CYTOLOGICAL OBSERVATIONS ON FERN GENUS *Pteris* IN THE BOGOR BOTANIC GARDENS

Observasi sitologi pada paku genus *Pteris* di Kebun Raya Bogor

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Abstract

Pengamatan sitologi dan kromosom somatik dilakukan pada 23 individu dari enam jenis tumbuhan paku genus *Pteris* (Pteridaceae) yang tumbuh meliar di Kebun Raya Bogor. Hasil pengamatan menunjukkan bahwa jumlah kromosom dasar pada keenam jenis *Pteris* yang diamati konsisten, yaitu x = 29. Ras diploid dengan 2n = 58 ditemukan pada *P. biaurita, P. fauriei* dan *P. tripartita*. Ras triploid dengan 2n = 87 terdapat pada *P. fauriei* dan *P. multifida.* Sedangkan ras tetraploid dengan 2n = 116 ditemukan pada *P. ensiformis, P. tripartita,* dan *P. vittata*. Informasi tentang *P. tripartita* ras diploid dan *P. multifida* ras triploid dilaporkan pertama kali untuk kawasan Malesia. Sedangkan informasi kromosom *P. fauriei, P. multifida,* dan *P. vittata* merupakan catatan sitologi baru untuk Pulau Jawa. Jenis-jenis yang memiliki lebih dari satu sitotipe diamati morfologinya dan dibahas secara singkat.

Key words: cytology, *Pteris biaurita, P. ensiformis, P. fauriei, P. multifida, P. tripartita, P. vittata*

INTRODUCTION

Pteris is a large fern genus, consisting of about 300 species in the world (Copeland, 1947). Hassler & Swale (2001) recorded 330 species and 3 hybrids. It is distributed in all tropical and subtropical countries, and is endemic in every continent except Antartica. A few of *Pteris* species extend into the warm temperate regions of the world. It is a genus of diverse ecology. Most of the species occur in primary forests, but frequently also in secondary forests, in opening or along rocky stream banks (Copeland, 1947).

Taxonomically, *Pteris* is a complex group. Many species of the genus are interfertile and hybridization often occurs (Walker, 1962). Hybrids are often becoming stabilized as species through the occurrence of apogamy. These, to some extent, result in the difficulty of recognizing hybrids and in satisfying the species concept as well as species distinction within the genus (Tryon & Kramer, 1990). In a few cases hybrid swarms are even known. Polyploidy also plays a part (Walker 1962), and races with different ploidy levels may occupy different areas (Nakato, 1981). Cytologically, *Pteris* is one of the best known ferns genera due to the studies

of Walker (1958, 1960, 1962) on species over a large geographic range. Polyploidy, apogamy and hybridization are evidently widespread system in *Pteris* and have an important role in speciation. The basic chromosome numbers with x = 29 range from diploid (2n = 2x = 58) to octoploid (2n = 8x = 232). Lovis (1977) reported that 85 species observed cytologically showed ploidy levels as follows: 2, 3, 4, 6, 8 and 10. Diploid, triploid, and tetraploid races encompass apomictic forms. About 55% of cytological data recorded are polyploid with apogamy of about one third (Tryon & Tryon, 1982).

Bogor Botanic Gardens is an artificial forest having a "natural ecosystem" which enables various wild plants species, including *Pteris*, to grow and develop. Six species of wild *Pteris* are found growing naturally in its habitat in Bogor Botanic Gardens. Chromosome survey on wild ferns in Bogor Botanic Gerdens firstly conducted by Praptosuwiryo and Darnaedi (1995) indicated that the diversity of *Pteris* in this ecosystem is not only limited in species level, but also in ploidy level. Three species observed, *P. tripartita*, *P. ensiformis*, and *P. biaurita*, showed different ploidy levels. The first two species were tetraploid, while the last one was diploid.

The aim of this observation is to observe the occurrence of ploidy levels within species of *Pteris* for further analysis to examine the genetic diversity of the wild species of *Pteris* growing naturally in Bogor Botanic Gardens. The information gathered may be of useful contribution to enhanche the understanding of genetic diversity, polyploidy mechanism and biology of ferns.

MATERIALS AND METHOD

Chromosomal observation was conducted in a Biosystematic Laboratory of Herbarium Bogoriense. Voucher specimens were housed in the herbarium of Bogor Botanic Gardens. Materials used in this study were wild species of *Pteris* growing naturally in Bogor Botanic Gardens.

Bogor Botanic Gardens, which was formerly known as *s'Lands Plantetuin te Buitenzorg* and played an important role in the introduction of economically important plant species to Indonesia, is displaying Indonesian floral diversity. The Gardens is specified for conserving species from wet, lowland habitats. It is located at the altitude of about 260 m above sea level with humidity ranges from 80 – 90 %, temperature 25 – 28 °C and average annual rainfall 3,000 – 4,000 mm.

Cytological investigations were carried out on 23 individuals of six species of *Pteris,* with one to 12 individuals for each species (Table 1), by observing the somatic chromosomes of the root tips following a procedure developed by Manton (1950) and modified by Darnaedi (1992). Root tips were pretreated with 0.002 M 8-hydroxyquinolin for 4 – 6 hrs at 18 – 20 °C, and then fixed with 45 % acetic acid for 10 minutes. The fixed roots were macerated with a mixture of 45 % acetic acid and 1 N HCl (1 : 3) for 3 – 4 minutes at 60 °C. They were squashed in a 2 % aceto-orcein stain solution. Chromosomes counting were carried out under a light microscope (Nikon AFX-IIA) with 1000x magnification, and photographs were taken using Nikon Camera (Nikon FX-35WA).

Reproduction type was recognized by spore counting for each sporangium as described by Manton (1950). Under a binocular microscope, a fresh mature sporangium was broken gently on an objective glass, covered with a covering glass and the spores were then counted. Minimally, ten sporangia for every individual were observed. Plants with 64 normal spores in each sporangium were treated as sexual race, while those with 32 spores per sporangium were considered as apogamous races.

RESULTS

Cytological records on six wild species of *Pteris* in Bogor Botanic Gardens are presented in Table 1 and Figure 1. All the species observed had basic chromosome number x = 29. One individual of *P. ensiformis* in *Vak* (block) XVB showed tetraploid type with 2n = 116. The same situation was also observed in *P. vittata* that was found in *Vak* XIVA (Fig 2).

Four diploid individuals were found in *P. biaurita* (Fig. 1a). Two individuals of *P. biaurita* examined, WF14042003-1 from *Vak* XL and Pb-1 from *Vak* XIVA (Fig.2.), had different morphological appearances. The

first individual, which had apogamous race with 32 spores in each sporangium, appeared bigger than the other. Unfortunately, we were fail to examine the second individual, whether it was sexual or apogamy, because the plant was still in an infertile condition. The appearance of the second individual was significantly different in size and texture of both laminas and stipes. The apomic one had double the size of the second individual with coarse and glossy lamina.

Pteris fauriei was found to have two ploidy levels, diploid and triploid. Diploid type was found growing in "Mexico Garden" (*Vak* IIA), while the triploid relatives were in *Vak* XVA. The distance between the two cytotypes was less than 1 km. Unfortunately, we did not able to make further observation into the morphological differences of the two cytotypes, because the triploid type were still very young and they were died before developing into mature sporophytes due to dry season.

Table 1. Somatic Chromosome, ploidy level and type of reproduction of wild *Pteris* in Bogor Botanic Gardens.

No	Species	Somatic Chromosomes (2n)	Ploidy level	Types of Reproduction (*)	Vouchers Specimens & Localities
1.	*Pteris biaurita* L.	58	Diploid	-	TNgP s.n. (April 2004-1), *Vak* XIVA
		58	Diploid	Apogamous	TNgP s.n. (14 April 2003-1), *Vak* XL
		58	Diploid	-	TNgP s.n. (7 April 2004-1, *Vak* XIVA
		58	Diploid	Apogamous	TNgP s.n. (7 April 2004-2), *Vak* XIVA
2.	*P. ensiformis* N.L. Burm.	116	Tetraploid	-	TNgP s.n. (2003), *Vak* XVB
3.	*P. fauriei* Hieron	58	Diploid	-	TNgP s.n. (May 2003)-1, *Vak* XVA
		87	Triploid	Apogamous	TNgP s.n. (26 May 2003), *Vak* IIA
4.	*P. multifida* Poir	87	Triploid	Apogamous	Pm-1, *Vak* XIVA
		116	Tetraploid	-	WF30052003-1, *Vak* XIIIA
		116	Tetraploid	Sexual	WF28052003-1, *Vak* XIIIA
5.	*P. tripartita* Sw.	58	Diploid	-	Pt-1, *Vak* XIVA
		58	Diploid	Sexual	Pt-2, *Vak* XIVA
		58	Diploid	-	Pt-3, *Vak* XIVA
		58	Diploid	-	TNgP1003, XIVA
		58	Diploid	-	WF09042003-1, Nursery
		58	Diploid	-	WF14042003-1, Nursery
		116	Tetraploid	-	WF03042003-1, *Vak* XIVA
		116	Tetraploid	Sexual	FW15052003,
		116	Tetraploid	-	WF13052003, *Vak* XL
		58	Diploid	-	WF21052003, *Vak* XL
		58	Diploid	-	WF03052003-1, *Vak* XL
		58	Diploid	-	WF03062003-1, *Vak* XL
6.	*P. vittata* L.	116	Tetraploid	-	Pv-1, *Vak* XIVA

Note: (*) Type of reproduction: Plant with 32 normal spores in each sporangium was considered as apogamous form, whereas those with 64 normal spores per sporangium was treated as sexual form.

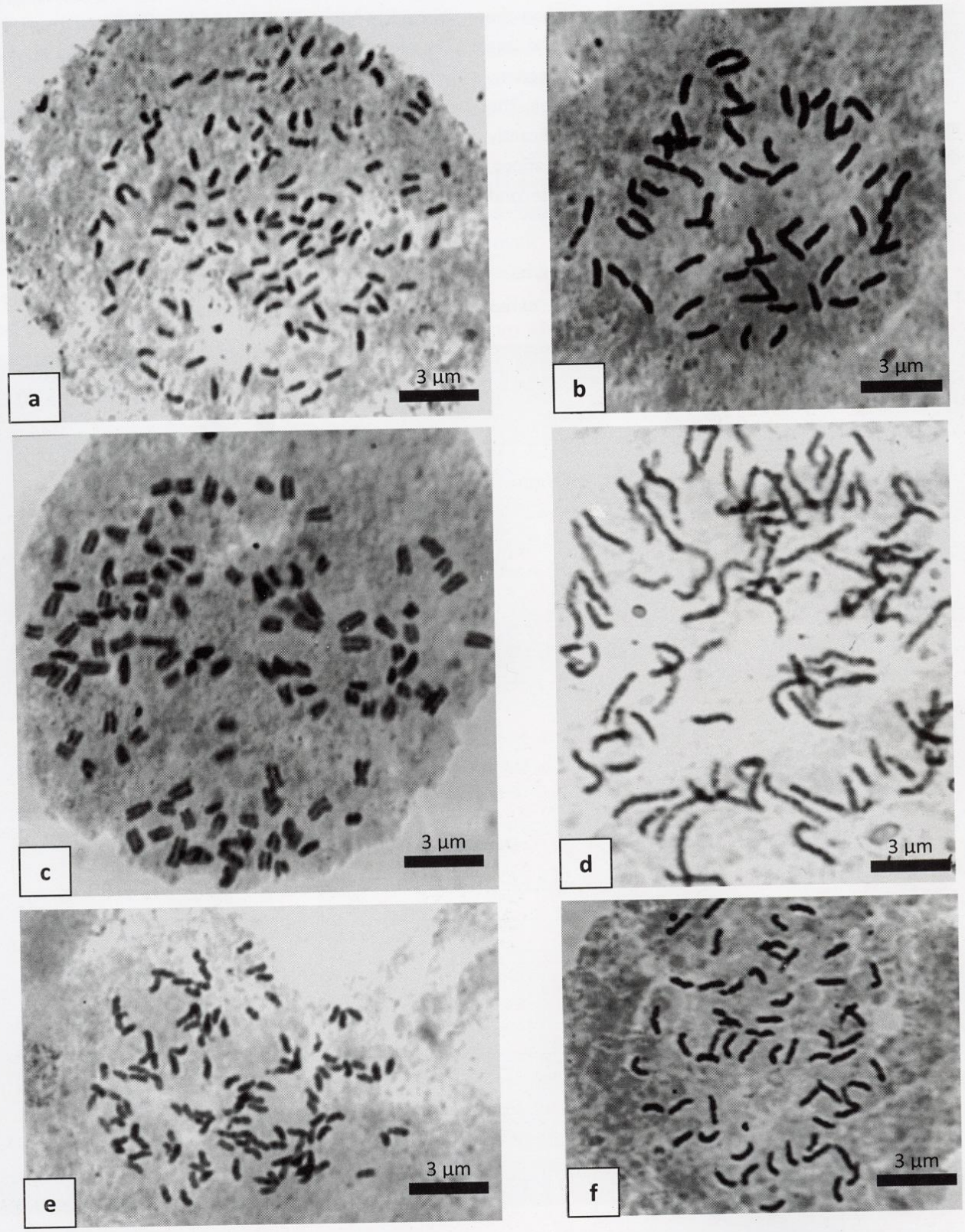

Figure 1. Somatic chromosome of *Pteris*: (a) *P. vittata*, 2n = 116, (b) *P. biaurita*, 2n = 58, (c) *P. tripartita*, 2n = 116, (d) *P. fauriei*, 2n = 87, (e) *P. multifida,* 2n = 116, (f) *P. tripartita*, 2n = 58.

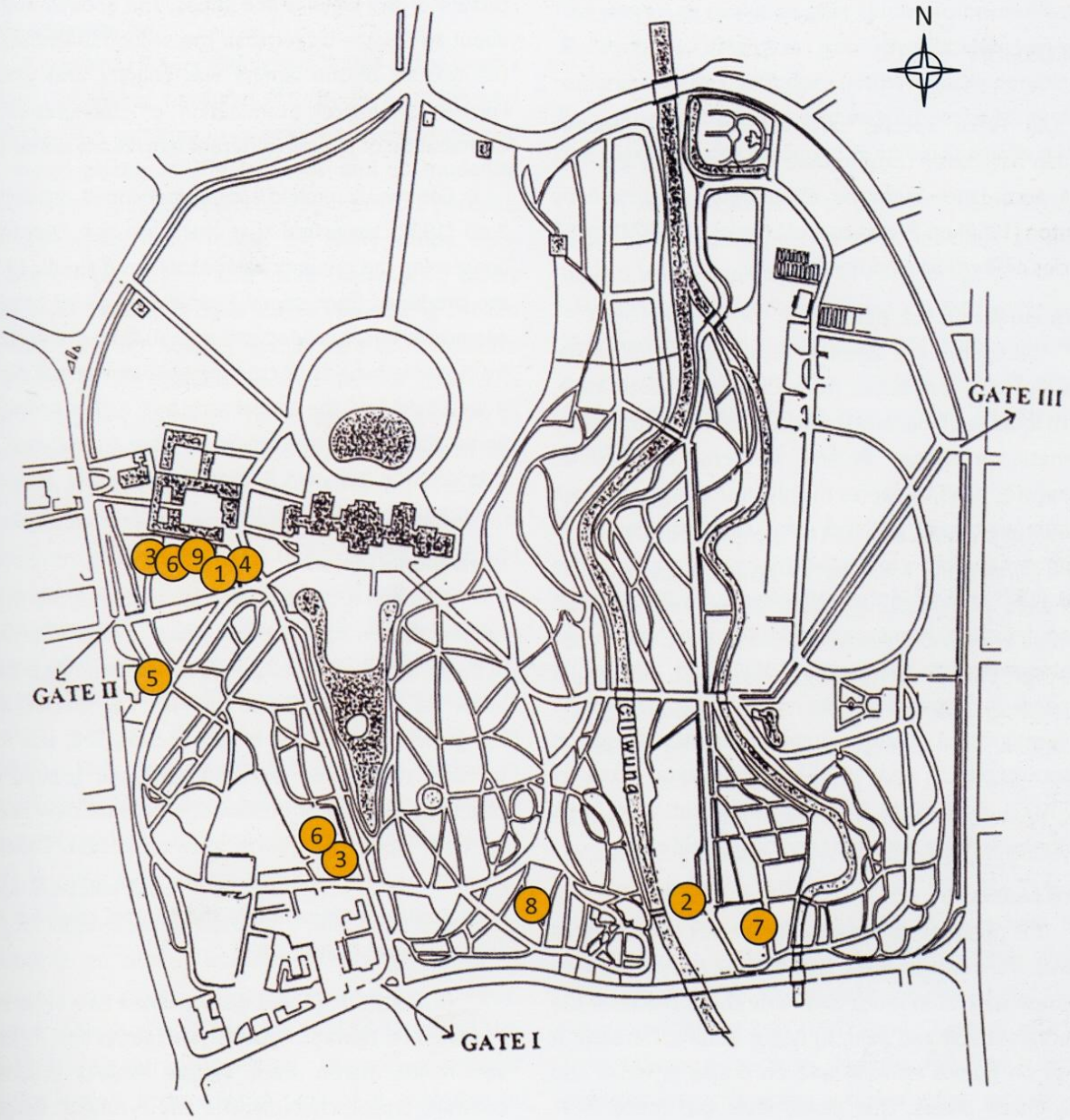

Figure 2. Map of Bogor Botanic Gardens showing the distribution of cytotypes of wild *Pteris* in the garden: *P. biaurita* diploid (1), *P. ensiformis* tetraploid (2), *P. tripartita* diploid (3), *P. multifida* triploid (4), *P.multifida* tetraploid (5), *P. tripartita* tetraploid (6), *P. fauriei* diploid (7), *P. fauriei* triploid (8) dan *P. vittata* tetraploid (9).

Two ploidy levels were also found in *P. tripartita*, diploid (Fig. 1d) and tetraploid (Fig. 1c). Seventy five percent of individuals of *P. tripartita* observed in Bogor Botanic Gardens were diploid. Diploid and tetraploid types grew side by side within the range of 5 – 10 m in *Vak* XIVA, nursery and propagation areas (Fig. 2). Apparently, diploid type of *P. tripartita* dominated the tetraploid type in Bogor Botanic Gardens.

Two ploidy levels of *P. multifida* are observed, one triploid individual and two tetraploid individual (Fig 1e). The tetraploid type was found in *Vak* XIIIA, while the

triploid type was in *Vak* XIVA (see Map on Fig 2). The distance was not more than 0.5 km.

DISCUSSION

All *Pteris* species observed in Bogor Botanic Garden have basic chromosome number of x = 29. This is in accordance with the observation conducted by Manton (1950) on *P. cretica* and Love *et al.* (1977) on 93 species of *Pteris* around the world.

***Pteris ensiformis* N.L. Burm.**

This species is a common fern found in Indonesia. Globally, it is distributed from Ceylon to South India, North India to China, and through Malesia, Australia and Polynesia. In Bogor Botanic Gardens, the species propagates itself by spores mainly in shady places, both on humus-rich soil and rocky soil. One individual of *P. ensiformis* showed a tetraploid type with 2n = 116. Love *et al.* (1977) reported that tetraploid *P. ensiformis* were found in Ceylon, Himalaya and South India. Meanwhile, Praptosuwiryo & Darnaedi (1995) and Melani & Darnaedi (in preparation) also reported the tetraploid *P. ensiformis* from Bogor Botanic Gardens. Based on "Cytotaxonomical Atlas of the Pteridophyta" (Love *et al.*, 1977), it is clear that the tetraploid type of *P. ensiformis* is more common than the diploid one.

***Pteris biaurita* L.**

It is regarded as a pantropic species, growing well in Java, from East to West Java. In East Java, it is a very common species in moist and fairly shady places in the mountain forest and field. In Bogor Botanic Gardens, it grows on humus rich-soil and on drains in moist and fairly shady places. One ploidy level was found in *P. biaurita*, viz. diploid. The diploid *P. biaurita* was also found in Gede-Pangrango National Park (Darnaedi, 1992), Bogor Botanic Gardens (Praptosuwiryo & Darnaedi, 1995) and some locations in East Java (Zubaidah, 1998). Diploid type of *P. biaurita* outside Java was reported by Abraham *et al.* (1962) and Manton & Sledge (1954). Two individuals of *P. biaurita* examined showed different morphological appearances. The first individual which was apogamous with 32 spores in each sporangium, was bigger and stouter than the other. Unfortunately, we were unable to determine whether the second individual was sexual or apogamous, since the plant was still in infertile state. However, the second individual was apparently very different in size and texture of the laminas and stipes. The apomic one was about two times bigger than the second individual and the texture of the lamina was coarser and glossier. Further cytological examination of the species and examination of the two different individuals are needed.

Commonly, diploid apomic individuals are hybrids. Kato (1992) suggested that there are two hypotheses concerning the origin of agamosporous diploids: (i) they are produced from sexual diploid species by acquiring agamosporous reproduction; and (ii) they are derivated from sterile hybrids of crossing beetwen sexual diploid. If we could find the sexual race and gather more data about the species we would be able to assumpt that hybridization between *P. biaurita* and other species of *Pteris* have been taking place.

***Pteris fauriei* Hieron.**

P. fauriei is distributed in Japan, China, the Ryukyu Islands, Taiwan and Vietnam (Shieh, 1975). In Java, *i.e.* in Bogor Botanic Gardens, *P. fauriei* commonly grows in moderate shade under trees or bush. The species, which can grow on humus soil or rather rocky soil, was found to have two ploidy levels, diploid and triploid. The results of cytological observation provide new records for both types of *P. fauriei* growing in Java. Diploid *P. fauriei* was first reported by Roy and Holttum (1965) from Southern China, while the triploid relatives were found in Japan (Mitui, 1968).

Huang *et al.* (2006) distinguished two varieties of *P. fauriei* in Taiwan: *P fauriei* var fauriei and *P. fauriei* var. *minor* Hieron. Each variety exhibits a distinct, constant cryptic characteristic and cytotype. Based on the diameter of spores and spore number per sporangium from the type specimens, Huang *et al.* (2006) confirmed that *P. fauriei* var *minor* is sexual diploid and that *P. fauriei* var *fauriei* is apogamous triploid. Further, Huang *et al.* (2007) has identified two varieties of *P. fauriei*, one diploid (193 plants) and one triploid (323 plants), from 32 localities in Taiwan. The number of spores per sporangium and the size of spores are reliable indicators of ploidy. Diploid *P. fauriei* have 64-spore sporangia and the spores are significantly smaller than spores of triploid plants, which have 32-spore sporangia. In general, diploid plants occur more

frequently in warmer habitats than triploid plants and the triploids grow at higher elevations than diploids.

***Pteris tripartita* Sw.**

P. tripartita is very widely distributed in the tropics of the Old World (including Africa) to Australia and Polynesia. It is common in open ground, in well-drained but moist places, in the lowlands and at moderate elevations on the mountains. In Bogor Botanic Gardens, it often grows from spores in sunny sandy soils, nurseries, moist drains, and other places. Two ploidy levels were found in *P. tripartita*, *i.e.* diploid (Fig 1d) and tetraploid (Fig 1c). Seventy five percent of *P. tripartita* examined in the garden showed diploid race. The diploid race is new record for *P. tripartita* growing in West Malesia region. The record of diploid race of this species was first reported by Holttum & Roy (1965) on species from New Guinea, East Malesia, despite most species occurs throughout the tropics of the Old World were found to be tetraploid. Similarly, all cytological observations of *P. tripartita* outside Malesia region showed tetraploid type occurance (Walker, 1962; Wagner, 1963; Gomez-Pignataro, 1971). Tetraploid *P. tripartita* was also reported to be endemic in Java (Walker, 1962; Praptosuwiryo & Darnaedi, 1995). In Bogor Botanic Gardens, however, we found that diploid type grow predominantly to their tetraploid relatives.

***Pteris multifida* Poir.**

In Bogor Botanic Gardens, *P. multifida* is growing commonly on drains, wall-side or among stones. It propagates by spores. Two ploidy levels were recorded, *i.e.* one individual was showing triploid type and the other two were tetraploid (Fig 1e). This present paper is the first report upon triploid type of *P. multifida* in Malesia region. Most individuals of *P. multifida* found in region outside Malesia, *e.g.* Himalaya (Mehra & Verma, 1960), South India (Abraham *et al.*, 1962), Japan (Kawakami *et al.*, 1995; Mitui, 1965), and South China (Roy & Holttum, 1965) were reported to be tetraploid.

The existence of triploid and tetraploid *P. multifida* poses a question whether the species is originated from hybridization. Experimental study on apogamous sporophyte formation in *P. multifida* (2n=4x=116) taken by Kawakami *et al.* (1995) resulted in haploid individuals (n=2x=58). These haploid plants showed abnormality in terms of meiotic division and spores sterility. Based on these data, they suggested two kinds of hypotheses: (i) *P. multifida* has structurally changed its chromosomes in the process of evolution after chromosome doubling; (ii) *P. multifida* originates from hybridization between different species, so it does not have many homologous chromosomes. Further studies are needed to elucidate the speciation mechanism of *P. multifida* and its status.

The two cytotypes of different ploidy level, *i.e.* triploid and tetraploid type of *P. multifida* as well as diploid and tetraploid type of *P. tripartita* do not show any distinct morphological characteristic differences. This contributes to the difficulties of understanding the polyploidy mechanism that occurred in these species, besides the limitation in the number of specimens examined.

***Pteris vittata* L.**

The Brake Fern or *P. vittata* is a fairly common fern in open sunny places in rocky soil, or growing on the wall crevices, varies widely in size. In dry places, the plant are quite small, but where moisture is abundant the plants may attain large sizes. Backer and Posthumus (1939) reported that the species may grow on an elevation up to 2000 m above sea level in Java, but predominantly below 1200 m. In Bogor Botanic Gardens, *P. vittata* grows on the wall crevices of drains or sandy soil in open sunny places. One individual of *P. vittata* was tetraploid, 2n = 116 (Fig 1b). Most of cytological observations on *P. vittata* have been tetraploid (Manton & Sledge, 1954; Manton, 1954; Wagner, 1963; Mitui, 1968). The apogamous diploid and triploid were reported by Tsai (1992) and Chang *et al.* (1992) from Taiwan, respectively. In India, this fern is actually reported as a 'species complex' that includes five cytotypes, *viz.* diploid, triploid, tetraploid, pentaploid and hexaploid with the basic chromosome number being 29 (Khare & Kaur, 1983).

Apparently, polyploidization has also played an important role in the distribution of *P. vittata* (Srivastava *et al.*, 2007). Other cytotypes have been reported in different regions. Diploid cytotype was first reported from Nainital (recorded as forma *brevipinna*) Kodaikanal, upper Kothayar, Tirunelveli (Mehra, 1961). Diploid has also been reported from a few regions of China (Sichuan, Yunnan, Guizhou, Hubei and Hunan) (Wang, 1989).

SUMMARY

Three out of six *Pteris* species found in Bogor Botanic Gardens (i.e. *P. fauriei*, *P. multifida*, and *P. tripartita*) have series of polyploidy. This investigation showed that wild ferns in general, and wild *Pteris* in particular, in Bogor Botanic Gardens, were not only confined in species level, but also in ploidy level. Based on these series of ploidy data and the fact that the different ploidy level existed in each species, we assume that one of significant aspects of fern evolution, the polyploidy, has been working in a very narrow "natural ecosystem" such as in Bogor Botanic Gardens. But, we should have a critical question whether all the ploidy levels are naturally living in the garden or being introduced from outside the garden.

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Plate 1. Morphological appearances of six *Pteris* species found in Bogor Botanic Gardens: **A.** *P. ensiformis* N.L. Burm., **B.** *P. biaurita* L., **C.** *P. fauriei* Hieron., **D.** *P. tripartita* Sw., **E.** *P. multifida* Poir., and **F.** *P. vittata* L.