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Distribution and threat status of the cytotypes of *Pteris vittata* L. (Pteridaceae) species complex in India

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In India, *Pteris vittata* L. is very common and widely distributed. This fern is reportedly a 'species complex' and includes five cytotypes, viz. diploid, triploid, tetraploid, pentaploid and hexaploid with the basic number being 29 chromosomes. A survey of the literature indicated that some of the cytotypes were reported and collected only once or twice and never thereafter. In order to determine the current availability of these cytotypes, several collection excursions were undertaken, and a number of plants were collected from all over India and maintained in the fernery at National Botanical Research Institute (NBRI), Lucknow, India. In the absence of clear morphological characters specific to any cytotype, only the chromosome analysis of these plants confirmed their ploidy status. It was observed that only the tetraploid form was collected from different places. The hexaploid first reported in South India was not traceable during subsequent collection trips to this region. Similarly, in none of our collection trips did we come across any diploid form. In case of the triploid and pentaploid cytotypes, the only specimen available are those maintained in the NBRI fernery ever since their first report. The cytological abnormalities and reproductive failures along with other factors are probably responsible for the extinction of the cytotypes in their natural habitats. Thus in India, only the tetraploid cytotype is abundant while diploid and hexaploid are probably extinct. The triploid and pentaploid cytotypes, represented only by the limited specimens maintained at NBRI fernery, must also be considered as extinct in natural habitats.

Keywords: Brake fern, cytotypes, polyploidy, *Pteris vittata*, species complex.

THE common fern *Pteris* L., an almost exclusively tropical genus with 330 species and 3 hybrids, grows in both xeric and moist shady habitats as terrestrial perennials[1]. In India, about 45 species of *Pteris* have been reported, however, no comprehensive survey and analysis of diversity and biosystematics of the genus have been reported[2]. The Brake fern or *Pteris vittata* L. is a very common and widely distributed species occurring at higher altitudes up to 1600 m. The fern is also highly valued as an ornamental. It has recently been reported that *P. vittata* can accumu-

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late extremely large concentrations (up to 23,000 mg kg^{-1}) of arsenic in its above ground biomass[3]. This has given considerable importance to this fern species as a potential arsenic phytoremediator as well as for cleaning up groundwater arsenic contamination[4]. The fern is actually reported as a 'species complex' in India and includes five cytotypes, viz. diploid, triploid, tetraploid, pentaploid and hexaploid with the basic number being 29 chromosomes[5].

The pteridophytes, amongst the oldest existing land plants, include the largest proportion of polyploid species[6,7]. These have probably used polyploidy to a great advantage for the success not only in long survival from more than 360 million years ago till date, but also for their global distribution. Polyploidy helps the pteridophytes to overcome not only the negative effects of inbreeding due to isolation of populations but also the buffering effects for variations, and this in turn helps in successful colonization by the plants[8,9]. Several mechanisms, including somatic doubling, gametic non-reduction and triploid bridges, have been identified as the methods for generation of the polyploids, yet the polyploids are not necessarily generated by the same mechanism in all plants[7,10].

Polyploidy, a dominant characteristic of the pteridophytes, accounts for about 50–60% of the cytotypes in most of the large pteridophyte families and in fact, as many as 95% of all ferns and fern allies are estimated to be polyploid[11,12]. In case of the genus *Pteris* L. also, a study of cytology and breeding structure of as many as 82 species showed that apomixis and polyploidy were common[13]. Polyploidization has also apparently played an important role in the distribution of *P. vittata*, and other cytotypes have been reported in different regions of India. The diploid cytotype was first reported (Table 1) from Bhujiaghat (Nainital) and Kodaikanal and Kothayar hills. Elsewhere in the world, diploid has been reported only from a few regions of China (Table 1). Triploid and pentaploid cytotypes were reported only from Lucknow as hybrids and while these are currently maintained at the National Botanical Research Institute (NBRI) fernery, these have never again been reported from natural distribution and habitats in India. Similarly, the hexaploid, though reported by two groups within a span of 15 years from South India (Table 1), is also rare since there is no report of a hexaploid from anywhere in the world since 1977 when it was last reported[14]. Only the tetraploid has been reported (Table 1) from a large number of regions in India and elsewhere in the world. We need to carry out a detailed survey of occurrence and distribution of the cytotypes in this species complex in India and assess their biodiversity and biosystematic status. In this communication, we confirm the distribution and present day abundance of *P. vittata* L. based on our collection excursions spread over a period of six years from 2000 to 2006.

A number of plants were collected from all over India (Table 2) and many of these are maintained in the NBRI fernery. The different polyploid cytotypes with the exception of perhaps the diploid cytotype do not differ from each other in obvious morphological characters, so that it is difficult to identify the ploidy types in natural habitats simply by a visual examination. Only the chromosome analysis enables clear identification of the ploidy status. Therefore, for most of the collected specimens, chromosome analysis was carried out to determine their ploidy status. Additionally, we also recorded observations of obvious cytological irregularities, if any. For the chromosome studies young sporophylls were fixed in 3 : 1 (v/v) absolute alcohol and acetic acid (Carnoy's fixative) for at

Table 1. Different cytotypes of *Pteris vittata* and the regions or locales where reported to occur

Ploidy	Locations/habitat in India	Reference	Locations/habitat in other countries	Reference
Diploid	Nainital (recorded as forma *brevipinna*)	26	Sichuan, Yunnan, Guizhou, Hubei and Hunan, China	22
	Kodaikanal, Upper Kothayar, Tirunelveli	27		
Triploid*	Lucknow	5	NR	
Tetraploid	Mussoorie, Dehradun, Amritsar Botanical and Company Gardens, Meerut Gandhi Garden	28	Ceylon (Sri Lanka)	29
	Western Himalayas	30	Ghana, Cameroons, West Africa	11
	Mahabaleshwar, Lonavala, Khandala	31	Philippines, New Guinea	11
	Lucknow	5	New Caledonia	32
	Kanyakumari, Kothayar	33	Florida, USA	34
	Tanakpur, Haldwani, Nainital, Ramnagar, Lohaghat, Pithoragarh, Almora, Ranikhet, Dharchula, Munsiyari, Nagling	35	Taiwan	36, 37
			Kathmandu Valley, Nepal	11, 38
			Guangdong, Guangzhou, Yunnan, Hainen, China	22
			Japan	39
			Agat, Guam. (Introduced species)	40
Pentaploid*	Lucknow	5	NR	
Hexaploid*	Shevaroy Hills, Kollapully	19, 12	NR	

NR, Not reported in any other country. *Irregular meiosis, abnormal spores and other morphological peculiarities have been reported in these cytotypes.

Table 2. Summary of different collection excursions and plants collected during 2000–05. Almost all plants collected were wild and the cultivated or semi-wild collections are indicated in the footnote

Date of collection (month, year)	Locale/habitat (State)	Voucher number[a]	Live plants at NBRI fernery	Ploidy
September 2000	Kainchee Nainital, Uttarkhand	54252	Yes	Tetraploid
November 2000	Almora, Uttarkhand	221690		Tetraploid
August 2001	Gurana, Pithoragarh, Uttarkhand	222581	Yes	Tetraploid
May 2002	Bindsar, Almora, Uttarkhand	223207		Tetraploid
February 2003	Kolkata, West Bengal[b]		Yes	Tetraploid
March 2003	Lohali, Pithoragarh, Uttarkhand	223252, 223254		Tetraploid
March 2003	Niglar, Pithoragarh, Uttarkhand	223254		Tetraploid
June 2003	Coimbatore, Tamil Nadu[b]		Yes	Tetraploid
September 2003	Gorakhpur, Uttar Pradesh		Yes	Tetraploid
May 2004	NEHU Shillong, Meghalaya[b]		Yes	Tetraploid
May 2004	Cherrapunji, Meghalaya		Yes	Tetraploid
May 2004	BSI Itanagar, Arunancahal Pradesh[b]		DNS	Tetraploid
May 2004	Shillong Peak, Meghalaya		Yes	Tetraploid
May 2004	Andheri Falls, Pakyong Road, Gangtok, Sikkim		DNS	Tetraploid
August 2004	RFRS Pune, Maharashtra[b]		DNS	Tetraploid
August 2004	Lonavala, Maharashtra		DNS	Tetraploid
September 2004	Calicut, Kerala		Yes	Tetraploid
September 2004	Palayamkottai, Tirunelveli, Tamil Nadu[b]		Yes	Tetraploid
November 2004	Tippajharia, Sonebhadra, Uttar Pradesh	223337		Tetraploid
November 2004	Shaktinagar, Sonebhadra, Uttar Pradesh	223332, 206679		Tetraploid
November 2004	Shaktinagar (Jwalamukhi), Sonebhadra, Uttar Pradesh		Yes	Tetraploid
November 2004	Karahiyanala, Sonebhadra, Uttar Pradesh	223330		Tetraploid
November 2004	Enroute Tippajharia, Sonebhadra, Uttar Pradesh	223334		Tetraploid
November 2004	Ballia nala, Sonebhadra, Uttar Pradesh	223335		Tetraploid
February 2005	Keonjhar, Orissa		Yes	Tetraploid
April 2005	Tezpur, Jorhat, Guwahati, Assam	227842, 227843	Yes	Tetraploid
October 2005	Modern College, Pune, Maharashtra[b]		Yes	Tetraploid
October 2005	Kempty Falls, Mussoorie, Uttarkhand		Yes	Tetraploid
February 2006	Anpara, Uttar Pradesh		Yes	Tetraploid
OLD	NBRI Fern House, Lucknow, Uttar Pradesh[b]	9 different plants, provenance not known or recorded	Yes	Pentaploid
OLD	NBRI Fern House, Lucknow, Uttar Pradesh[b]	1 plant, provenance not known or recorded	Yes	Triploid

[a]Voucher numbers are for those specimens with a Herbarium voucher deposited in NBRI Herbarium (LWG).
[b]These plants are semi-wild in the sense that they are cultivated at the location where collections were made, but their provenance is unknown.
DNS, Live plants were collected and brought back to NBRI fernery. However, these did not survive beyond a month or two.
OLD, These are old plants, maintained at the NBRI fernery for more than two decades. Their provenance and details of original collection are not known or recorded.

least 24 h. The fixed sporophylls were squashed in 2% (w/v) acetocarmine solution for the study of meiotic chromosomes[15]. We observed that only the tetraploid form is found in abundance almost all over the natural distribution range, while the hexaploid first reported in South India was not traceable during our collection trips to this region. Similarly, in none of our collection trips did we come across any diploid form and as for the triploid and pentaploid cytotypes, the only specimen available are those maintained in the NBRI fernery ever since their first report[3,16]. In fact it was reported earlier also that except for tetraploid, diploid and hexaploid were not collected again even in the previously reported habitats[17]. Similarly, though they have also reported the triploid and pentaploid for the first time, since then, no other report of collection of these two cytotypes in natural habitats is available. It is possible that the early reports of different cytotypes could be a result of serendipitous observations. However, the fact that other than tetraploid, triploid and pentaploid plants do exist, it does appear that the different polyploids must occur in nature, however, these were not collected again subsequent to the first reports.

Thus, the present survey is a confirmation of the earlier observations of distribution of the cytotypes, as well as the non-availability of the other polyploids than tetraploids, in natural habitats. These surveys taken together indicate that at least four cytotypes, namely diploid, triploid, pentaploid and hexaploid are threatened and must now be considered as extinct in their native habitats and only the tetraploids have continued to proliferate in abundance. These observations also indicate the robustness

and reproductive success that this cytotype (tetraploid) has, as a result of which it has been able to perhaps colonize over widespread areas, including urban and cosmopolitan habitats. We have collected *P. vittata* from several additional localities such as Thiruvananthapuram in Kerala, Chennai in Tamil Nadu, Mahabaleshwar in Maharashtra and a few others than those originally reported (Tables 1 and 2), and all collected plants were tetraploid, thereby clearly showing the wide distribution of this cytotype.

In case of triploid, pentaploid and hexaploid, morphological as well as cytological irregularities have been reported[3,17–20]. The diploid cytotype is also an enigma since its first reported occurrence in India was in 1961 and thereafter, it has never been collected or reported again. In fact till 1990 (Verma, pers. commun.)[21], even the original plant from which the diploid cytotype, identified as *P. vittata* L. forma *brevipinna* (*P. vittata* L. subsp. *Vermae* Fras-Jenk.) was not found again anywhere including at the original site of collection from Bhujiaghat, Nainital, Uttarakhand. Moreover, even the Chinese diploid and tetraploid cytotypes[22] are probably the same as the Indo-Himalayan types reported[21], though there is no further report about the current status of the different cytotypes in China. The diploid is not likely to have a problem of any cytological irregularity, but in comparison to the tetraploid perhaps it was less robust and so may have become extinct. Here it is also possible to consider a mechanism similar to that in case of *Adiantum pedatum* L., where incipient polyploid speciation has been reported[23]. According to this mechanism unreduced spores in diploid sporophytes result in diploid gametophytes from where either tetraploids are produced by the intragametophytic selfing or triploids are produced by the intergametophyte crossing or extinction of the gametophytes occurs if neither of the above two modes of crossing can take place. Thus invoking such a mechanism for *P. vittata* diploids it is possible that, all diploids that did not give rise to higher polyploids may have finally become extinct. Such irregularities will have resulted in drastic losses in population strengths in nature due to reproductive failures. It is well known that small and isolated populations of species are more vulnerable to demographic, environmental and genetic flux as a result of which, these are at the greatest risk of extinction in that region[24]. This situation has recently been shown for *Isoetes sinensis* Palmer, where four different cytotypes have restricted distribution in China and Japan[25]. Losses in population sizes of cytotypes can thus accelerate the extinction and this could have happened in *P. vittata* too. Triploid and pentaploid cytotypes are maintained in the NBRI fernery for nearly two decades and are thriving well there. This indicates that in favourable natural habitats these cytotypes could still survive despite having several irregularities. However, the rapid urbanization of large areas (including forest areas) and human activities mean that it will be a great challenge to set up protected and favourable habitats for these different cytotypes to survive and proliferate. Failing this, besides the diploid and hexaploid that must now be considered as extinct in India, even the triploid and pentaploid will meet the same fate once the NBRI fernery populations dwindle. Furthermore, a more comprehensive survey in other locales, especially those with similar climate and natural conditions to that of localities where diploid and hexaploid cytotypes were reported from earlier, may perhaps result in the discovery of additional surviving populations of these cytotypes. At the same time, efforts must also be made to disseminate triploid and pentaploid types to their natural habitats by increasing the populations in the NBRI fernery either through natural or *in vitro* cultures.

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Assessment of land subsidence phenomenon in Kolkata city, India using satellite-based D-InSAR technique

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In this study, one of the latest space-based techniques for measuring sub-centimetric ground displacement, Differential Synthetic Aperture Radar Interferometry (D-InSAR) has been used to assess the potential land subsidence phenomenon of Kolkata city, India. The occurrence of a thick surface clay layer with an average thickness of ~40 m and above, raises a question on the land subsidence phenomenon in Kolkata city. At this juncture, the D-InSAR-based study will help to ascertain the actual land subsidence scenario of Kolkata city. We have identified an area in Kolkata city in and around Machhua Bazar, Calcutta University and Raja Bazar Science College, which had been undergoing subsidence during the period of observation, i.e. 1992–98 with an estimated rate of 5 to 6.5 mm/yr.

Keywords: D-InSAR, land subsidence, Kolkata city.

PRESENTLY, Differential Synthetic Aperture Radar Interferometry (D-InSAR) has been found to be an efficient technique for measuring land subsidence in a number of studies[1–6]. D-InSAR is used for two broad purposes: (i) identification of land subsidence phenomenon in an area, and (ii) quantitative analysis and modelling of deformation phenomenon with particular emphasis on precision of measurements. In the latter case, the D-InSAR technique should be accompanied by precise GPS monitoring and/or ground-based levelling. In this study, an attempt has been made primarily to identify the subsiding areas in Kolkata city, India (Figure 1), and the approximate rate of subsidence during 1992–98. In Kolkata city, due to over-drafting of groundwater under confined aquifer condition, potential land subsidence phenomenon has been reported by a number of workers[7,8] and by the local media. In confined aquifer condition, over-extraction of groundwater causes lowering of piezometric pressure, which results in the development of tensional forces in overlying confining layer material. Consequently, compaction of overlying confining layer material takes place and land

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