Genus *Kobresia* (Cyperaceae) Collected from Nepal Himalaya

Okihito Yano¹, Hiroshi Ikeda^{1*}, Mark F. Watson²,
and Keshab R. Rajbhandari³

¹ The University Museum, The University of Tokyo, 7–3–1 Hongo, Bunkyo-ku, Tokyo
113–0033, Japan

² Royal Botanic Garden Edinburgh, 20a Inverleith Row, Edinburgh EH3 5LR, Scotland, U.K.

³ G.P.O. Box 9446, Kathmandu, Nepal

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Summary Chromosome numbers of 4 species of the genus *Kobresia* (Cyperaceae) collected from the Nepal Himalaya are reported. Chromosome numbers of *K. curvata* Kük. ($2n=50$), *K. esenbeckii* (Kunth) Noltie ($2n=66$), and *K. duthiei* C. B. Clarke ($2n=ca. 84$) are reported for the first time. *Kobresia nepalensis* (Nees) Kük. showed an unusually high chromosome number, $2n=ca. 114$. It is concluded that chromosomal evolution in the unispicate *Kobresia* species may have been caused by both polyploidy and aneuploidy.

Key words Aneuploid, Cyperaceae, Himalaya, *Kobresia*, Nepal, Polyploid.

The genus *Kobresia* Willd. is distributed mainly in the Northern hemisphere, and comprises 50–70 species (Rajbhandari and Ohba 1991, Goetghebeur 1998, Zhang 2001, 2004). High levels of species level diversity is found in *Kobresia* across the high mountain ranges from the Himalayas to South–West China. Rajbhandari and Ohba (1991) recognized 24 taxa in *Kobresia* from the Nepal Himalaya, and Zhang (2004) recognized 37 taxa from Tibet. Cytological studies have proven useful for understanding the diversification of several plant groups in the Himalayas (Wakabayashi and Ohba 1988, Akiyama *et al.* 1992, Ikeda and Ohba 1999). Roalson (2008) enumerated chromosome numbers of Cyperaceae, and reported a few chromosome counts for the genus *Kobresia*. Previous cytological studies of 7 species of *Kobresia* have reported chromosome numbers of $2n=32$, *ca.* 36, 52–56, 58, 60–66, 70–76, 80, *ca.* 122 (Flovik 1943, Holmen 1952, Löve and Löve 1956, Jørgensen *et al.* 1958, Knaben and Engelskjøn 1967, Johnson and Packer 1968, Zhukova 1969, Krogulevich 1971, Poghosian *et al.* 1971, Zhukova and Petrovsky 1976, 1980, Mehra and Sachdeva 1975, 1976, Probatova and Sokolovskaya 1988, Hoshino *et al.* 1993, 2000, Roalson 2008). However, most of these cytological works used materials collected from the circumpolar regions, and only 2 of the species examined were collected from the Himalayas. Mehra and Sachdeva (1975, 1976) reported chromosome numbers of $2n=80$ for *K. royleana* (Nees) Boeck., from Kashmir Himalaya in North–West India, and Hoshino *et al.* (2000) reported $2n=ca. 122$ for *K. nepalensis* (Nees) Kük. from Langtang Himal, in central Nepal. However, in order to understand the evolution and diversification of this genus in the Himalayas, additional karyomorphological studies are needed.

This paper reports chromosome numbers for 4 additional *Kobresia* species collected from the Manaslu Himalaya and adjacent areas in central Nepal, with the aim to elucidate the chromosomal evolution of this genus.

* Corresponding author, e-mail: h_ikeda@um.u-tokyo.ac.jp

Table 1. Species, localities, voucher specimens, and chromosome numbers of 4 species of genus *Kobresia* collected from the Mt. Manaslu and adjacent regions in Nepal Himalaya

Species, locality and voucher specimen	Chromosome number (2n)
<i>Kobresia curvata</i> Kük. Samagaon, on way to Manaslu Base Camp, 4040 m (Ikeda <i>et al.</i> 20814085)	50
<i>K. esenbeckii</i> (Kunth) Noltie Samagaon, 3500 m (Ikeda <i>et al.</i> 20814080)	66
<i>K. duthiei</i> C. B. Clarke Samagaon, on way to Manaslu Base Camp, 4220 m (Ikeda <i>et al.</i> 20814088)	ca. 84
<i>K. nepalensis</i> (Nees) Kük. Lho-Samagaon, 3070 m (Ikeda <i>et al.</i> 20814064)	ca. 114

Materials and methods

Karyomorphological observations were conducted on 4 *Kobresia* species of the mostly collected from the Manaslu Himalaya and adjacent areas in central Nepal (see Ikeda and Watson 2010). Voucher specimens and localities of the material are listed in Table 1. The methods used for preparing the plant material for chromosome observation followed Yano *et al.* (2010). Voucher specimens are deposited in the Herbarium at the University of Tokyo (TI), with duplicates available in the National Herbarium of Nepal (KATH) and the Royal Botanic Garden Edinburgh (E).

Results and discussion

The chromosome numbers determined in this study are shown in Table 1. Somatic metaphase chromosomes of these 4 species were very small, less than $1.2\mu\text{m}$ in length, and they were polycentric, *i.e.* primary constriction was not observed in any of the species. Chromosome numbers of $2n=50$ for *K. curvata* Kük., $2n=66$ for *K. esenbeckii* (Kunth) Noltie, and $2n=\text{ca. } 84$ for *K. duthiei* C. B. Clarke, were determined for the first time (Fig. 1). The chromosome number of $2n=\text{ca. } 122$ had previously been reported for *Kobresia nepalensis* by Hoshino *et al.* (2000), and our count of $2n=\text{ca. } 114$ (Fig. 1) is somewhat different.

In the genus *Kobresia*, inflorescence structure, paniculate *versus* unispicate, is used as an important character in intrageneric classification (Kükenthal 1909, Ivanova 1939, Egorova 1983, Rajbhandari and Ohba 1991, Zhang 2001, 2004). Recently, Starr and Ford (2009) reported the monophyly of unispicate species of the genus *Kobresia* with strong supports in the tribe Cariceae, based on DNA sequence data. In our study, *K. esenbeckii* ($2n=66$), *K. duthiei* ($2n=\text{ca. } 84$), and *K. nepalensis* ($2n=\text{ca. } 114$) have unispicate inflorescence. From previous studies, chromosome numbers of unispicate *Kobresia* species were: $2n=32$ for *K. schoenoides* (C. A. Mey.) Steud., $2n=\text{ca. } 36, 52-56, 58, 60-66$ for *K. myosuroides* (Vill.) Fiori & Paol., $2n=\text{ca. } 54, 58, 62$ for *K. sibirica* (Turcz. ex Ledeb.) Boeck., and $2n=\text{ca. } 122$ for *K. nepalensis* (Table 2). The unispicate *Kobresia* species are seen to exhibit a polyploidy series, as well as aneuploids, which might be common in Cyperaceae plants with diffuse centromeric chromosomes. Himalayan species showed high polyploidy, especially the chromosome numbers of $2n=\text{ca. } 114$ and $\text{ca. } 122$ for *K. nepalensis*, which is the highest yet reported for the genus. Our results suggest that *K. nepalensis*, with the numbers $2n=\text{ca. } 114$ and $\text{ca. } 122$, may have arisen through polyploidization from ancestors such as *K. myosuroides* or *K. esenbeckii* as they have the number of $2n=52-56, 58$, and $60-66$. Mehra and Sachdeva (1976) suggested that chromosome numbers in the genus *Kobresia* showed ploidy series on 3 basic numbers, $x=8$ ($2n=32, 40, 72$), $x=9$ ($2n=54, 72$), and $x=13$ ($2n=52, 78$), and other chromosome numbers, $2n=58$ and 76 originating from secondary aneuploidy. In the present study, our results also suggested that the chromosomal evolution in unispicate *Kobresia* species may have

Fig. 1. Photomicrographs of somatic metaphase chromosomes of 4 species in the genus *Kobresia*. A. *K. curvata* ($2n=50$). B. *K. esenbeckii* ($2n=66$). C. *K. duthiei* ($2n=ca. 84$). D. *K. nepalensis* ($2n=ca. 114$). Bar= $5\ \mu m$.

Table 2. Chromosome numbers of genus *Kobresia*

Species	Chromosome number ($2n$)	Reference
Unispicate inflorescence species		
<i>K. esenbeckii</i>	66	Present study
<i>K. duthiei</i>	ca. 84	Present study
<i>K. myosuroides</i>	ca. 36	Krogulevich (1971; as <i>K. bellardii</i> (All.) Degl.)
	52–56	Jørgensen <i>et al.</i> (1958)
	56	Hoshino <i>et al.</i> (1993; as <i>K. bellardii</i>)
	58	Johnson and Packer (1968), Zhukova and Petrovsky (1976; as <i>K. bellardii</i>)
	60–66	Holmen (1952)
	60	Löve and Löve (1956; as <i>Elyna myosuroides</i> (Vill.) Fritsch)
<i>K. nepalensis</i>	ca. 112	Present study
	ca. 122	Hoshino <i>et al.</i> (2000)
<i>K. schoenoides</i>	32	Poghosian <i>et al.</i> (1971)
<i>K. sibirica</i>	ca. 54	Johnson and Packer (1968; as <i>K. hyperborea</i> A. E. Porsild)
	58	Zhukova (1969)
	62	Zhukova and Petrovsky (1980)
Paniculate inflorescence species		
<i>K. curvata</i>	50	Present study
<i>K. filifolia</i>	ca. 60	Probatova and Sokolovskaya (1988)
<i>K. royleana</i>	80	Mehra and Sachdeva (1975, 1976)
<i>K. simpliciuscula</i>	58	Löve and Löve (1956; as <i>Carex bipartita</i> All.)
	60	Löve and Löve (1956; as <i>C. bipartita</i>)
	64	Flovik (1943; as <i>C. bipartita</i>)
	70–75	Jørgensen <i>et al.</i> (1958)
	72–76	Johnson and Packer (1968)
	76	Knaben and Engelskjøn (1967)

been caused by both polyploidy and aneuploidy.

Previously reported chromosome numbers of *Kobresia* species with paniculate inflorescences are: $2n \approx 60$ for *K. filifolia* (Turcz.) C. B. Clarke, $2n = 58, 60, 64, 70-76$ for *K. simpliciuscula* (Wahlenb.) Mack., and $2n = 80$ for *K. royleana* (Table 2). In our study, *K. curvata* ($2n = 50$) has a paniculate inflorescence. Starr and Ford (2009) demonstrated that *Kobresia* species with paniculate inflorescences were not monophyletic within the tribe Cariceae. With such a mixed phylogenetic history it is currently difficult to recognize chromosome relationships of paniculate *Kobresia* species only from chromosome numbers. Future cytological and molecular phylogenetical studies are needed including wider sampling in order to fully understand phylogenetics and chromosomal evolution of the genus *Kobresia*.

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