

## Karyological studies of *Parapteropyrum* and *Atraphaxis* (Polygonaceae)

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**Abstract** — Chromosomal data are important for elucidating intergeneric relationships and delimitating infrafamilial tribes in plants. In this study, we firstly reported karyological data of two genera of the Atraphaxideae in the Polygonaceae: *Parapteropyrum* and *Atraphaxis*. The former is a monotypic genus and occurs only in southwest Tibet while the latter with about 20 species is widely distributed in the dry habitats of central Asia. The chromosome number and karyotype of *P. tibeticum* are formulated as  $2n = 48 = 16m + 16sm + 16st$ , belonging to 2B type. Both *A. manshurica* and *A. bracteata* have the same chromosome number of  $2n = 22$  and similar karyotypes (22m, belonging to 1A). The basic chromosome number of another genus (*Calligonum*) of the Atraphaxideae is  $x = 9$ . Because the basic chromosome numbers of these three genera are different, suggesting that aneuploid evolution may have played an important role in the early diversification of the Atraphaxideae or this tribe is not monophyletic. The origin of the Qinghai-Tibetan Plateau endemic *Parapteropyrum* might have undergone a complex evolutionary history through aneuploid increase of the basic number and the following polyploidization.

**Key words:** Atraphaxideae, *Atraphaxis*, chromosome number, karyotype, *Parapteropyrum*

### INTRODUCTION

The Atraphaxideae of Polygonaceae comprises four genera: *Parapteropyrum*, *Pteropyrum*, *Atraphaxis*, and *Calligonum* and differs from other tribes with woody stems (GROSS 1913; LI 1981). *Parapteropyrum* is monotypic and the only species, *P. tibeticum*, occurs sparsely in the dry valleys along the Yalongzhangbo river in Tibet (LI 1981). This species is similar to *Pteropyrum*, but distinguished by the racemous inflorescences, flattened filaments and yellow anthers (BAO and LI 1993). These three characteristics were also shared by *Atraphaxis*. Both *Pteropyrum* (four species) and *Atraphaxis* (around 27 species) are disjunctly distributed in the central Asia (Pakistan, Iran and Oman), and all species of the former genus and 11 species of the latter genus were recorded in China (BAO and LI 1993). The fourth genus *Calligonum* with around 30 species occurs widely in the arid habitats of Asia, Europe and Africa, and 20 of them are recorded in China (MAO *et al.* 1983). The intergeneric relationships and systematic

position of the Atraphaxideae remain unsolved. GROSS (1913) suggested that *Calligonum* is the most primitive and *Pteropyrum* and *Atraphaxis* are closely related to each other. However, BAO and LI (1993) assumed that *Atraphaxis* diverged earliest, followed by *Parapteropyrum* and the third group comprising *Calligonum* and *Pteropyrum* originated at last. Based on the chloroplast *rbcL* sequence data, LAMB FRYE and KRON (2003) found that *Atraphaxis* nested deeply within *Polygonum* and the sampled species of a few taxonomic tribes failed to comprise as monophyletic lineages. In addition, CARLQUIST (2003) suggested that the woody habits within the Polygonaceae might have undergone repeated evolution and independently originated from the divergent herbal lineages.

Karyological data are of great importance for systematic and evolutionary studies of plants and have been widely used to elucidate inter-familial and/or generic relationships and delimitate generic or familiar circumscriptions (STEBBINS 1971; RAVE 1975; HONG 1990). Most chromosomal researches on the Polygonaceae were centered on the Polygonaceae *sensu lato*, for example, *Polygonum* (SIMMONDS 1945; MAUDE 1939), *Fagopyrum* (CHEN 1999) and *Koenigia* (HEDBERG 1997), and the Rumiceae, i.e., *Oxyria* (MAUDE 1939), *Rumex* (BALDISBERGER and WIDMER 2006) and *Rheum* (HU

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*et al.* 2007). However, within the Atraphaxideae, only *Calligonum* has been karyologically studied and the studied species were found to have the basic chromosome number of  $x = 9$  (MAO *et al.* 1983). Up to now, the chromosomal data of the other three genera in this tribe remain unknown. In this study, we explored the chromosomal characteristics of *Parapteropyrum* and *Atraphaxis* for the first time and further discussed the tribal delimitation of the Atraphaxideae and the intergeneric relationships within this tribe based on the available chromosomal and morphological data. We also summarized the origins of the Sino-Himalayan endemic genera at the chromosomal level.

### MATERIAL AND METHODS

The seeds of *P. tibeticum* were collected in the natural population in Lang county, Tibet (N 29°01.636', E 93°03.469', Alt. 3174m) while seeds

of both *A. manshurica* and *A. bracteata* from the artificial cultivation in the Minqin Desert Botanical Garden, Minqin, Gansu (N 37°27.913', E 104°59.741', Alt. 1500m). They were germinated in petri dishes lined on gauze with moist condition. The root tips were pretreated in colchicines (0.2% w/v) for 2-3 h when they grow up to a length of 0.5 cm, then fixed with Carnoy's fluid (absolute alcohol: glacial acetic acid 3: 1) at 4°C for at least 30 min. The fixed roots were hydrolysed in 1 mol/L hydrochloric Acid at 60°C for 10 min, then washed with water, stained with carbol fuchsin and squashed for observations. The metaphase chromosomes of at least 20 cells of 5 plant roots were counted. Photographs of chromosome spreads were enlarged and the homologous chromosomes were paired by their similarity in size and shape. For each chromosome pair, the short arm, long arm, and total chromosome size were calculated. The degree of karyotype asymmetry was calculated following STEBBINS (1971).

Table1 — Parameters of mitotic metaphase chromosomes of three species.

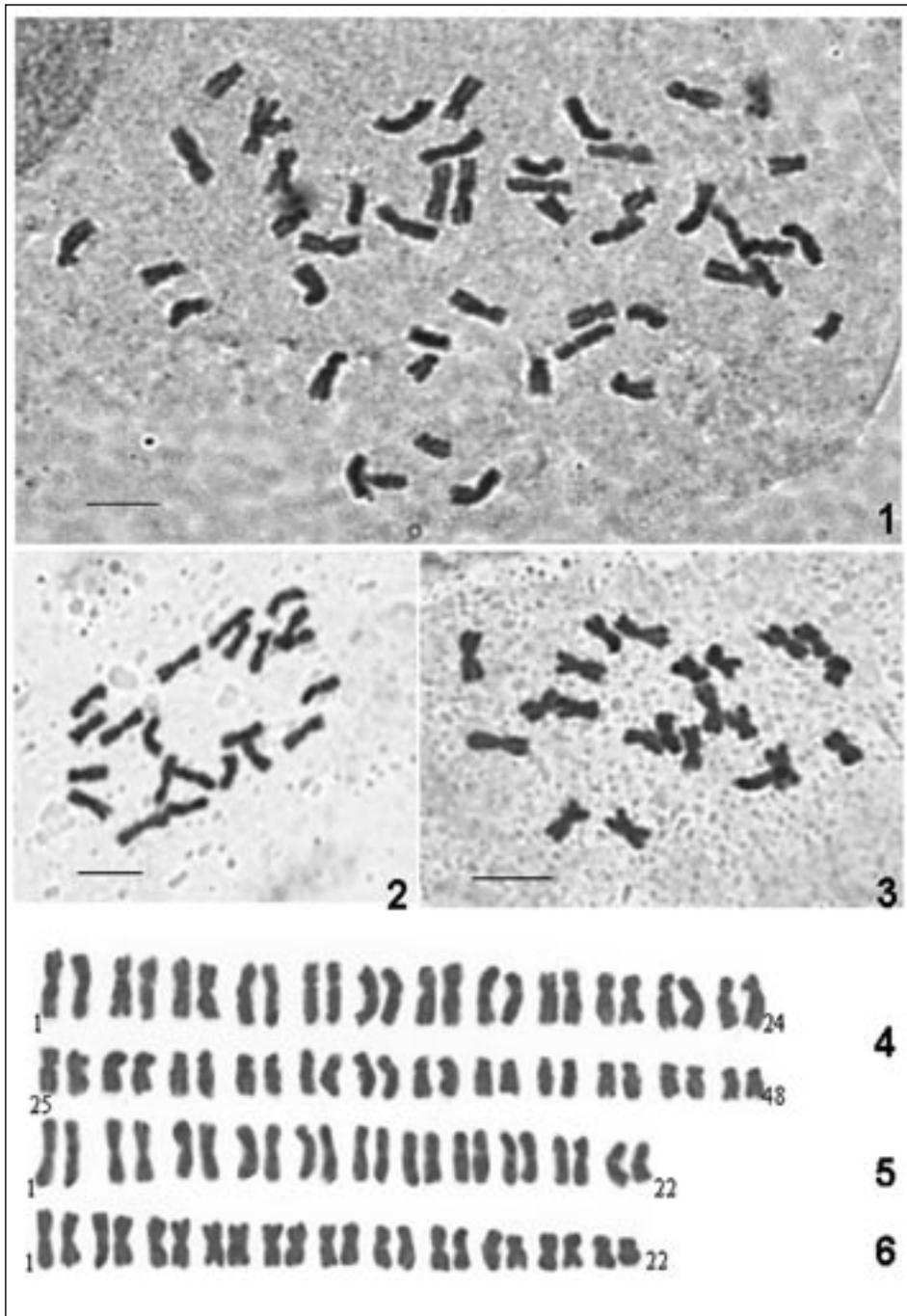
<i>Parapteropyrum tibeticum</i>								
	NO	RL	AR	T	NO	RL	AR	T
1		6.15	1.32	m	13	3.87	1.23	m
2		5.62	1.73	sm	14	3.81	1.18	m
3		5.30	1.1	m	15	3.77	3.2	st
4		5.14	1.72	sm	16	3.67	3.02	st
5		4.98	1.48	m	17	3.57	1.88	sm
6		4.93	1.26	m	18	3.57	2.22	sm
7		4.81	1.33	m	19	3.57	3.30	st
8		4.66	1.86	sm	20	3.34	4.66	st
9		4.61	1.81	sm	21	3.18	3.35	st
10		4.61	1.11	m	22	2.94	3.89	st
11		4.28	2.35	sm	23	2.66	3.08	st
12		4.28	2.74	sm	24	2.66	3.13	st
<i>Atraphaxis bracteata</i>					<i>Atraphaxis manshurica</i>			
	NO	RL	AR	T	NO	RL	AR	T
1		11.41	1.10	m	1	11.77	1.10	m
2		10.54	1.06	m	2	10.69	1.03	m
3		9.54	1.09	m	3	10.21	1.11	m
4		9.35	1.29	m	4	9.39	1.11	m
5		9.35	1.08	m	5	9.17	1.15	m
6		8.86	1.35	m	6	9.04	1.14	m
7		8.81	1.41	m	7	9.01	1.08	m
8		8.80	1.29	m	8	8.46	1.09	m
9		8.10	1.34	m	9	8.40	1.54	m
10		7.84	1.11	m	10	7.60	1.09	m
11		7.39	1.14	m	11	6.25	1.18	m

RL, relative length; AR, arm ratio L/S (length of long arm/length of short arm); m, median region; sm, submedian region; st, subterminal region.

**RESULTS**

The metaphase chromosome morphology of the studied species is shown in figs 1-6. *P. tibeticum* has a chromosome number  $2n = 48$ , and its karyotypic formula is  $2n = 48 = 16m + 16sm + 16st$  (Table 1), belonging to 2B type according

to STEBBINS (1971). Both *A. mansburica* and *A. bracteata* are diploid, with the same chromosome number  $2n = 22$  and their karyotypes are very symmetrical, consisted of metacentric chromosomes. Their karyotypes are formulated as  $2n = 22 = 22m$  (Table 2), belonging to 1A type.



Figs. 1-6 — Cytological features. Fig 1-3 Chromosomes of metaphases. Fig. 1. *Parapteropyrum tibeticum*. Fig. 2. *Atraphaxis bracteata*. Fig. 3. *A. mansburica*. Fig 4-6 Karyotypes. Fig. 4 *P. tibeticum*. Fig. 5 *A. bracteata*; Fig. 6 *A. mansburica*. Bars = 10µm.

Table 2 — The basic chromosome number of Rumiceae, Polygoneae and Atraphaxideae (Polygonaceae).

Tribe/genus	Basic number	Reference
Rumiceae		
<i>Oxyria</i>	X = 7	MAUDE 1939
<i>Rumex</i>	X = 9	BALTISBERGER & WIDMER 2006
<i>Rheum</i>	X = 11	HU <i>et al.</i> 2007
Polygoneae		
<i>Polygonum</i>	X = 10 or 11	SIMMONDS 1945; MAUDE 1939
<i>Pteroxygonum</i>	X = 10	SUN <i>et al.</i> 2008
<i>Fagopyrum</i>	X = 8	CHEN 1999
<i>Fallopia</i>	X = 11	BEERLING <i>et al.</i> 1994
<i>Koenigia</i>	X = 7	HEDBERG 1997
Atraphaxideae		
<i>Calligonum</i>	X = 9	MAO <i>et al.</i> 1983
<b><i>Atraphaxis</i></b>	<b>X = 11</b>	<b>Present research</b>
<b><i>Parapteropyrum</i></b>	<b>X = 12</b>	<b>Present research</b>

## DISCUSSION

Our results as well as the previous report (MAO *et al.* 1983) suggest that the basic chromosome numbers of three genera of the Atraphaxideae are totally different:  $x = 9$  for *Calligonum*,  $x = 11$  for *Atraphaxis* and  $x = 12$  for *Parapteropyrum* (Table 2). In addition, we find that the karyotypes are distinct between *Atraphaxis* and *Parapteropyrum*: the former has the most symmetrical karyotypes (1A) while 2B was revealed for the latter despite they share racemous inflorescences, flattened filaments and yellow anthers (BAO and LI 1993). It remains elusive whether *Parapteropyrum* is closely related to *Pteropyrum* because both chromosomal and molecular evidence are lacking for the latter genus. However, most morphological characteristics shared by them are also found in the other genera, for example, inconspicuously reticulate leaf vein and reticulate-foveolate pollen exine ornamentation (BAO and LI 1993). Only fascicled phyllotaxy is shared by them. These comparisons suggested that they may not be closely related to each other. The available chromosomal results for the Atraphaxideae have two alternative systematic implications. First, it seems likely that aneuploid variation of the basic chromosome number may have played an important role in the intergeneric divergence in this tribe. In fact, such a scenario prevails in the other tribes of the Polygonaceae: almost each of the traditionally circumscribed tribes has more than one of the basic chromosome numbers (for example, Rumiceae and Polygoneae, Table 2). Second, this tribe and the other tribes delimited based on the morphological characters are not monophyletic. This possibility is consistent with the recent molecular phylog-

eny research that the sampled *Atraphaxis* species grouped with a few *Polygonum* species with high bootstrap supports and the traditional tribes were not supported as monophyletic lineages (FRYE and KRON 2003). Under this scenario, the woody habits of all species in the Atraphaxideae may have acquired independently (CARLQUIST 2003). Because only a few genera were involved in the previous molecular researches based on *rbcL* data and the statistical supports for a few phylogenetic nodes are also weak, it is necessary to sequence more DNA fragments and more genera representing all tribes in order to construct a more convincing phylogenetic tree. The final robust molecular phylogeny undoubtedly will test which of the two alternative assumptions can account for the diverse basic chromosome numbers of the Atraphaxideae and further illustrate the evolutionary pathways of the chromosome numbers within the Polygonaceae.

The Qinghai-Tibetan Plateau (QTP), as one of the global biodiversity hotspots, is noted for production of endemic species. It is estimated that more than 25% of the totally recorded species are endemic to this region (WU 1987), and more than 40 endemic genera are found (WU 1987; WU *et al.* 1995). All of the QTP endemics were assumed to have diverged from their sympatric progenitors (WULF 1943) or from their closely related relatives in adjacent regions (WU 1987). Because of the extensive uplifts of the QTP since late Pliocene and the Quaternary climatic oscillations, allopatric speciation at the diploid level with the same basic chromosome number through repeated fragmentations is assumed to occur common in the production of the endemic alpine species in this region

(LIU 2004; NIE *et al.* 2005). However, aneuploidy and polyploidization played a minor role during the species differentiation and following speciation. Up to now, a total of eight endemic genera to the QTP and adjacent alpine regions have been karyologically studied (LIU and TIAN 2007). Most studied genera diverged with their closely related genera at the diploid level except for a few with aneuploid variations. Six endemic genera, i.e., *Sinadoxia* (Adoxaceae) (LIU *et al.* 1999), *Pomatosace* (Primulaceae) (KONG and LIU 1999), *Sinacalia* (Asteraceae) (LIU 2000), *Solms-laubachia* (Brassicaceae) (YUE *et al.* 2004), *Tibetia* (Fabaceae) (NIE *et al.* 2002) and *Soroseris* (Asteraceae) (ZHANG *et al.* 2007), have the same chromosome numbers with their closely related genera. In addition, the karyotypes between each of them and its related genera are similar, without distinct asymmetrical variation of karyotypes. However, the basic chromosome numbers of the other two endemic genera, *Lomatogoniopsis* (Genetianaceae) (LIU *et al.* 2002) and *Milula* (Liliaceae) (TANG *et al.* 2005), diverged karyologically from their closely related genera. During the aneuploid evolution, the karyotypes also showed a distinct asymmetry variation. Another monotypic endemic genus, *Przewalskia* (Solanaceae) is a polyploid species with a different basic chromosome number from its sister genus (TU *et al.* 2005). The origin of *Parapteropyrum* might be similar to that of *Przewalskia*, through aneuploid variation of the basic number and the following polyploidization because this basic chromosome number was not recovered for the other genus of the Polygonaceae. The polyploids have higher fitness than the diploids and are commonly found in the arid habitats (GRANT 1981; SOLTIS *et al.* 2003; CUI *et al.* 2008). Overall, these data together suggest that the origins of the QTP endemic genera are diverse at the chromosomal evolutions. In addition, these endemic genera originated *in situ* or *ex situ* rather than uniformly locally differentiated (LIU and TIAN 2007). Molecular calibrations of a few endemic genera also suggested that these endemic genera diverged from their sister groups during the different timescales, from 20 to less than 2 millions of years ago (LIU and TIAN 2007). The origins of more endemic genera occurring in the QTP need to be explored from both karyological and molecular approaches.

**Acknowledgements** — This research was supported by National Natural Science Foundation of China (30725004) and the Program for New Century Excellent Talents, Ministry of Education of China (NCET-05-0886).

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Received 17<sup>th</sup> August 2008; accepted 11<sup>th</sup> August 2009