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ORIGINAL ARTICLE

Distribution of basic diploid and polyploid species of *Isoetes* in East Asia

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ABSTRACT

Aims *Isoetes* L. is an ancient, primitive genus of heterosporous lycopsids characterized by a strongly reduced plant. The genus occupies a unique position in plant evolution as the closest relatives of the famous tree lycopods. Hybridization and allopolyploid speciation as well as habitat preference are regarded as having played an important role in the evolution of the genus *Isoetes*. However, it is still uncertain what factors have impacted upon hybridization and allopolyploid speciation of the genus *Isoetes*. Our understanding of the origin, evolution and modern distribution pattern of *Isoetes* in East Asia would benefit from investigations into the relationship between the unique geographical location and topographic features and the existing fossil record in the region.

Methods The chromosome number was determined from known 81 populations of 10 species of *Isoetes* in East Asia. The altitude was obtained in location of every population. *T*-test was used to evaluate the altitude difference between diploid and polyploid populations. Data of Japanese *Isoetes* from Takamiya *et al*. was used in the study.

Results There are 10 known species of *Isoetes* with four diploids and six polyploids in East Asia. The four diploid species ($2n = 22$) are found at high altitudes (mean altitude $= 2649.67 \pm 1396.16$ m, $n = 18$) and isolated from each other. In contrast, the six polyploid species widely occur in low altitude regions (mean altitude $= 182.62 \pm 181.44$ m, $n = 63$) and are partially sympatric. Highly significant differences ($P < 0.001$) were observed between the mean altitude of locations of populations of diploids and polyploids.

Main conclusions The polyploidy speciations of *Isoetes* in East Asia might originate and develop from Holocene (Quaternary). The present pattern of distribution of *Isoetes* in East Asia is the result of allopatric speciation derived from vicariance, dispersal and combinations of both, and significantly correlative with the geological history and geographical changes. The change of altitude might have played an important role in allopolyploid speciation and the pattern of distribution of the genus *Isoetes* in East Asia by exerting a considerable influence on dispersal and hybridization opportunities of *Isoetes* species.

Keywords

***Isoetes*, East Asia, altitude, dispersal and vicariance, hybridization and allopolyploidy, chromosome, origin and evolution.**

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INTRODUCTION

Isoetes L. is a small, cosmopolitan genus of heterosporous lycopsids with an estimated 150 extant species (Taylor & Hickey, 1992). The genus is recognized as the only remaining living representative of ancient taxa which were characterized by a strongly reduced plant body and occupies a unique position in plant evolution as the closest relatives of the famous tree lycopods (Takhtajan, 1956; Pigg, 1992, 2001).

Due to a series of habitat adaptations that have resulted in simplicity of form, morphological convergence and allopolyploidy, the determination of *Isoetes* species and species

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relationships has always been difficult (Hickey *et al.*, 1989; Taylor & Hickey, 1992). In recent years, these difficulties have somewhat been reduced, owing largely to chromosome counts that distinguish diploids from polyploids (Taylor *et al.*, 1993). The evidence from chromosome numbers and other cytological data promises to be of considerable significance in understanding the evolution of *Isoetes* species (Hickey, 1984; Hickey *et al.*, 1989; Taylor & Hickey, 1992).

Chromosome counts have been reported for many *Isoetes* species. The basic chromosome number of the genus *Isoetes* is x = 11 in most species (Löve *et al.*, 1977; Kott & Britton, 1980; Hickey, 1984), except x = 10 for *I. histrix* Dur. (Manton, 1950) and x = 12 for *I. pantii* Goswami & Arya and *I. muricata* Dur. (Bhu & Goswami, 1990). A polyploid series ranges from $2n = 22$ to $2n = 132$.

Of the 10 species of *Isoetes* reported from East Asia, four occur in China, six in Japan and four in Korea. *Isoetes hypsophila* Hand.-Mazz., *I. yunguiensis* Wang & Taylor and *I. sinensis* Palmer occur on the Chinese Mainland and *I. taiwanensis* De Vol occurs on the Taiwan Island of China (De Vol, 1972a,b; Liu *et al.*, 2002; Wang *et al.*, 2002). The Japanese species are *I. sinensis* Palmer, *I. japonica* A. Br. and *I. asiatica* (Makino) Makino (Takamiya *et al.*, 1994; Iwatsuki, 1995). Recently, according to different cytotypes, Takamiya *et al.* (1997) and Takamiya (1999, 2001) recognized *I. sinensis* Palmer as two taxa I. *sinensis* var. *sinensis* ($2n = 44$) and *I. sinensis* var. *coreana* ($2\ n = 66$), and *I. japonica* A. Br. as three taxa *I. japonica* A. Br. ($2\ n = 66$), *I.* × *michinokuana* M. Takamiya, M. Watanabe & K. Ono ($2n = 77$) and *I. pseudojaponica* M. Takamiya, M. Watanabe & K. Ono ($2\ n = 88$). In addition, *I. asiatica* (Makino) Makino, *I. japonica* A. Br., *I. sinensis* var. *sinensis* and *I. sinensis* var. *coreana* are reported from Korea (Takamiya *et al.*, 1997; Takamiya, 2001). Published studies on *Isoetes* in Mainland China are very limited. However, recently, the genus has received considerable attention from researchers. Wang *et al.* (2002) and Liu *et al.* (2002) reported chromosome counts for the three *Isoetes* species on Mainland China as well as information on their distribution and habitat characteristics. A new basic diploid species endemic to China *I. yunguiensis* Wang & Taylor was described by Wang *et al.* (2002) from material which previously had been erroneously identified as the polyploid species *I. japonica* A. Br. Chromosome counts of all 10 known *Isoetes* species in East Asia have revealed four diploids and six polyploids (Takamiya *et al.*, 1997; Liu *et al.*, 2002; Wang *et al.*, 2002). However, origins of the diploid species and their relationships with the polyploid species are still unclear.

This paper employs biogeography to discuss the geographical distribution pattern of *Isoetes* species and its causes in East Asia. This knowledge will be useful in understanding the origin and evolution of the genus in East Asia.

MATERIALS AND METHODS

Chromosome counts of extant populations of the four known *Isoetes* species in China were made. The counts were made in several metaphase cells from 12 individuals in each extant population. Root tips were prepared for observation of somatic chromosomes following the methods of Takamiya *et al.* (1994). A strong correlation exists between spore size and chromosome number in *Isoetes* (Cox & Hickey, 1984; Britton & Goltz, 1991; Small & Hickey, 2001). It was found that mean spore size in each species of *Isoetes*, even for dried herbarium material, generally reflected the ploidy level (Kott & Britton, 1983). Cytotype in *Isoetes* could therefore be reliably determined using the morphological characteristics of mega- and microspores from herbarium specimens (Watanabe *et al.*, 1996; Small & Hickey, 2001). Distribution maps for each taxon were reconstructed using information obtained from both dried herbarium specimens and live material (Takamiya *et al.*, 1997). Based on comparative analysis of data on spore surface morphology, spore size, habitats and geographical distribution obtained from both field and cultivated populations and from records on herbarium labels of voucher specimens, identifications and chromosome numbers of presently extirpated populations were deduced.

Classification of the materials followed Takamiya *et al.* (1997), Liu *et al.* (2002), Wang *et al.* (2002) and Huang *et al.* (1992). Terminology for megaspore surface morphology followed Lellinger & Taylor (1997), and Hickey (1986a).

The altitudes of locations of *Isoetes* species populations in China were obtained using an Ever Trust altimeter (Tokyo Compass MFG. Co. LTD, Tokyo, Japan). Population means were worked out using Microsoft Excel 2000. A *T*-test was used to evaluate the differences in altitude between the populations. The level of significance was set at $P < 0.05$.

Data on chromosome number and altitude of locations of populations of *Isoetes* species in Japan from Takamiya *et al.* (1996, 1997) were used in the study.

RESULTS

Chromosome numbers, spore characteristics and elevations of localities of *Isoetes* species in East Asia are summarized in Tables 1–3. The basic chromosome number of the 10 known species of *Isoetes* from East Asia is x = 11. A chromosome count of $2n = 22$ was made for *Isoetes taiwanensis* DeVol and is consistent with previous reports (De Vol, 1972a,b; Liu *et al.*, 2002). *Isoetes sinensis* Palmer from China has a chromosome count of $2n = 44$. *Isoetes yunguiensis* Wang & Taylor from the Yunnan-Guizhou Plateau in China which had previously been identified as *I. japonica* A. Br. has been shown to have a chromosome number of $2n = 22$. *Isoetes hypsophyla* has a chromosome number of $2n = 22$.

The geographical distribution of ploidy level and mean elevation of occurrence for each *Isoetes* species are presented in Figs 1 and 2. In view of their small number and the unavailability of information on the altitude at their locations, *Isoetes* populations from Korea were not included. The four basic diploid species are distributed in regions of comparatively higher elevation (mean altitude = 2649.67 ± 1396.16 m, $n = 18$). In contrast, the six polyploid species occur at low

Table 1 Materials of extant *Isoetes* from China used for the present study

Species	Population code	Vouchers and collector or Presenter	Location	Altitude (m)	Habitat	Megaspore surface morphology	Microspore surface morphology	Chromosome count 2*n*
I. yunguiensis	PB1	WH 2001166. Liu *et al.*, 2001; HGAS 75043. Wang, 1975	Pingba, Guizhou	1280	Valley swamp	Reticulate	Levigate	22
I. sinensis	XN1	WH 20011023027. Liu *et al.*, 2001; NSA 2405. 1959	Xiuning, Anhui	360	Abandoned rice field	Cristate	Echinate	44
	JD1	WH 20011019006. Liu *et al.*, 2001	Jiande, Zhejiang	134	Freshwater intertidal zone	Cristate	Echinate	44
	JD2	WH 20011919007. Liu *et al.*, 2001	Jiande, Zhejiang,	134	Freshwater intertidal zone	Cristate	Echinate	44
	GL1	Xue, Y.G., 2002	Guilin, Guangxi	150	Stream	Cristate	Echinate	44
I. hypsophila	DC1	WH 2001141. Liu *et al.*, 2001	Daocheng, Sichuan	4400	Pond in riverside meadow	Levigate	Perforate	22
	DC2	WH2001137. Liu *et al.*, 2001	Daocheng, Sichuan	4330	Pond in riverside meadow	Levigate	Perforate	22
	DC3	WH2001138. Liu *et al.*, 2001	Daocheng, Sichuan	4320	Pond in riverside meadow	Levigate	Perforate	22
	DC4	WH2001139. Liu *et al.*, 2001	Daocheng, Sichuan	4220	Pond in riverside meadow	Levigate	Perforate	22
	DC5	WH2001140. Liu *et al.*, 2001	Daocheng, Sichuan	4050	Pond in riverside meadow	Levigate	Perforate	22
	ZD1	WH20030808001. Liu *et al.*, 2003	Zhongdian, Yunnan	3300	Pond in meadow	Levigate	Perforate	22
I. taiwanensis	TW1	DeVol, 1972; Huang	Taipei, Taiwan	1100	Shallow pond	Tuberculate	Echinate	22

elevation (mean altitude = 182.619 ± 181.44 m, $n = 63$). The difference in mean altitude of localities between diploid and polyploid species is highly significant ($P < 0.001$).

DISCUSSION

Hybridization is widespread in the genus *Isoetes*, and allopolyploid speciation has been regarded as an important factor in the evolution of the genus (Hickey *et al.*, 1989; Taylor & Hickey, 1992; Small & Hickey, 2001). However, widespread interspecific hybridization and allopolyploid speciation may have resulted in morphological convergence in *Isoetes*, which makes it difficult to identify species and species relationships based on morphology alone (Hickey, 1986a; Taylor & Hickey, 1992; Hoot & Taylor, 2001). Hybridization is likely a common source of megaspore variation, in particular, as a source of intermediate spore morphologies (Hickey, 1986a). *Isoetes* hybrids are virtually always found in association with their putative parents and generally characterized by intermediate megaspore morphology between the parental species (Taylor & Luebke, 1988; Taylor & Hickey, 1992; Britton & Brunton, 1995; Musselman *et al.*, 1995; Brunton & Britton, 1999; Small & Hickey, 2001). Megaspore characters are useful for delimiting natural species alliances in concert with other vegetative features and habitat preference (Hickey, 1986a,b). Hickey (1986a) proposed megaspore terminology for *Isoetes* describing 12 texture types. On the basis of this, Lellinger & Taylor (1997) published a system of terminology for classification of ornamentation in the pteridophyta. Although the number and evolutionary relationships of spore ornamentation patterns in basic diploids are not clear, derived intermediate spore ornamentation patterns in polyploids could have evolved through interspecific hybridization and allopolyploidy. The evolutionary history of *Isoetes* polyploids probably involves a combination of diverse ancestry, multiple origins, divergence and gene silencing (Caplen & Werth, 2000). Therefore, the relationship between spore ornamentation types and chromosome number might be helpful in understanding the evolution of *Isoetes* species.

The parental species of the *Isoetes* polyploids in East Asia could not be conclusively confirmed, but features of these polyploids, including megaspore and microspore textures, chromosome counts, meiotic behaviour and habitats, indicate an origin of hybridization and possible close relationships with some of the diploids (Tables 1–3; Takamiya *et al.*, 1994, 1996, 1997; Takamiya, 1999, 2001; Liu *et al.*, 2002; Wang *et al.*, 2002). A hypothesis that the tetraploid *I. sinensis* with cristate megaspore was derived through hybridization between diploid *I. yunguiensis* with reticulate megaspore and diploid *I. taiwanensis* with tuberculate megaspore is supported by data from *LEAFY*, ITS, chromosome counts and morphological studies (W.C. Taylor, Q.F. Wang, X. Liu, N. Napier, S.B. Hoot & A.R. Lekschas, unpubl. data). The cristate megaspore pattern, which is one of the intermediate spore morphologies, could possibly have been formed by hybridization not only between reticulate-spored species and echinate-spored species

Table 2 Materials of extinct *Isoetes* from China used for the present study

Species	Population code	Vouchers and collector or Presenter	Location	Altitude (m)	Habitat	Megaspore surface morphology	Microspore surface morphology	Estimated Chromosome number 2 *n*
I. yunguiensis	KM1	KUN 0002883. Qiu, 1957. Kunmin Institute of Botany, 2002	Heilongtang, Kunming, Yunnan	2000	Artificial pond	Reticulate	Levigate	22
	KM2	KUN 0002885. Qiu, 1957	Songhuaba, Kunming, Yunnan	2000	Reservoir	Reticulate	Levigate	22
	KM3	KUN 0002888. Li, 1981	Xiaoshao, Kunming, Yunnan	2000	Rice-field by stream	Reticulate	Levigate	22
	KM4	KUN 0002886. Qiu, 1981; Wang *et a*l., 2002	Shuangshao, Kunming, Yunnan	2160	Rice field by stream	Reticulate	Levigate	22
	XD1	KUN 65680. Shong, 1980	Tianshengqiao, Xundian, Yunnan	2080	Rice field by stream	Reticulate	Levigate	22
	GY1	HGAS 7906. Wang, 1990	Guiyang, Guizhou	1060	Valley swamp	Reticulate	Levigate	22
I. sinensis	NJ1	NAS 00072025. Steward, 1922; Palmer, 1927	Xuanwuhu, Nanjing, Jiangsu	100	Eutrophied lake	Cristate	Echinate	44
	GL2	IBK 30688. Fang, 1952	Yanshan, Guilin, Guangxi	180	Rice field by stream	Cristate	Echinate	44
	DX1	Chen, 1998	Anjiashan, Dongxiang, Jiangxi	49	Artificial pond	Cristate	Echinate	44
	HZ1	ZJFC 0188	Jiuxi, Hangzhou, Zhejiang	10	Artificial pond	Cristate	Echinate	44
	TX1	Dao, 1990	Tunxi, Huangshan, Anhui	180	Polluted river	–	–	44
	JD3	Dao, 1990	Meicheng, Jiande, Zhejiang	117	Artificial pond	–	–	44
	ZJ1	Dao, 1990	Wuxie, Zhuji, Zhejiang	260	Reservoir	–	–	44
	TT1	Ding, 2001; Liu, *et al.*, 2002	Huading, Tiantai, Zhejiang	820	Artificial pond	Cristate	Echinate	44
I. hypsophila	LJ1	Handel-Mazetti., 1923	Lijiang, Yunnan	3600	Swamp	Reticulate	Perforate	22
	DC6	CDBI, 322-1. Li	Xinyichuo, Daocheng, Sichuang	4300	Plateau Lake	Reticulate	Perforate	22

Table 3 Summary of chromosome counts, habitat and megaspore surface morphology of *Isoetes* in East Asia

Species*	No. of populations	Ploidy	Altitude (m) (mean ± SD)	Habitat	Distribution	Megaspore surface morphology	Microspore surface morphology
I. hypsophila	8†	2x	4605 ± 401.50	Marsh or pond	Southeastern Tibetan Plateau of China	Levigate	Perforate
I. yunguiensis	7‡	2x	1797.14 ± 437.02	Pond or stream	Yunnan-Guizhou Plateau of China	Reticulate	Levigate
I. taiwanensis	1	2x	1100 ± 0.00	Pond	Taiwan of China	Tuberculate	Levigate
I. asiatica	2	2x	747 ± 233	Lake or pond	Northern Honshu and Hokkaido of Japan and Korea	Echinate	Levigate
I. sinensis	12§	4x	207.83 ± 213.34	River, marsh or pond	South-eastern China	Cristate	Echinate
I. sinensis var. *sinesis*	5	4x	140 ± 131.30	River, marsh	Kyushu and Chubu District of Japan and Korea	Cristate	Echinate
I. sinesis var. coreana	10¶	6x	180 ± 198.12	River, marsh	Honshu, Shikoku and Kyushu District of Japan and Korea	Cristate	Echinate
I. japonica	22**	6x	228.23 ± 212.90	River, marsh or Pond	Honshu and Shikoku of Japan and Korea	Reticulate	Levigate
I. × *michinokuana*	3	7x	60 ± 0.00	Marsh	Miyagi Prefecture of Japan	Reticulate	Echinate
I. pseudojaponca	11††	8x	137.27 ± 117.06	Marsh	Honshu and Shikoku of Japan	Reticulate	Echinate
Totals	81						

*Following Tables 1 and 2, Huang *et al.* (1992), Takamiya *et al.* (1996, 1997, Liu *et al.* (2002) and Wang *et al.* (2002).

†Includes two extinct populations.

‡Includes six extinct populations.

§Includes eight extinct population.

¶Includes two populations with $2n = 65$ and one population with $2n = 68$.

**Includes one population $2n = 67$.

††Includes one population $2n = 87$.

(Taylor *et al.*, 1985; Hickey, 1986b) but also between reticulate-spored species and tuberculate-spored species. Likewise, tetraploid *I. sinensis* var. *sinensis* could have been formed by the same hybridization and chromosome doubling events as *I. sinensis*. Observation that the hexaploid *I. japonica* consistently formed 33 bivalents at diakinesis and in metaphase I in both micro- and megaspore mother cells led Takamiya *et al.* (1996) to conclude that the hexaploid *I. japonica* could have arisen from chromosome doubling in an as yet unknown triploid hybrid formed between a tetraploid (AABB) and a diploid (CC). On the basis of similarities in the reticulate and levigate microspore characteristics and habitats of *I. yunguiensis* and the hexaploid *I. japonica* Liu *et al.* (2002) and Wang *et al.* (2002) suggested that the former species could be the putative parent of the latter species. Recently the analysis of nucleotide sequences has shown that *I. taiwanensis* has the closest phylogenetic relationship with *I. japonica* (Hoot & Taylor, 2001). The diploid *I. asiatica* is characterized by echinate megaspores and levigate microspores. *I. japonica* occurs in the region between the ranges of *I. sinensis* var. *sinensis* and *I.asiatica*. Its range overlaps with those of *I. sinensis* var. *sinensis* and *I. asiatica* (Fig. 1; Takamiya *et al.*, 1997, Figs 31, 32, 35). On the basis of the foregoing observations, we suggest that *I. japonica* is an allopolyploid derived through hybridization between the tetraploid *I. sinensis* var. *sinensis* and the diploid *I. asiatica*. We do not concur with Takamiya *et al.* (1994, 1997) in proposing that *I. asiatica* is probably not the ancestral diploid of the polyploids occurring in Japan. The two hexaploid *I. sinensis* var. *corean* and *I. japonica* both form 33 bivalents during metaphase (Takamiya *et al.*, 1996). Both the hexaploid *I. sinensis* var. *corean* and the tetraploid *I. sinensis* var. *sinensis* are characterized by cristate megaspores and echinate microspores and are almost indistinguishable except for the variation in their mean microspore length. In Japan the geographical range of *I. sinensis* var. *corean* partly overlaps with that of *I. sinensis* var. *sinensis* in Kyushu District and in *I. sinensis* var. *corean* one locality in Miyazaki Prefecture both varieties were found occurring together (Fig. 1; Takamiya *et al.*, 1997, Figs 35, 36). *Isoetes sinensis* var. *sinensis* could well be the ancestral species of *I. sinensis* var. *corean*, however, its own diploid ancestry is uncertain. Studies of meiotic behaviours suggested that the octaploid condition arose from chromosome doubling in a tetrapoild hybrid between a tetraploid (AABB) and a tetraploid (DDEE), and the heptaploid condition is derived through hybridization between a hexaploid (AABBCC) and an octaploid (AABBDDEE) (Takamiya *et al.*, 1996). The origin of the tetraploid and hexaploid conditions is still uncertain and calls for further research. However, it is established that hybridization and allopolyploidy are significant speciation mechanisms in the polyploid *Isoetes* species in East Asia.

Hagerup (1932) suggested that polyploids are more tolerant of extreme ecological environments than their diploid relatives. In general, there is a higher proportion of polyploids in high altitude and high latitude regions than in low altitude and low latitude regions (Stebbins, 1950). The increasing polyploid frequency is thought to have resulted from a selective advantage of heterozygous polyploids in unstable environments (Stebbins, 1950), but it has been shown that both diploid and polyploid species can adapt to a broad range of ecological conditions (Stebbins, 1971).

The basic diploid species of *Isoetes* in East Asia occur at high elevation while the polyploid species occur at low elevation. In addition, the polyploid species have wider geographical distributions and a higher frequency of occurrence within their distributional ranges than the diploids. It would therefore appear that in East Asian species of *Isoetes*, diploids might have a greater tolerance for environmental extremes than the polyploids, but that the polyploids could be more competitive within their geographical ranges.

Dispersal of *Isoetes* spores is often accomplished via floating leaves (Small & Hickey, 1997). A change in topography could split a continuous water system into isolated systems and thus limit gene flow among populations. Higher elevation means spore leaves moving in rivers from high altitude to low altitude. Consequently, spores from populations isolated in high altitude regions could come together in low altitude regions through the downstream movement of floating leaves. A survey indicates that young plants of *Isoetes* in China are swept away from the headstream on mountains to lower reaches of a river by floods. Therefore, greater opportunity for hybridization and allopolyploid speciation may exist in low altitude regions. It has been suggested that migrating waterfowl could have had an impact on the dispersal of spores (Taylor & Hickey, 1992). However, if this were the case, a regular distribution of species along the migration line should exist. This has, however, not been found on Mainland China.

The evolution of the isoetalean lycopsids spans much of the history of vascular plants, from Late (or possibly Middle) Devonian to the modern-day genus *Isoetes* (Pigg, 1992, 2001). The Triassic was suggested as a time of important radiation and change in several key morphological characters prior to the appearance of the modern-day, reduced *Isoetes* forms (Pigg, 2001). Two kinds of isoetalean lycopsids widely prevailed in the Triassic, the *Pleuromeia*-type and the *Annalepis*-type, the latter including a plexus of loosely related genera (Pigg, 1992). Comparative studies using new macromorphological and ultrastructural data suggest that both genera are interconnected and closed related to *Isoetes* and that *Annalepis* is more probably ancestral to *Isoetes*, via *Isoetitles* (Grauvogel-Stamm & Lugardon, 2001).

Specimens of *Pleuromeia* and *Annalepis* have been found from the Middle Triassic Series in the Yangtze River valley of South China and described (Meng, 1998, 1999; Meng *et al.*, 2000). The comparison between *Annalepis* and *Isoetes* indicates that *Annalepis* is similar to the extant *Isoetes* in many aspects, such as the herb with a corm, more or less tufted sporophylls, presence of a ligule on the sporophyll, monolete ray microspore of *Aratrisporites*-type and trilete rays, etc. Meng (1998) and Meng *et al.* (2000) noticed the similarities of the features of the two genera, and suggested that *Annalepis*, especially

A. brevicystis Meng with its long-narrow sporophylls and sporangia all situated at the base of sporophylls, might be the ancestor of modern-day *Isoetes*.

In general, *Isoetes* species are characterized by geographically restricted ranges and by a great many locally derived endemics, and their patterns of distribution, at least at the species group level, have their basis in vicariant biogeography (Hickey, 1986a; Taylor & Hickey, 1992). A combination of ITS and *atpB-rbcL* spacer data was used to evaluate relationship on a global scale and three major well-supported clades were recognized: an Old-World/California clade, an Asian clade and a North American clade (Hoot & Taylor, 2001). These results, coupled with the extensive distribution of the *Annalepis*-type fossils from Lower Triassic of Russia and Australia, Middle Triassic of China, Germany and France, Upper Triassic of USA, Lower Cretaceous of Southern Tunisia and Portugal and Tertiary of North America (Grauvogel-Stamm & Lugardon, 2001), suggest that vicariance is a more likely explanation for the current disjunct distribution of *Isoetes* species.

Isoetes taiwanensis has previously been suggested as having the closest affinity to the diploid *I. kirkii* A. Ar. from New Zealand (Britton & Brunton, 1991). Recent studies based on ITS data indicate that *I. hypsophila* is a basal in the Asian clade and that a clade consisting of *I. japonica*, *I. sinensis*, *I. yunguiensis* and *I. taiwanensis* is sister to a clade containing *I. kirkii* and *I. brevicula* (Hoot & Taylor, 2001; Taylor *et al.*, unpubl. data). Taiwan Island and Japan did not divide from Mainland China until Pliocene. That is relatively recently on the geological time-scale. The origins of their flora have a discernible relationship to each other (Zeng, 1994; Ming & Zhang, 1996). These results suggest a strong affinity between at least some East Asian and Australian *Isoetes* species. Likewise, it is possible that the *I. hypsophila*, *I. yunguiensis* and *I. taiwanensis* originated from *Annalepis* in the Yangtze River valley of South China and that *I. kirkii* originated from *cylostrobus* and *skilliostrobus* in Australia via allopatric speciation. *Isoetes asiatica* which is distributed in Kamchatka, Sakhalin, the Kuriles and Japan, is regarded as a member of the *I. echinospora* Durieu species complex (Löve, 1962). The inclusion of two Californian species, *I. nutallii* and *I. orcuttii*, within the well-supported Old-World/California clade suggests that this group of *Isoetes* may have previously had a distribution throughout Asia and into North America (Hoot & Taylor, 2001). The time and space pattern of the fossils distribution of *Tomiostrobus* from Lower Triassic of East Siberia and South Siberia of Russia and *Lepacyclotes* from Upper Triassic of USA indicate simultaneously a dispersal direction throughout Asia into North America. Therefore, it is probable that the two diploid species originated via allopatric speciation. *Isoetes asiatica* might have been derived from a dispersal of *Tomiostrobus* from East-South Siberia of Russia to Kamchatka, Sakhalin, the Kuriles and Japan. *Isoetes echinospora* might be a result of another dispersal of *Tomiostrobus* from East-South Siberia of Russia to North America. Likewise, *I. asiatica* might be a key intermediate species between Asia and North America species and more ancient than *I. echinospora*. East Asia is probably an intersection of the *Annalepis*-type from Lower Triassic Russia (*Tomiostrobus*) in the north-eastern coast of Tethys and Lower Triassic Austinia (*Cylostrobus* and *Skilloistrobus*) in the south-east coast of Tethys.

Vicariance patterns are often obscured by dispersal but hybrid zones may indicate overlapping species areas (Wolf *et al.*, 2001). If the putative diploid or low chromosome type ancestry of a particular allopolyploid are now found in regions remote from each other, the inference if they could hybridize to form the allopolyploid it must be that in some former period in the earth's history they had a different geographical distribution and occurred together (Stebbins, 1950). Therefore, the origin of diploid *Isoetes* species is a result of vicariance, however, the hybridization and allopolyploid speciation should occur in the overlapping areas where the diploids or low chromosome type ancestry could come across by dispersal.

The present pattern of distribution of *Isoetes* in East Asia is the result of allopatric (geographical) speciation derived from vicariance and dispersal and combinations of both factors. However, area cladistics indicates that vicariance, dispersal and allopatric speciation occur in response to geological changes either directly, by geographical boundaries, or indirectly, at the level of ocean currents (Ebach & Humphries, 2002). The modern distribution pattern of *Isoetes* in East Asia must therefore be in response to the geological history and geographical changes.

From the distribution pattern in time and space of the *Annalepis*-type fossils (Grauvogel-Stamm & Lugardon, 2001), coupled with the cladogram of extant *Isoetes* (Hoot & Taylor, 2001; Taylor *et al.*, unpubl. data), we are able to infer the dispersal direction and the evolutionary series of the *Annalepis*-type. The *Annalepis*-type at first originated simultaneously from East-South Siberia of Russia (*Tomiostrobus*) and Austinia (*Cylostrobus* and *Skilloistrobus*) in Lower Triassic. Then they might have migrated along the northern and southern coast of Tethys, and spread to China (*Annalepis*), Germany (*Annalepis*) and France (*Annalepis*) of the northern coast of Tethys in Middle Triassic. The cladogram (Hoot & Taylor, 2001; Taylor *et al.*, unpubl. data), suggesting a strong affinity between at least some East Asian and Australian *Isoetes* species, lends more support to the hypothesis that the *Annalepis* from Middle Triassic of China might derive from a dispersal of the *Annalepis*-type from Lower Triassic of Australia from south to north. Likewise, the Old-World/California clade suggesting a distribution throughout Asia to North America (Hoot & Taylor, 2001) supports the inference that the *Lepacyclotes* from Upper Triassic of USA might derive from a dispersal of *Tomiostrobus* from Lower Triassic of Russia from west to east. This could account for the origin of North American *Isoetes*. The evolutionary series '*Annalepis–Isoetites–Isoetes*' is very likely (Grauvogel-Stamm & Lugardon, 2001). The Old-World/California clade also indicates a strong affinity between at least some North African and European species (Hoot & Taylor, 2001). Therefore, the *Isoetites* from Lower Cretaceous of Portugal of the northern and southern coast of Tethys and

Southern Tunisia might derive from *Annalepis* occurring in Germany and France in Middle Triassic. The *Isoetites* from Tertiary of North America might have a strong affinity with the *Isoetites* from Lower Cretaceous of Portugal and Southern Tunisia. This should be another mode of origin for North American *Isoetes*. The analysis presented above supports the hypothesis that there are at least two origins of North American species of *Isoetes* (Hoot & Taylor, 2001). The lack of variation and the rapid radiation in the North America *Isoetes* species complex (Hoot & Taylor, 2001) indicate that North America is probably the best area to study the most recent evolution trends in *Isoetes*. Likewise, Russia and Australia might be the two ancient centres of origin of the modern-day *Isoetes*. On all accounts, the origin and evolution of extant *Isoetes* should have a significant correlation with the Tethys.

The Tethys retreat and Himalayas-Hengduanshan Mountains uplift (Qinghai-Tibet Plateau) not only had an important influence on the natural environment and climate of Asia (Coleman & Hodges, 1995; Zheng & Li, 1999; An *et al.*, 2001), but also affected significantly the origin and development of the East Asian flora (Sun, 2002a,b). The Qinghai-Tibet Plateau used to be a section of the Ancient Tethys Sea and began to uplift about 50 Ma (Eocene of Lower Tertiary) (Harrison *et al.*, 1992; An *et al.*, 2001). Tethys disappeared in the Qinghai-Tibet area due to the collision and combination of the Indian plate with the Eurasian plate at *c.* 40 Ma (Eocene of Lower Tertiary), and then the Qinghai-Tibet Plateau started stages of continental palaeo-geographical evolution (Harrison *et al.*, 1992; Coleman & Hodges, 1995; Zheng & Li, 1999; An *et al.*, 2001). As a result of the large-scale intense uplift of Qinghai-Tibet Plateau, the Chinese stepped land features were formed in three steps (Fig. 1; Zhang *et al.*, 2000). The first step is the Qinghai-Tibet Plateau with an altitude of >5200–4000 m. In the north of Qinghai-Tibet Plateau and the east of Hengduanshan Mountain is the second step consisting mainly of the Yunnan-Guizhou Plateau, the Loess Plateau, the Mongolia Plateau, the Sichuan Basin, the Tarim Basin and the Junggar Basin which rapidly descends to an altitude of 2000–1000 m. The third step includes the Northeast Plain, the North China Plain, the middle and lower Yangtze River plains, the south-east hill and the littoral plain with an altitude <1000–50 m in the east of Daxinganling, Tahang Mountain, Wu Mountain and the east side of Yunnan-Guizhou Plateau. The Yangtze and Yellow Rivers flow eastwards to the sea following the gradient of the Chinese stepped land features.

According to the distribution characteristics of the horizon-bearing *Annalepis* from east to west in the Yangtze River valley (Figs 1 and 2; Meng *et al.*, 2000), the *Annalepis*

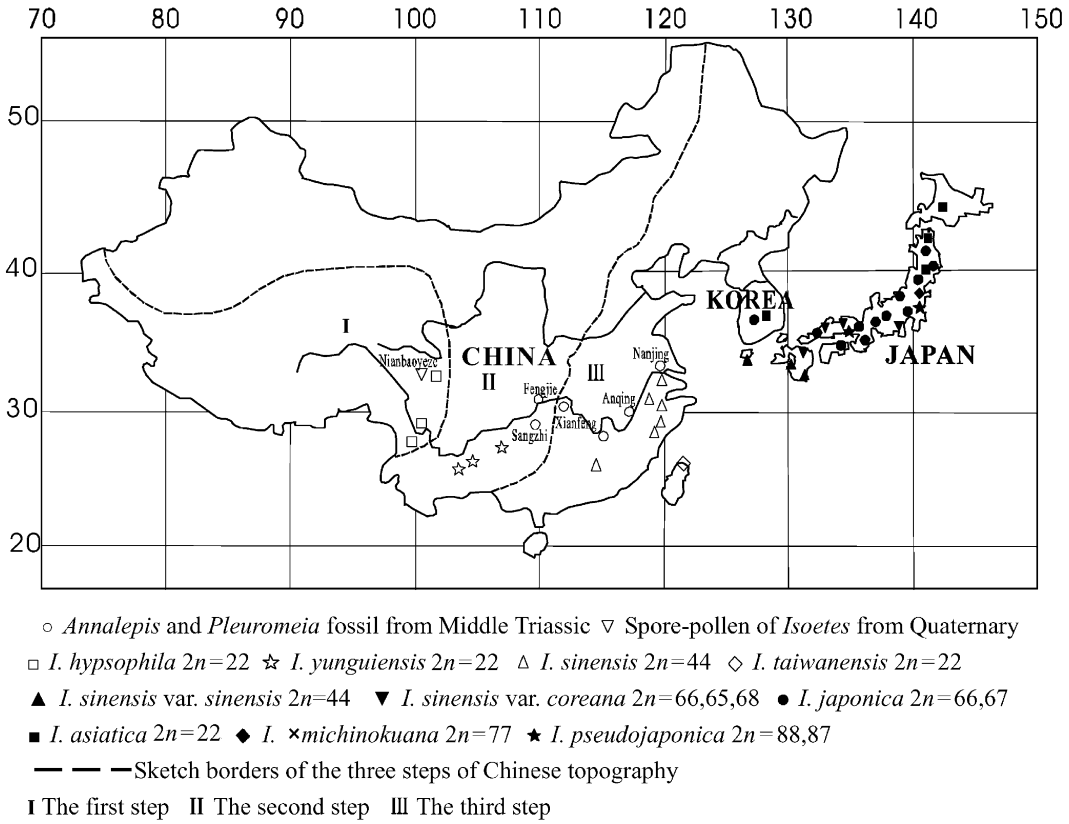

Figure 1 Distribution pattern of *Isoetes* species in East Asia (Data cited from Huang *et al.*, 1992; Takamiya *et al.*, 1997; Meng *et al.*, 2000; Liu *et al.*, 2002 and Wang *et al.*, 2002).

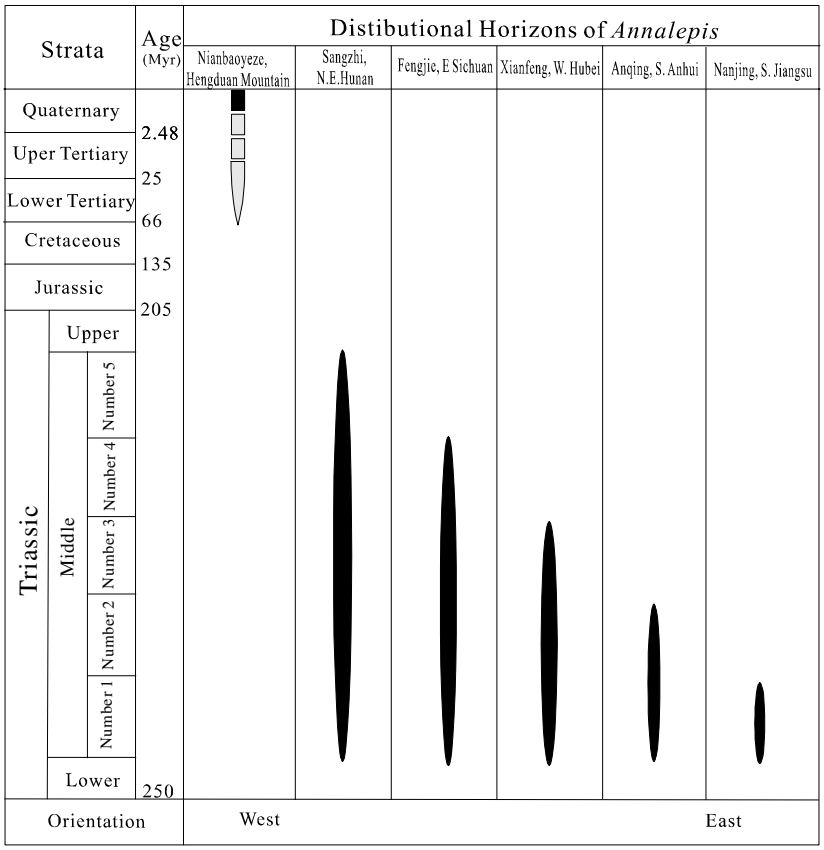

Figure 2 The distribution pattern of time and space of *Annalepis* in the Yangtze River valley (Data cited from Bai *et al.*, 1994 and Meng *et al.*, 2000).

appeared almost simultaneous in the Yangtze River valley during early Anisian (early Middle Triassic), but its distribution shrunk gradually from east to west during middle Anisian–Ladinian (middle-late Middle Triassic), in response to the marine regression that occurred from east to west on the Yangtze Platform during that period (Figs 1 and 2; Meng, 1998; Meng *et al.*, 2000). An analysis of spore-pollen from a peat section from the Nianbaoyeze indicates that *Isoetes* occurred on Hengduanshan Mountains in the Holocene (Quaternary) (Figs 1 and 2; Bai *et al.*, 1994). However, the altitude in this region reached as high as 3000 m in the Quaternary period (Zhang *et al.*, 2000) and dispersal by floating leaves is impossible from low to the high elevation. The *Annalepis* had appeared in Germany and France of Northwestern coast of Tethys in Middle Triassic and the *Isoetites* had occurred in Portugal of Northwestern coast of Tethys and Southern Tunisia of Southwestern coast of Tethys in Lower Cretaceous. *Isoetes* species in China display a space order of the distribution pattern *I. hypsophila–I. yunguiensis–I. taiwanensis–I. sinensis* from high altitude to low altitude and from west to east (Figs 1 and 4). The cladogram (Hoot & Taylor, 2001; Taylor *et al.*, unpubl. data) in which *I. hypsophila* is basal in the Asian clade, displays a similar time order of origin. Consequently, the ancestors of *Isoetes* could have reached the Qinghai-Tibet region before the beginning of the uplift of Qinghai-Tibet Plateau (Eocene of Lower Tertiary) in response to the retreat of Tethys and the change of Chinese geomorphology from east to west (Figs 1 and 2). These ancestral forms migrated from the high altitude to the low altitude areas during the generation of the stepped Chinese landscape via water flow from west to east (Figs 1 and 3). The polyploidy speciations of *Isoetes* in East Asia might originate and develop from Holocene (Quaternary), therefore, the retreat of Tethys and uplift of the Qinghai-Tibet Plateau are to a large extent responsible for the modern distribution pattern of *Isoetes* in East Asia.

Based on the analysis presented above, the causes for the modern distribution pattern of *Isoetes* in East Asia are inferred as follows: (1) ancestral basic diploids which originated in low altitude regions and which spread to high altitude regions (for example the Qinghai-Tibet Plateau and the Yunnan-Guizhou Plateau) before the land was uplifted; (2) ancestral basic diploids in high altitude regions being dispersed to low altitude regions through water systems after the land was uplifted; (3) hybridization and allopolyploid speciation occurring only in low altitude regions; (4) polyploids exhibiting greater adaptability than diploids when the two cytotypes occur sympatrically; (5) diploids are a relict in high altitude regions and were outcompeted in low altitude regions by polyploids which were generated through hybridization of diploids; and (6) Each diploid species has evolved independently subsequent to altitude shifts.

With nearly 60% of the known *Isoetes* species being polyploid, allopolyploidy may be a significant speciation

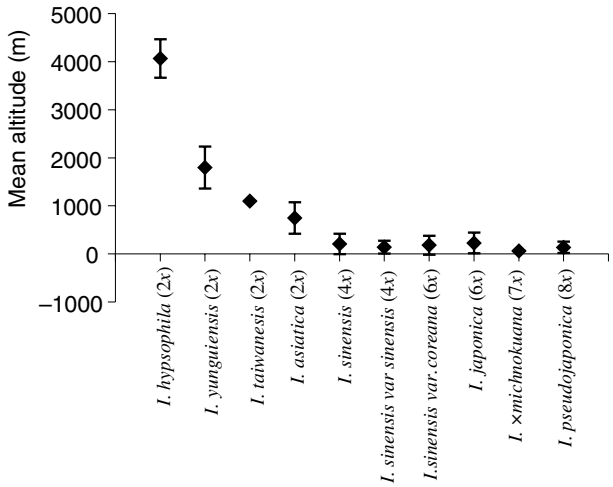

Figure 3 Mean altitude of distribution locations of every *Isoetes* species in East Asia.

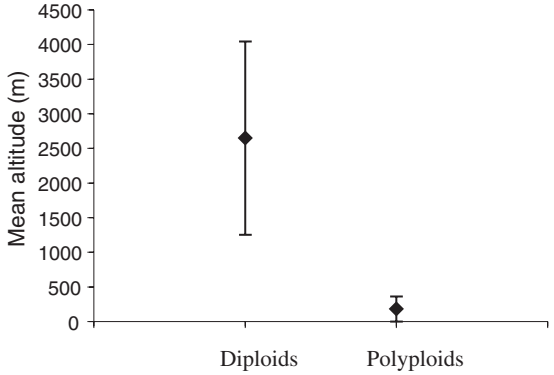

Figure 4 Mean altitude of distribution locations of diploids and polyploids of *Isoetes* species in East Asia.

mechanism in genus *Isoetes*, and it has been suggested that habitat preference has played a major role in the evolution of *Isoetes* (Hickey, 1986a; Taylor & Hickey, 1992; Takamiya *et al.*, 1996). The extant aquatic species can be characterized as being highly polyploid, in contrast to terrestrial taxa which are generally diploid (Taylor & Hickey, 1992). Terrestrial habitats result from changes in topography as well as elevation of land. Chromosome doubling either in the zygote or in the apical meristem and the formation of an unreduced spore from microspore and megaspore mother cells of a hybrid seem to be two of the possible mechanisms of polyploidy in *Isoetes* (Takamiya *et al.*, 1996). The present study suggests that the upland and lowland aquatic habitats could have had an important impact on dispersal and hybridization opportunities of *Isoetes* species. However, further research is required into the process of how diploids form allopolyploids through interspecific hybridization and chromosome doubling under the influence of geographical and geological changes.

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