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Karyomorphology of Sinacfoxa (Adoxaceae) and its systematic significance

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Abstract — Karyomorphology of *Sinadoxa corydalifolia* and the karyotype of the diploid *Adoxa moschatellina* are investigated for the first time. Karyomorphological data indicate that *Sinadoxa* is directly akin to other two genera of the Adoxaceae, rather than related to Araliales; and three genera of the Adoxaceae should be a natural group. The karyomorphological combinations of the Adoxaceae delimit it from all taxa assumed to be related to it. The karyotype characteristics of all taxa of the Adoxaceae are compared and summarized. The mechanisms of speciation in the Adoxaceae are discussed.

INTRODUCTION

Until recently only a single species *(Adoxa moschatellina)* of the Adoxaceae was known. The characteristics of *Adoxa* are so unique that FRITCH (1891) concluded that "there is no genus in the plant kingdom with which *Adoxa* is closely related" and he therefore placed it in a monotypic Adoxaceae. The morphological features of *Adoxa* have suggested a wide variety of possible relationships, including with Araliaceae, Caprifoliaceae, and even Fumariaceae and Ranunculaceae (ADANSON 1763; CANDOLLE 1830; HOOKER 1873; SPAGUE 1927; WARMING and MOBIUS 1929; HUTCHSON 1973). The systematic position of Adoxaceae has been an interesting issue in the phylogeny of the an-giosperms (DONOGHUE et al. 1992; ERBAR 1994; ROELS and SMETS 1994; DONOGHUE 1995; ERIKSSON and DONOGHUE 1997; RONSE DECRAENE *et al.* 1998). However, two genera under the Adoxaceae from China (Wu 1981; Wu *et al.* 1981) were established in 1981 and the second species under *Adoxa* was described from the Russia by NEPOMNYASHCHAYA (1984). According to the recent revision (LiANG and Wu 1995), the Adoxaceae comprises 3 genera with four species: *Tetradoxa omeiensis* (Hara) C. Y. Wu, endemic to the vicinity of Mt. Omei, Sichuan Province of China, *Adoxa moschatellina* L., distributed in temperate and boreal regions around the Northern Hemisphere, *A. orientalis,* found in the Russia and Northeastern China, and *Sinadoxa corydalifolia* C. Y. Wu, Z. L. Wu et R. F. Huang, endemic to the Southern Qinghai Province of China.

Sinadoxa corydalifolia, with less than 500 individuals based on our field expedition, is one of the exceedingly endangered species in the Qinghai-Xizang Plateau. Because of the difficulty of obtaining the materials, *Sinadoxa* was poorly studied. Although it differs morphologically with *Adoxa* and *Tetradoxa* in the characters of rhizome, leaf, calyx and ovary, especially in the highly complicated inflorescence like a spike with several glomerate interrupted clusters, most Chinese researchers accepted *Sinadoxa* as a natural member of the Adoxaceae (Li and NING 1987; LIANG and Wu 1995). However, DONOGHUE *et al.* (1992) pointed out that *"Sinadoxa* differs in many respects and may be more closely related to Araliales than to *Adoxa".*

Among the four species of the Adoxaceae, except *Sinadoxa corydalifolia,* other 3 species have been karyomorphologically investigated. *Tetradoxa omeiensis* was found to be 2n=36 with the basic chromosome number of x=18 while *A. orientalis* to be 2n=108, a hexaploid of x=18 by LIANG (1986, 1993a, b). The chromosome numbers of *Adoxa moschatellina* were reported to be 2n=36 from Europe

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(SOKOLOVSKAJA 1966; ZHUKOVA 1967; JOHNSON and PACKER 1968; PACKER 1968), 2n=36, 54 from China (LIANG 1993a), and 2n=45, 54 from Japan (KARA 1956; NOGUCHI and KAWANO 1974). Only the population from Japan with 2n=54 was karyomorphologically investi-gated and found to be a triploid (NOGUCHI and KAWANO 1974); nevertheless, the karyo-type of the diploid *Adoxa moschatellina* has not been reported yet.

The present paper aims (1) to describe the karyomorphology of the monotypic *Sinadoxa;* (2) to analyze the karyotype of the diploid *Adoxa moschatellina* with the chromosome number of 2n=36; and (3) to determine if *Sinadoxa* is a natural member of the Adoxaceae based on the karyomorphological data comparison with other taxa of the family.

MATERIALS AND METHODS

The materials of *Sinadoxa corydalifolia* were collected from two counties of the Southern Qinghai Province. In Yushu county, only one individual was found in the scree area of the XiaLaXiu Pass at a altitude of 4700 m. In Nangchian county, along the Xianglonggou valley about 15 km long at altitudes between 3800 and 3850m, three small populations were found. In each of three small populations, five individuals were selected. For each selected individual, 3-8 root tips were dug and picked up. The materials of *Adoxa moschatellina* were obtained from Xining of Qinghai Province with a altitude of 2200m. The voucher specimens of *Sinadoxa corydalifolia* (Liu Jianquan 598) and *Adoxa moschatellina* (Liu Jianquan 625) are deposited in the Herbarium of the Northwest Plateau Institute of Biology, the Chinese Academy of Sciences (HNWB).

The excised root tips were pretreated in a mixture of 1:1 (v/v) 8-hydroxyquinoline (0.002% w/v):colchicine (0.05% w/v) for 14 h, and fixed and stored in 3:1 ethanol-acetic acid. After being brought back to the laboratory, they were macerated with 1N HC1 for 10 m at 60°C, stained and squashed with Carbol Fuchsin. The chromosomes of at least 50 cells were counted and the measurements of 15 cells were completed. The karyomorphological classifications of the resting nuclei and mitotic-prophase chromosomes followed TANAKA (1977). Nomenclature for the centromeric positions of chromosome introduced by LEVAN *et al.* (1964) were followed. The karyotype classification of STEBBINS (1971) and the karyotype asymmetry indices (A_1=the intrachro-mosomal asymmetry and A_2=the interchromosomal asymmetry index) defined by ROMERO ZARCO (1986) were adopted.

RESULTS

Sinadoxa corydalifolia C.Y. Wu, Z.L. Wu et R.F. Huang.

The resting nuclei and the prophase chromosomes of all individuals investigated are similar. The resting nucleus (Fig. 1) belongs to the diffuse type, characterized by the dark staining chromatins without conspicuous aggregation. In the mitotic-prophase stage (Fig. 2), the chromosomes stain darkly and homogeneously and no conspicuous heterochromatic or euchromatic segment can be detected. The chromosomes of the mitotic-prophase are classified as the continuous type.

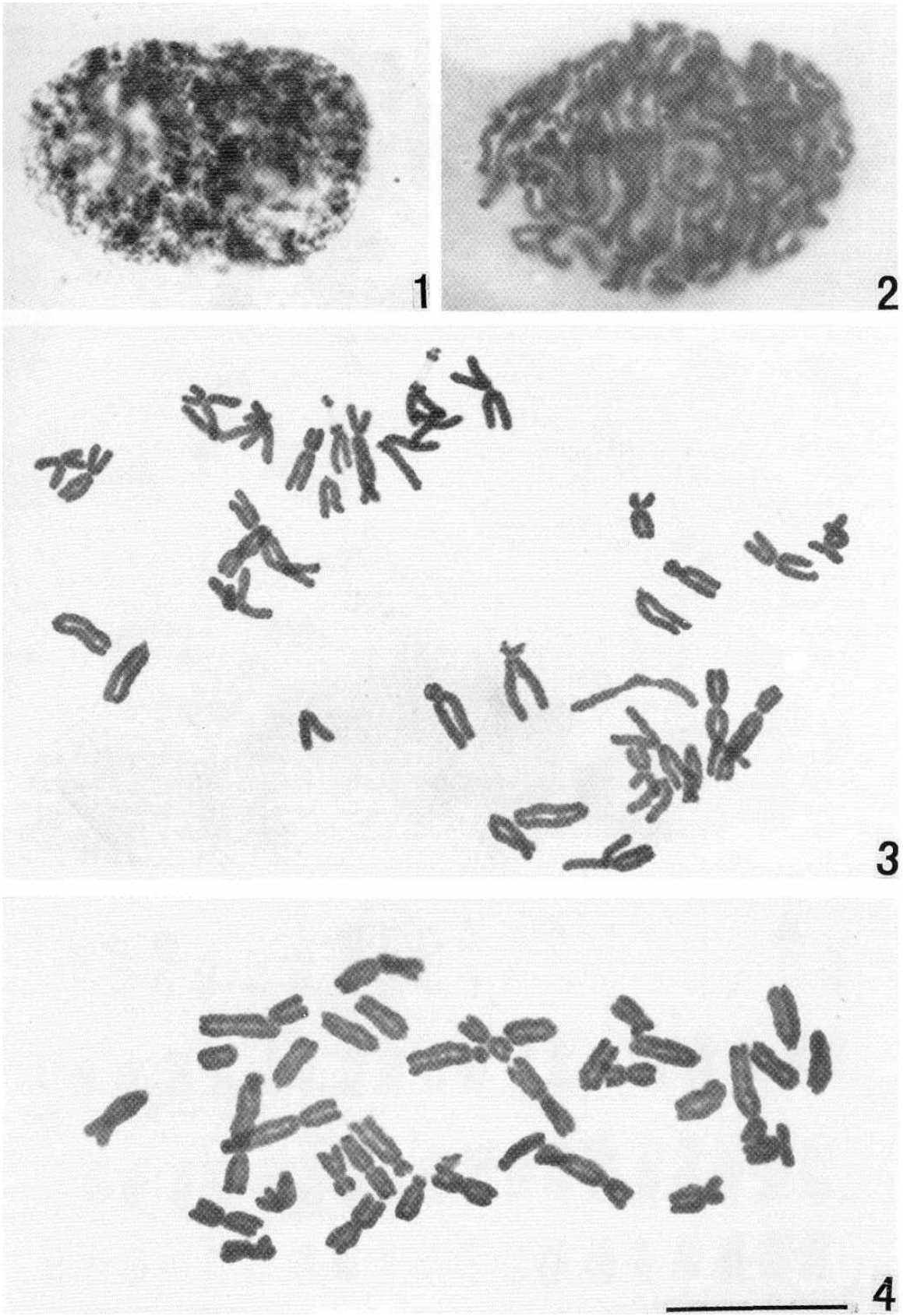
The chromosome number and the karyotype are stable among individuals and populations (Figs. 3 and 4). The karyotype is formulated as 2n=36=8M+12SM+4ST(4SAT)+12T (Fig. 8). Two pairs of ST chromosomes always exhibit satellites although sometimes they are indistinct and invisible at metaphase. Asymmetry of the karyotype is categorized to be 3B type. The karyotype complement shows it should be a diploid with the basic chromosome number of x=18.

Adoxa moschatellina L.

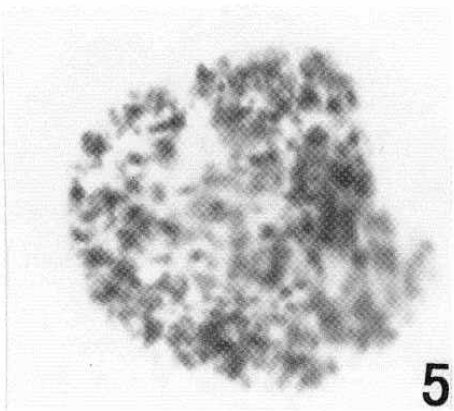
The resting nucleus and the prophase chromosomes (Figs. 5 and 6) belong to the diffuse type and the continuous type respectively, similar to those of *Sinadoxa corydalifolia.* The chromosome number of the population from the Xining of Qinghai province in the present study is found to be 2n=36 (Fig. 7). The karyotype formula is 2n=36=8M(2SAT)+12SM+4ST (4SAT)+12T (Fig. 9). Two pairs of ST chromosomes and one pair of M chromosomes have satellites (sometimes indistinct and invisible) on the their short arms. Asymmetry of the karyotype is estimated to be the 3B type. The karyotype constitution indicates it should be a diploid with x=18.

DISCUSSION

Most taxa of Araliales have the basic chromosome number of x=12 or x=l1, 10, aneu-



Figs. 1-4: *Sinadoxa corydalifolia.* Fig. 1 — The resting nucleus. Fig. 2 — Prophase chromosomes. Fig. 3 and Fig. 4 — Metaphase chromosomes. Figs. 1, 2, 4 from Nangchan population and Fig. 3 from one individual of Yushu population. Bar=10μm.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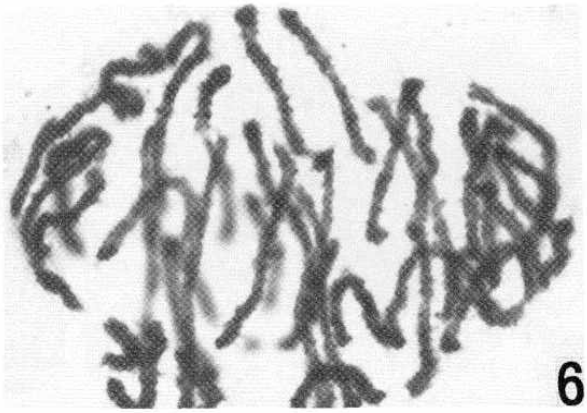
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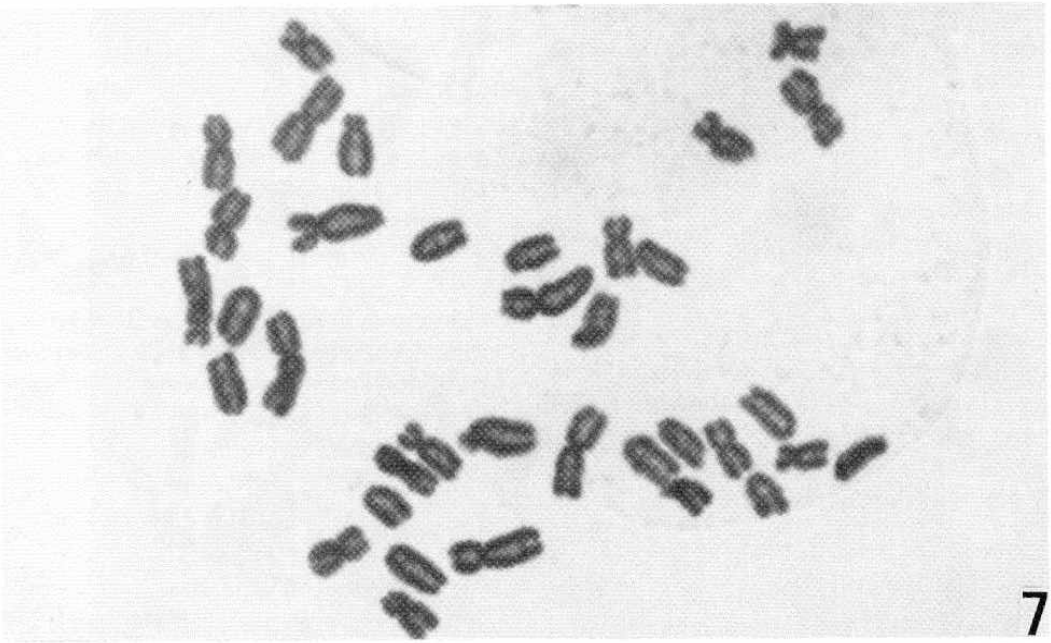
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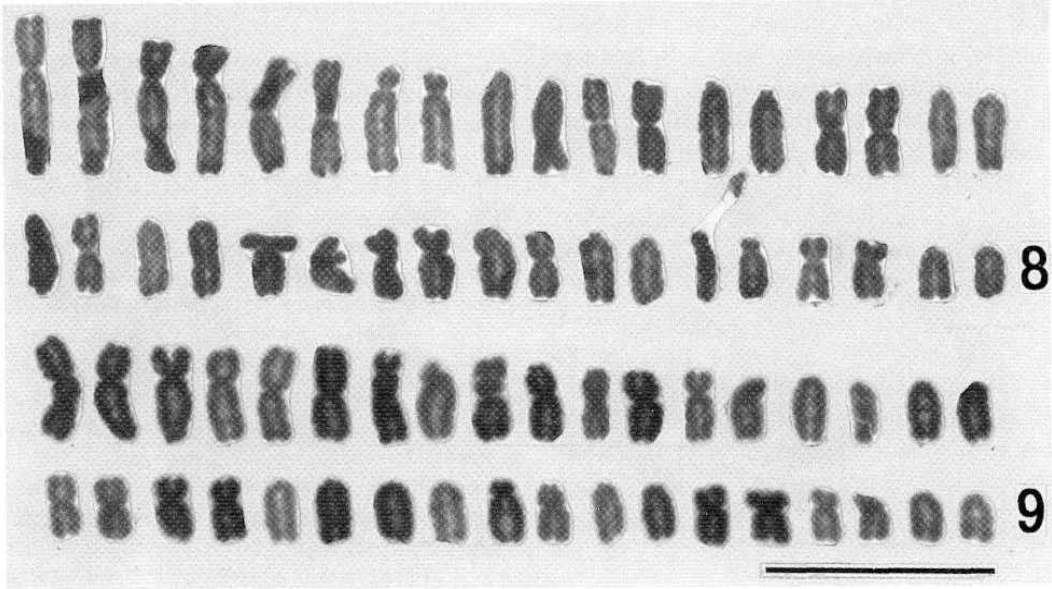
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TABLE 1 — Karyotype structure of the Adoxaceae

Taxon	Chromosome number ($2n$)	Ploidy level	Karyotype Structure					Type	Asymmetrial index		References
			M	SM	ST	T	SAT		A_1	A_2	
Adoxa moschatellina	36	2x	8	12	4	12	6	3B	0.62	0.23	Present research
	54	3x	18	9	6	21	Ca. 21	3B	0.63	0.24	NOGUHI and KAWANO 1974 *
A. orientalis	108	6x	32	24	20	32	0	3B	0.61	0.26	LIANG 1993b *
Sinadoxa corydalifolia	36	2x	8	12	4	12	4	3B	0.62	0.30	Present research
Tetradoxa omeiensis	36	2x	6	14	4	12	0	3B	0.62	0.30	LIANG 1986 *

* Karyotypic data are calculated based on the published measurements of chromosomes or idiogram.

ploidy dropping from x=12 (HoNG 1990), while the basic number of *Sinadoxa* was here found to be x=18. *Sinadoxa* differs basically with Araliales in the basic chromosome number. It can not be closely related to Araliales as suggested by DONOGHUE *et al.* (1992). In fact, karyomorphological data indicate that *Sinadoxa* should be a natural member of the Adoxaceae. It shares commonly with the other taxa of the Adoxaceae (LIANG 1986, 1993) the following karyomorphological characters: the diffuse type of the resting nuclei, the continuous type of the prophase chromosomes, the basic chromosome number of x=18 and the 3B karyotype constantly with 2 pairs or sets of subtelecentric (ST) and 6 pairs or sets of tel-ecentric (T) chromosomes. Furthermore, such combinations of karyomorphological features are only found in and can serve as the chromosomal markers of the Adoxaceae, by which the family is cytologically distinguishable from all the taxa (e.g. Araliales, see HONG 1990; *Sambucus* and *Viburnum* of Caprifoliaceae, see BENKO-ISEPPON and MORAWETZ 1993) assumed to be related to it.

The karyotype characteristics of all taxa of the Adoxaceae are compared and summarized in Table 1. The karyotype structures of the Adoxaceae are similar except for the trivial difference of the asymmetrical indices, chromosome types of SM-M and the number of the satellites among species and populations. Therefore, it is difficult to illustrate the interrelationship and evolutionary stage on the basis of karyotype data.

The mechanisms of speciation in the Adoxaceae could be gradual and rapid. At the early diploid level differentiation of three genera of the Adoxaceae, main cause of speciation is probably gene mutations or occasionally chromosome structural changes based on reciprocal translocation, because all diploid species of three genera have the same number and similar karyotypes. Although polyploidization supplies the possibility of rapid speciation, it only occurs in *Adoxa.* Its occurring in *Adoxa* might be directly connected with the successfully asexual reproduction through rhizomes in this genus (LiANG and Wu 1995). Polyploidization and asexual reproduction together accelerate the speciation and adaptation of *Adoxa.* This could explain why *Adoxa* has more cytotypes, species and wider distribution while the other two genera are monotypic, narrowly distributed and exceedingly endangered.

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Figs. 5-7, 9: *Adoxa moschatellina.* All from Xining population. Fig. 5 — The resting nucleus. Fig. 6 — Prophase chromosomes. Fig. 7 — Metaphase chromosomes. Fig. 9 — Karyotype. Fig. 8 — Karyotype *of Sinadoxa corydalifolia.* Bar=10μm.

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