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ORIGINAL ARTICLE

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Apomixis in the interspecific triploid hybrid fern *Cornopteris christenseniana* (Woodsiaceae)

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Abstract *Cornopteris christenseniana* is a "sterile" interspecific triploid hybrid of diploid *C. crenulatoserrulata* and tetraploid *C. decurrenti-alata*. Morphological and cytological studies show that, of 41 young plants of *Cornopteris* that have been propagated naturally in the Fern Garden of the Botanical Gardens, University of Tokyo, 30 plants are the sterile *C. christenseniana*, 10 are fertile *C. decurrenti-alata* and 1, fertile *C. crenulatoserrulata*. This proportion supports the view that the young plants of *C. christenseniana* are derived from spores of reproductively mature plants of the species cultivated. Cytogenetic observations and culture experiments show that *C. christenseniana* produces normal spores in various proportions in some sporogenetic pathways that are aberrant from the ordinary process in sexual and apomictic ferns. Under culture conditions, normal spores germinate in rough proportion to the frequency of normal spores, and sporophytes are apogamously produced in rough proportion to the frequency of spore germination. As a whole, the rates of spore germination and apogamous sporophyte development vary according to the specific plant. Taken together, these observations suggest that *C. christenseniana* is an incipient apomict.

Key words Apomixis · Chromosome numbers · *Cornopteris christenseniana* · Hybrid fern · Polyploidy (triploid) · Sporogenesis

Introduction

In plants, hybridization between populations or species is common and provides variation that facilitates adaptation (Stebbins 1950; Grant 1981). Hybrids with highly heterozygous genotypes or enriched gene pools may possess heterosis (hybrid vigor), an advantage with which to potentially proliferate, perpetuate and colonize newly available (e.g., hybridized or open) habitats or even evolve into new species (Dobzhansky et al. 1977; Grant 1981; Arnold 1997). Interspecific hybridization generally results in offspring sterility. It can be overcome by polyploidization, which retrieves homologous chromosome pairs allowing meiosis, or by apomictic or asexual reproduction (Manton 1950; Stebbins 1950; Grant 1981; Mogie 1992; Richards 1997; Ramsey and Schemske 1998; Kato and Nakato 1999). Apomixis (in a broad sense; agamospermy, agamospory) is an asexual reproduction via chromosomal unreduction of spores (diplospores) and gametophytes, and the subsequent apogamous reproduction (in a strict sense), i.e., asexual reproduction of a sporophyte from somatic cells of the gametophyte. In this paper we use the two terms in these senses. About 10% of the world's fern species (Lovis 1977) and about 15% of Japanese pteridophytes are apomictic (Takamiya 1996, 1997). Apomixis is strongly associated with polyploidy (Asker and Jerling 1992; Richards 1997; Kato and Nakato 1999), and three-quarters of apomictic pteridophytes are triploid (Wagner and Wagner 1980).

The origin of apomictic species has been investigated for some ferns. From electrophoretic data of enzyme polymorphism, Gastony and Gottlieb (1985) suggested that apomictic tetraploids of *Pellaea andromedifolia* (Kaulf.) Fée arose as crosses between unreduced triploid sperms of apomictic triploids and reduced haploid eggs of sexual diploids. Watano and Iwatsuki (1988) and Suzuki and Iwatsuki (1990) also suggested that tetraploids of *Asplenium unilaterale* Lam. sensu lato and triploids of *Pteris cretica* L., respectively, were derived from hybrids between apomictic and sexual parents. Furthermore, Gastony and Gottlieb (1985), Watano and Iwatsuki (1988) and Suzuki and Iwatsuki (1990) showed that there are genetic variations in those apomictic species, suggesting multiple origins. Lin et al. (1995) demonstrated that the polymorphic *Dryopteris varia* (L.) O. Ktze complex has considerable genetic variation, suggesting highly multiple origins. In contrast, Gastony (1986, 1988) and Gastony and Windham (1989) argued that autopolyploidy is involved in the origin of apomictic plants

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of the *Pellaea glabella* Mett. ex. Kuhn complex and other ferns. Darnaedi et al. (1990) supported the view that the apomictic triploid *Dryopteris yakusilvicola* Kurata was derived from a hybrid of diploid and tetraploid sexual species, and, from absence of allozyme variation, suggested that speciation occurred once, relatively recently. Schneller et al. (1998) suggested a single origin with subsequent mutations in the apomictic triploid *Dryopteris remota* (Döll) Druce. From the results of culture experiments showing that a few sporophytes are produced by gametophytes that develop from occasionally produced, unreduced spores in some triploid hybrids of ferns, Morzenti (1967), Evans (1969), Vida and Reichstein (1975) and Pintér (1995) hypothesized that "sterile" hybrids may evolve into apomictic species. Thus, previous studies have suggested that apomictic ferns are derived from hybrids between apomictic and sexual parents, or from sterile hybrids between sexual parents. In hybridization experiments with *Pteris*, Walker (1962) demonstrated that apomictic reproduction is inherited dominantly. This evidence is consistent with the former pattern of origin of apomicts. In comparison, our understanding of the origin of apomictic reproduction in sterile hybrids is poor (Lovis 1977).

Cornopteris christenseniana (Koidzumi) Tagawa, like *C. crenulatoserrulata* (Makino) Nakai and *C. decurrenti-alata* (Hook.) Nakai, is a mesic fern preferring a shady humid habitat. The intermediacy of leaf morphology, sorus, chromosome number and distribution pattern suggests that *C. christenseniana* is a triploid interspecific hybrid ($2n = 120$) between diploid *C. crenulatoserrulata* ($2n = 80$) and tetraploid *C. decurrenti-alata* ($2n = 160$) (Kurita 1964; Mitui 1968; Hirabayashi 1970; Kato 1979, 1995; Kurata and Nakaike 1990). In their cytological studies Kurita (1964) and Mitui (1968) considered that *C. christenseniana* is sterile. Nonetheless, it often colonizes secondary *Cryptomeria japonica* D. Don forests and is distributed from Kyushu to Shikoku and Honshu in Japan and southern Korea including Cheju Island, a region that overlaps parts of the regions of the two parental species and is partly beyond them (Kurita 1964; Kurata and Nakaike 1990). It has also been recorded from China (Chu and He 1999). The wide distribution compared with sterile hybrids in general (Barton and Hewitt 1985; Kurata and Nakaike 1994) may suggest proliferation and expansion by an unknown method.

We were aware some years ago that young plants of *Cornopteris* grew naturally (that is, untended) in the Fern Garden of the Botanical Gardens, University of Tokyo, located in the center of Tokyo (Fig. 1). A preliminary identification showed that the young plants included *C. christenseniana*. The purpose of this study is to identify the young plants precisely and, if they include *C. christenseniana*, which is believed to be sterile, to investigate how it propagates.

Materials and methods

The Fern Garden of the Botanical Gardens, Graduate School of Science, University of Tokyo, is a facility to cultivate a variety of ferns including *Cornopteris christenseniana*, *C. crenulatoserrulata* and *C. decurrenti-alata* on a semiexcavate ground with three tiers of enclosing platforms under a shade, with daily sprinkling (Fig. 1). Young plants of some fern species grow in the soil among vertically piled rocks forming the platforms and hence the young plants of *Cornopteris* do not seem to have been derived from rhizome branches of the three species of *Cornopteris* that are planted together but isolated from the young plants by the rocks. Materials were collected from such young plants of *Cornopteris* in the Fern Garden twice in 1998 and 1999. In 1998, 13 plants were collected, and 14 plants of the 1999 collection included those that had not been collected in 1998 and 11 very young plants that seem to have arisen or have become enlarged after the 1998 collection (Table 1). We observed that 14 other young plants grew in the Fern Garden. The collected plants were transplanted to the nursery adjacent to the Fern Garden in 1998 and 1999 for further analysis (Fig. 1). Many of the transplanted plants grew and bore sporophylls, though they were still smaller in size than fully mature plants. Materials were also collected from 27 mature plants of *C. christenseniana*, *C. crenulatoserrulata* and *C. decurrenti-alata* that had been transplanted from various places in Japan to the Fern Garden and nursery (Table 1). Vouchers are deposited in the Herbarium, Botanical Gardens, Graduate School of Science, University of Tokyo (TI).

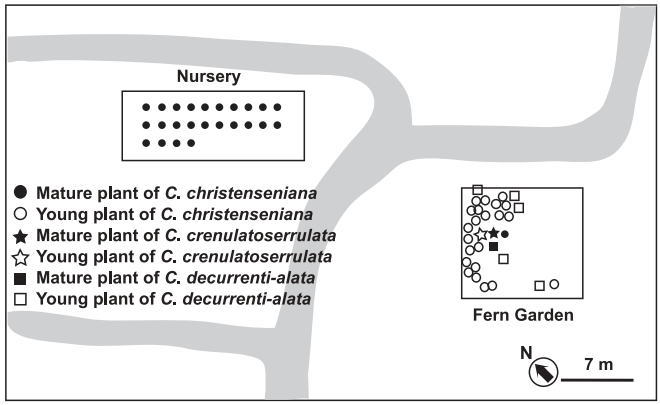

Fig. 1. Map showing Fern Garden and nursery in Botanical Gardens, University of Tokyo, where materials of *Cornopteris* were collected

For observing somatic chromosomes, root tips were pretreated with 2 mM 8-hydroxyquinoline at about 20°C for 4–6 h. They were fixed in 45% acetic acid at about 4°C for 30 min and then preserved in 70% ethanol at 4°C. The root tips were retreated by 45% acetic acid for 15 min just prior to examination, macerated in a mixture of 1 N HCl and 45% acetic acid (3 : 1) at 60°C for 1–3 min, and squashed in 2% aceto-orcein.

To examine meiosis, young sporangia were fixed with Carnoy's solution (absolute ethanol : acetic acid; 3 : 1) overnight at about 4°C and then squashed in 2% aceto-orcein. The frequency of normal spores was examined by counting spores in about five squashed sporangia in each of nearly 100 pinnules per plant. The number of spores in each

Table 1. Materials and somatic chromosome numbers of three species of *Cornopteris*

Species	Source	Voucher	Chromosome number (2*n*)
C. christenseniana (young plants)	Fern Garden[a]	BGFGH1[e] (Figs. 3a, 4a), BGFGH2[e], BGFGH3[e], BGFGH4[e], BGFGH5[e], BGFGH6[e], BGFGH7[e], BGFGH8[e] (Fig. 2a, b), BGFGH9[e], BGFGH10[e], BGFGH11[e], BGFGH34[e], BGFGH35[e], BGFGHN2[f], BGCHN3[f], BGFGHN5[f],BGFGHN6[f], BGFGHN7[f], BGFGHN8–2[f], BGFGHN9[f]	120
	Fern Garden[a]	BGFGHN8-1[f] (Figs. 3c, 4c)	121
C. christenseniana (mature plants)	Fern Garden[b]	BGFGHM1 (Figs. 3b, 4b, 5a, b)	120
	Honjo, Akita Pref.	BGCH33	120
	Mamurogawa, Yamagata Pref.	BGCHYMA1, BGCHYMA3 (Figs. 5e, 8d, e)	120
	Mamurogawa, Yamagata Pref.	BGCHYMB (Fig. 8c)	121
	Awano-cho, Tochigi Pref.	BGCHTO094	120
	Mt. Ozaku, Tochigi Pref.	BGCHOZ977, BGCHOZ31, BGCHOZ41	120
	Mt. Ozaku, Tochigi Pref.	BGCHOZ7 (Figs. 3d, 4d)	121
	Mt. Ozaku, Tochigi Pref.	BGCHOZC12 (Figs. 3e, 4e), BGCHOZC14	122
	Hanno, Saitama Pref.	BGCHSA044 (Fig. 5c), BGCHSA058	120
	Mt. Yahazu, Shizuoka Pref.	BGCH17	120
	Mt. Yahazu, Shizuoka Pref.	BGCH17A (Figs. 3f, 4f)	126
	Ono, Fukui Pref.	BGCHHU051	120
	Mt. Yoshino, Nara Pref.	BGCH29, BGCH32	120
	Mt. Otaki, Kagawa Pref.	BGCHK12 (Fig. 8a), BGCHK14	120
	Maebara–cho, Fukuoka Pref.	BGCH26, BGCH27 (Fig. 5f), BGCH30 (Figs. 5d, 8b)	120
	Maebara-cho, Fukuoka Pref.	BGCH19	121
C. crenulatoserrulata (young plant)	Fern Garden[a]	BGFGCN1[f] (Figs. 2e, f; 3g)	80
C. crenulatoserrulata (mature plant)	Fern Garden[c]	BGFGC1 (Fig. 3h)	80
C. decurrenti-alata (young plants)	Fern Garden[a]	BGFGDN2[f] (Fig. 2c, d), BGFGDN7[f] (Fig. 3i), BGFGDN7-1[f], BGFGDNB1[f], BGFGDNB3[f]	160
C. decurrenti-alata (mature plant)	Fern Garden[d]	BGFGD1 (Fig. 3j)	160

[a] Botanical Gardens, Graduate School of Science, University of Tokyo
[b] Originally collected from Mt. Ozaku (Tochigi Pref.) and planted in 1995
[c] Originally collected from Atsushio-kano (Fukushima Pref.) and planted in 1987
[d] Originally collected from Isahaya (Nagasaki Pref.) and planted in 1987
[e] Transplanted to the nursery in 1998
[f] Transplanted to the nursery in 1999

sporangium was counted to presume reproduction mode. In leptosporangiate ferns in general, 64 chromosomally reduced and 32 unreduced spores are produced in a sporangium in sexual and apomictic reproduction, respectively (Manton 1950; Lovis 1977; Walker 1979).

Sporophylls of *Cornopteris christenseniana* were collected from 25 plants cultivated in the Fern Garden and the nursery in September 1999 and October 2000, when spores were fully matured. They were kept in paper envelopes at 4°C in a refrigerator. Spores were sterilized with 0.9% sodium hypochlorite (5.25% sodium hypochlorite : distilled water; 1 : 5) in microcentrifuge tubes for 2 min and rinsed twice with distilled water. Morphologically normal spores were selected under a stereomicroscope for culture, and aborted or empty spores were excluded, because a preliminary sowing showed that abortive spores did not germinate. To determine germination rate, spores were sown in sterilized 0.1% HypoNex (Hyponex Japan, Tokyo) solution in Petri dishes of 90 mm in diameter and incubated at 25°C under continuous illumination with fluorescent and incandescent lamps (600–800 lx). Some of the spores were stained in 1% acetocarmine after 2 days culture. Germinated spores and spores that were just before germination exhibited definitely pink cytoplasm and densely stained nuclei in prothallial and rhizoidal cells. Germination rate was examined by scoring three random samples (Petri dishes) per plant, each containing about 200 spores.

To develop gametophytes and sporophytes, ungerminated and germinated spores used for counting germination rate were resown on 1% agar medium with sterilized 0.1% HypoNex solution in Petri dishes of 90 mm in diameter (density was about 50 spores per Petri dish). Cultures were maintained at 23–25°C under continuous illumination with fluorescent and incandescent lamps (600–800 lx). The frequency of apogamous sporophytes was determined by counting the number of sporophyte-bearing gametophytes to all gametophytes in five random samples (Petri dishes).

Results

Morphology

The young plants collected in the Fern Garden were further cultivated into reproductive maturity for accurate identification. They were identified based on diagnostic characters of the three species of *Cornopteris*, as described by Kato

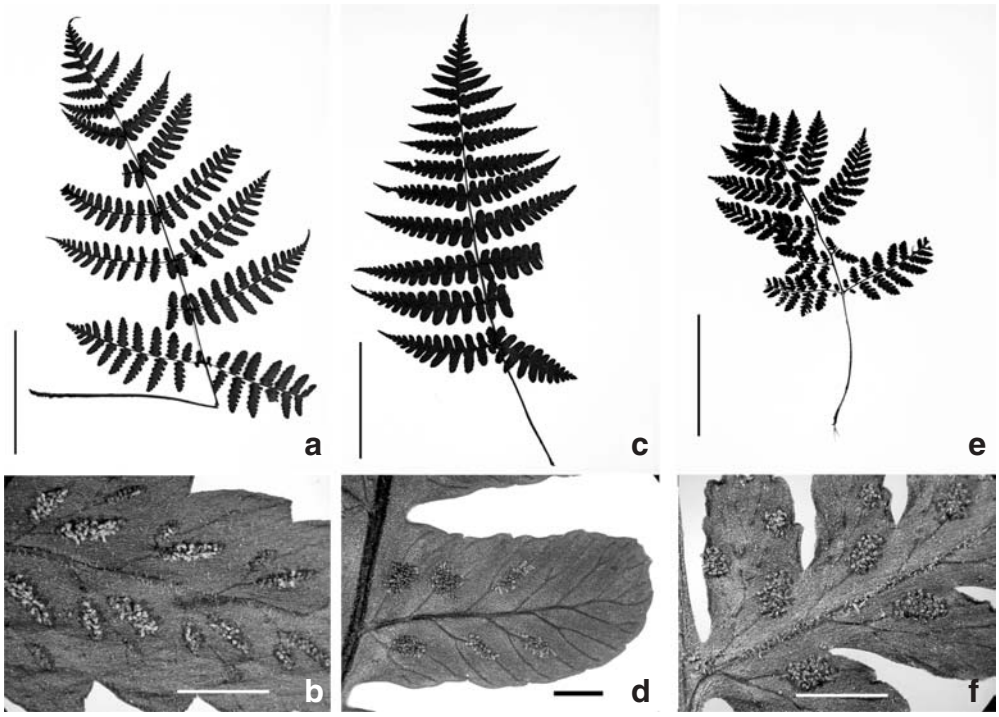

Fig. 2a–f. Leaves collected from young plants of *Cornopteris* that have been cultivated into reproductive maturity in the nursery. **a**, **b** *C. christenseniana* (BGFGH8). **c**, **d** *C. decurrenti-alata* (BGFGDN2). **e**, **f** *C. crenulatoserrulata* (BGFGCN1). **b**, **d** and **f** show parts of leaves with sori. *Bars* 10 cm in **a**, **c**, **e**; 2 mm in **b**, **d**, **f**

(1979). In *C. christenseniana* the leaves were tripinnatifid with pinnules lobed or pinnatifid, the segments subcartilaginous at the margin, the rachis and costae were hairy with unicellular hairs, and in sporophylls the sori were elliptic or linear (Fig. 2a, b). The plants of each of *C. decurrenti-alata* and *C. crenulatoserrulata* also exhibited the characters of the species at early and semimature stages (Fig. 2c–f; Kato 1979, 1995). Of the 27 young plants collected, 21 were identified as *C. christenseniana*, 5 as *C. decurrenti-alata*, and 1 as *C. crenulatoserrulata*. Of the 21 plants of *C. christenseniana*, 6 were very young when collected in 1999 and seemed to have germinated after the 1998 collection. All these plants corresponded well with specimens collected from natural populations of the species deposited in TI. Of the remaining 14 young plants still growing in the Fern Garden, 9 were *C. christenseniana* and 5 were *C. decurrenti-alata*.

Chromosome number

Somatic chromosomes were counted for a total of 54 plants of the three species including 27 young plants collected from the Fern Garden and 27 mature plants that were collected from various places in Japan and transplanted to the Fern Garden and nursery (Table 1). Of the 21 young plants of *Cornopteris christenseniana* examined, 20 plants had 120 somatic chromosomes (2*n*) and 1 plant had 121 chromosomes (Figs. 3a, c; 4a, c). Of 27 mature plants, 25 plants of *C. christenseniana* cultivated in the nursery and Fern Garden were divided into four cytotypes with 120, 121, 122, and 126 chromosomes, of which the cytotype with 120 chromosomes was the most common (19 plants; Figs. 3b, 4b) and aneuploids with 121, 122 and 126 chromosomes were rare (3, 2 and 1 plants, respectively; Figs. 3d–f, 4d–f). The aneuploids were found to occur in some populations together with the most common cytotype with 120 chromosomes (Table 1).

One young plant and one mature plant of *C. crenulatoserrulata* had 80 somatic chromosomes (2*n*) (Fig. 3g, h), and five young plants and one mature plant of *C. decurrenti-alata* had 160 somatic chromosomes (Fig. 3i, j). The mature plants (BGFGC1, BGFGD1) of both species are cultivated adjacent to *C. christenseniana* in the Fern Garden (Fig. 1).

The results of the three species, other than aneuploids of *C. christenseniana*, were in accordance with Kurita (1964), Mitui (1968), and Hirabayashi (1970).

Sporogenesis

The mature plants of *C. christenseniana* showed abnormal sporogenetic patterns in all of nearly 500 sporangia examined in each of 25 plants. The sporangia usually contained 16, rarely 14 or 12 spore mother cells (SMC; Fig. 5a), and no sporangium with 8 SMC, typical of Döpp-Manton system, was found. In the first meiotic anaphase, bivalents were regularly separated while univalents were isolated and scattered in the cell (Fig. 5b). We usually observed that SMC underwent two successive cytokineses completely or incompletely, resulting in tetrads and eventually 64 or fewer spores.

Cytogenetic observations at different stages of sporogenesis showed that *Cornopteris christenseniana* had different later sporogenetic patterns. In one pattern (sporogenesis 1), many or most of about 64 or fewer spores formed in single sporangia were aggregated in tetrads and none was normal

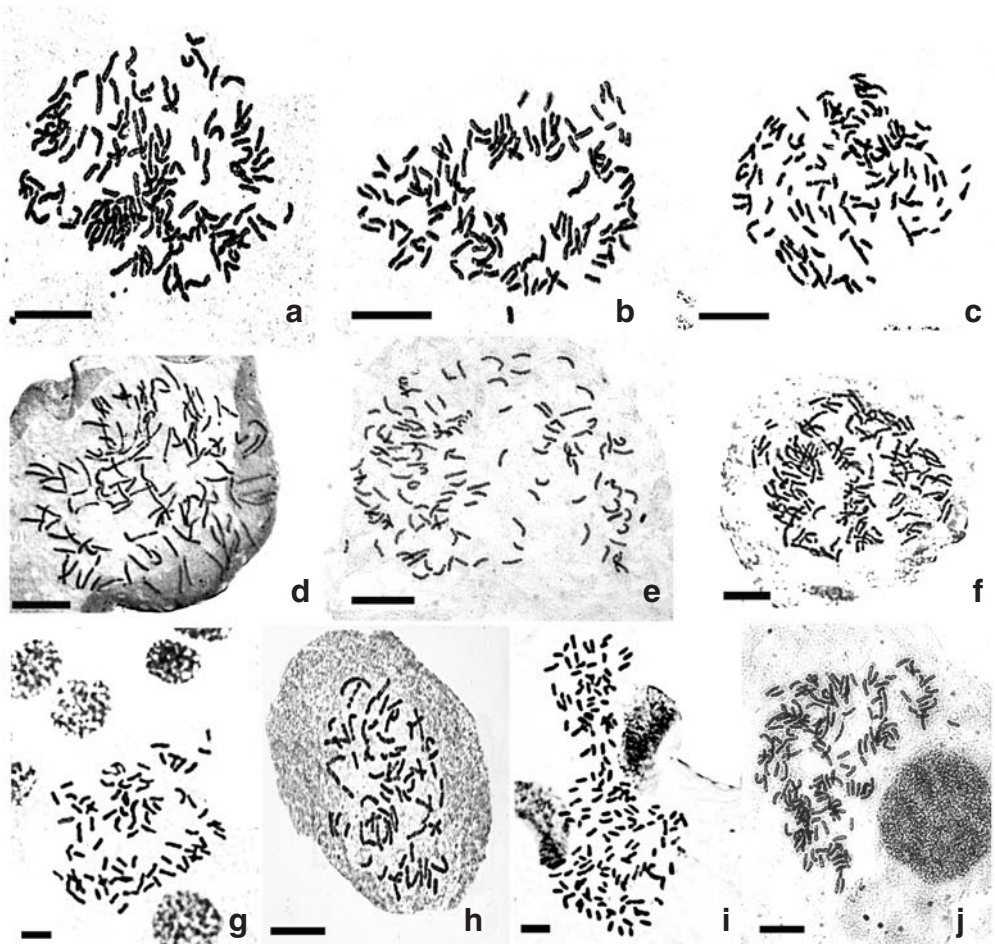

Fig. 3a–j. Somatic chromosomes of three species of *Cornopteris*. **a–f** *C. christenseniana*. **a** Young plant with 120 chromosomes (BGFGH1). **b** Mature plant with 120 chromosomes (BGFGHM1). **c** Young plant with 121 chromosomes (BGFGHN8-1). **d** Mature plant with 121 chromosomes (BGCHOZ7). **e** Mature plant with 122 chromosomes (BGCHOZC12). **f** Mature plant with 126 chromosomes (BGCH17A). **g**, **h** *C. crenulatoserrulata*. **g** Young plant with 80 chromosomes (BGFGCN1). **h** Mature plant with 80 chromosomes (BGFGC1). **i**, **j** *C. decurrenti-alata*. **i** Young plant with 160 chromosomes (BGFGDN7-1). **j** Mature plant with 160 chromosomes (BGFGD1). *Bars* 10 μm

(Fig. 5c). In another pattern (sporogenesis 2), most of about 64 spores were abortive and some were normal (Fig. 5d). In still another pattern (sporogenesis 3), about 64 spores were formed in tetrads and mostly normal (Fig. 5e). In an extreme pattern (sporogenesis 4), only about 16 spores were formed (Fig. 5f). They were mostly normal and larger than those of sporogenesis 3. The frequency of normal spores varied considerably from 1% to 92% with plants collected from different localities, although the average was low (Fig. 6). The frequency was exceptionally high in plant 3 in Fig. 6, and under 60% in all the others, being 1–2% in plants 5–17.

The frequency of normal spores depended on the frequency of the sporogenetic patterns. In 25 plants examined, sporogenesis 1 and 2 occurred in 16 plants from Akita (plant 1 in Fig. 6; see also Table 1), Tochigi (5–12), Saitama (13, 14), Shizuoka (15, 16), Fukui (17), and Nara Prefectures (18, 19), with very low or low frequencies of normal spores. Sporogenesis 3, along with sporogenesis 1 and 2, was observed in the remaining nine plants (2–4, 20–25). Furthermore, sporogenesis 4 was found in a plant from Yamagata (3), a plant from Kagawa (20) and two plants from Fukuoka (24, 25) among these nine plants. Some of those (22, 23) with sporogenesis 1–3 had low frequencies of normal spores, most (2, 4, 20, 21, 24, 25) with sporogenesis 1–3 or 1–4 had relatively high frequencies, and one (3) with sporogenesis 1–4 had very high frequency.

Spore germination and apogamous sporophyte development

Normal spores of *Cornopteris christenseniana* germinated at various frequencies (0–80%) with different plants (Fig. 7). The frequency ranged from 80% in plant 3 in Fig. 7, to 54–65% in plants 4, 20, 24 and 25, to 10–40% in plants 1, 2, and 21–23, and to 0% or nearly so in plants 5–17. The rate was roughly in proportion to the frequency of normal spores: plants with higher frequencies of normal spores had a general tendency toward higher frequencies of germination (cf. Figs. 6, 7). Germinated spores developed into protonemata about a week after sowing (Fig. 8a). They further developed into gametophytes of irregular forms and no archegonium and antheridium were formed in any plants examined (Fig. 8b). A sporophytic bud was apogamously formed on the upper (dorsal) side of the cushion 5–6 months after spore germination (Fig. 8c, d). The first leaf appeared first and became pinnate, and the second larger leaf and later leaves followed. A root was produced after the appearance of the second or later leaf. This develop-

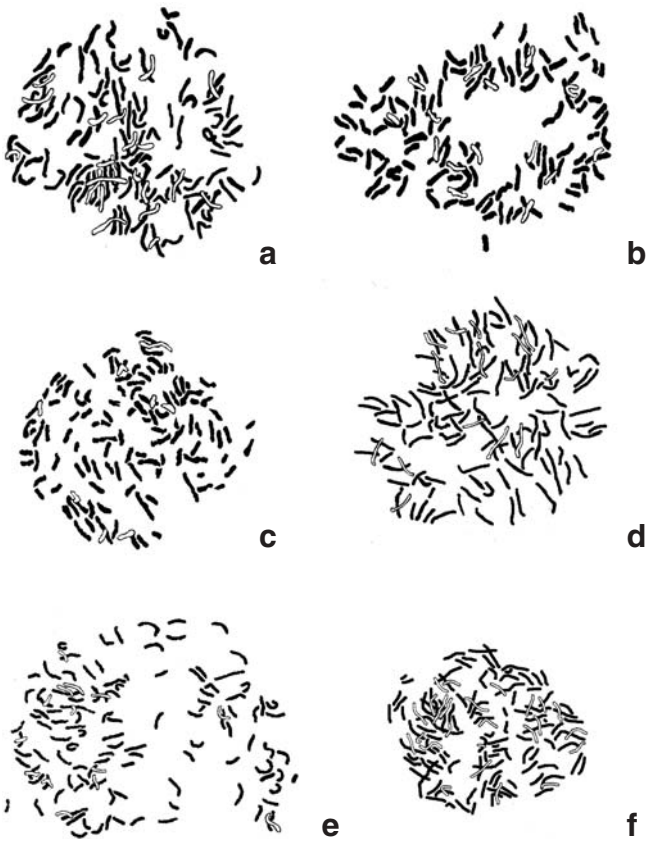

Fig. 4a–f. Explanatory drawings of Fig. 3**a–f**

mental pattern is nearly identical with that of obligatory apomictic species (Kanamori 1967, 1972; White 1979; Raghavan 1989). The somatic chromosome number of an apogamous sporophyte was $2n = 120$ (Fig. 8e, f), the same as the parental sporophyte (Table 1). This provides further evidence for apomixis and the continued maintenance of ploidy (3×) and chromosome number. Sporophytes were formed in 45% (or far fewer) of gametophytes that developed from spores of plants with relatively high frequencies of normal spores and spore germination (plants 1–4, 20, 21 23–25 in Fig. 9), while no sporophyte was formed on gametophytes from plants with no or low frequencies of these characteristics (plants 5–10, 18, 19, 22 in Fig. 9). The frequency of apogamy differed between plants.

Discussion

The morphological and cytological data obtained, along with field observations, show that the young plants of *Cornopteris christenseniana* are produced from air-dispersed spores in the Fern Garden, although it has been considered as a sterile triploid hybrid (Kurita 1964; Mitui 1968; Kato 1979). Propagation seems not to be rare, because six young plants probably arose during a short time between 1998 and 1999. It is noted that the life cycle of this fern from spore dispersal through sporophyte maturation proceeds naturally in an artificial environment in the Fern Garden, compared with apogamous reproduction under culture conditions reported for "sterile" hybrids in *Asplenium*, *Polystichum* and others (Morzenti 1967; Evans 1969; Pintér 1995).

Theoretically there are two possible ways in which the young plants of *Cornopteris christenseniana* are propagated in the Fern Garden. One is in situ hybridization of the cultivated plants of *C. crenulatoserrulata* and *C. decurrenti-alata*. However, this is unlikely (although not ruled out for some of the young plants of *C. christenseniana*) because *C. crenulatoserrulata* propagates naturally in the Fern Garden at an extremely low frequency, suggesting that its gametophyte population is too sparse to hybridize with *C. decurrenti-alata*. If interspecific in situ hybridization had yielded more than 20 young plants of *C. christenseniana*, intraspecific fertilization should have been frequent enough to produce many more young plants of *C. crenulatoserrulata* than the one plant actually produced. Molecular data (C.-H. Park et al., unpublished data) also do not support this way of propagation. The other, more likely, possibility is apomixis of mature plants of *C. christenseniana* cultivated in the Fern Garden and/or the adjacent nursery.

Sporogenesis of *Cornopteris christenseniana* is identical with that of sexual reproduction until 16 SMC are produced, as far as we observed. Rarely, 12 or 14 SMC may be produced in a manner similar to that described by Kurita (1981) in which imperfect nuclear restitution in some cells give rise to 9–15 SMC. However, the subsequent sporogenesis is variable. Nearly two-thirds of the *C. christenseniana* plants examined produced mostly abortive spores via disturbed meiosis but occasionally normal, viable, unreduced spores, as shown for "sterile" hybrids by Evans (1969) and Pintér (1995). In some sporangia of four plants, about 16 spores were also produced in single sporangia. This is reminiscent of the "pseudomeiotic" sporogenesis in which some "sterile" hybrids produce up to 16 viable spores ("mitospores") from 16 SMC (Morzenti 1962, 1967). In addition, most (ca. 60) normal spores are formed in sporangia in about one-third of the plants. However, our observations of a number of sporangia failed to document such mitotic divisions. The proportion of normal spores varies with plants, according to the frequency of sporogenetic patterns in each plant. Exceptionally, one plant (BGCHYMA3) produces, in most sporangia, about 64 spores most of which are normal like those of sexual species. Thus, in *C. christenseniana* no 32-spored sporangium typical of apomictic ferns was found. Variations in sporogenesis are also reported in apomictic ferns (Manton 1950; Mitui 1968, 1982; Walker 1979, 1985; Takamiya et al. 1999).

It has been argued (by e.g., Morzenti 1962, 1967; Evans 1969; Vida and Reichstein 1975; Pintér 1995) that sporadic unreduced spores produced in some "sterile" hybrids, like those of *Cornopteris christenseniana*, can play a significant role in speciation (Morzenti 1967; Evans 1969; Gastony 1986). However, there is a considerable difference between the sporadic and regular sporogenesis of unreduced spores in "sterile" hybrids and apomictic species, respectively. Endopolyploidization (resulting in restitution nucleus)

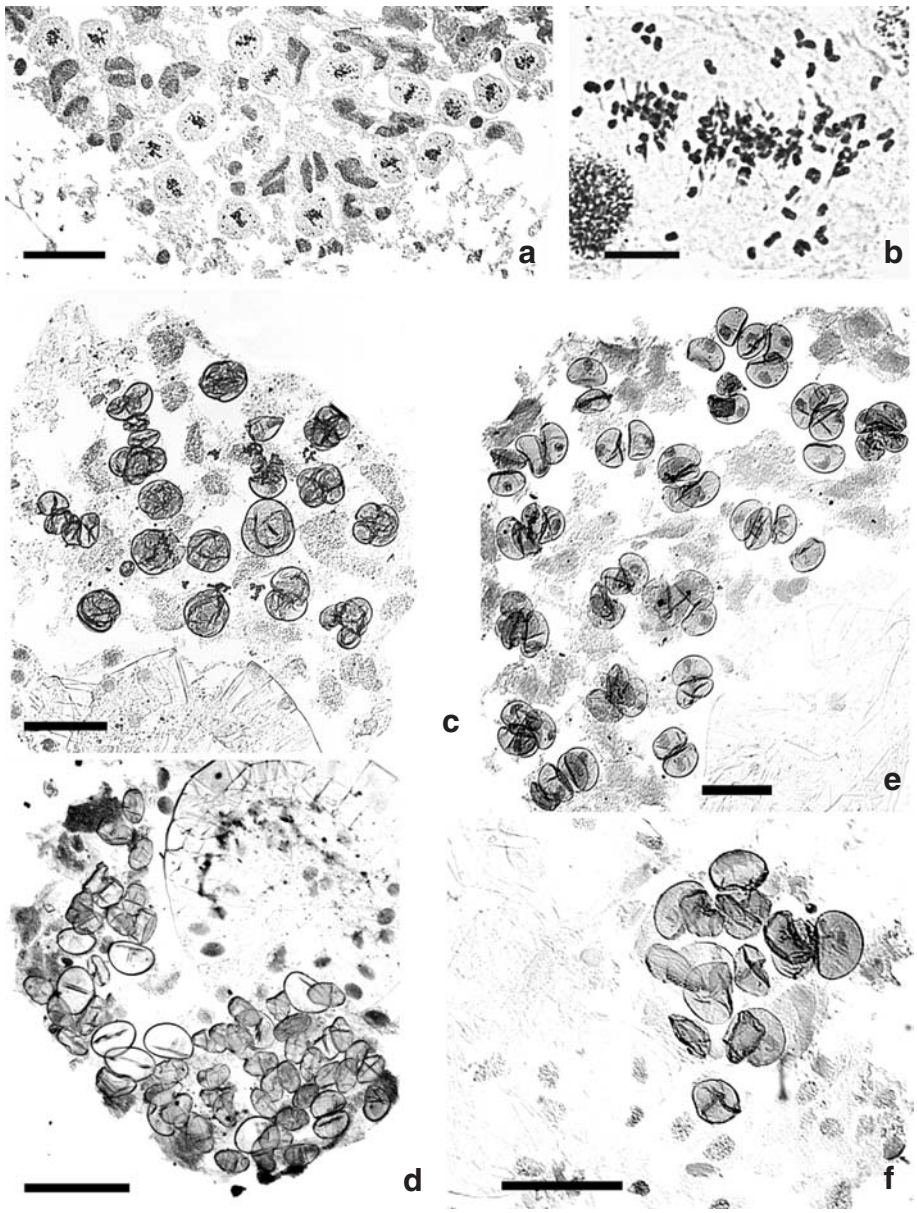

Fig. 5a–f. Spore mother cells (SMC) and young spores from single sporangia of *Cornopteris christenseniana*. **a** Sixteen SMC at anaphase (BGFGHM1). **b** Meiotic anaphase with bivalents separating and univalents scattered (BGFGHM1). **c** All abortive spores, often forming tetrads (BGCHSA044). **d** Most abortive and several normal spores of about 64 spores (BGCH30). **e** Most normal and a few abortive spores of about 64 spores, forming 16 tetrads (BGCHYMA3). **f** Most normal spores of 16 spores (BGCH27). *Bars* 10 µm in **b**; 100 µm in **a**, **c**–**f**

prior to SMC formation (Döpp-Manton system) or during meiosis (Braithwaite system) is a mechanism whereby apomictic ferns can produce unreduced spores regularly. Hickok (1977) reported that meiotic mutation results in restitution nuclei in a triploid hybrid of *Ceratopteris*, but sticky chromosomes are not found in apomictic ferns. The origin of regular endopolyploidization is an open question.

Morzenti (1967) and Evans (1969) reported that in some "sterile" hybrids, spontaneous viable spores germinated and developed into gametophytes, from which a few sporophytes developed apogamously. Pintér (1995) also reported that in the triploid hybrid *Polystichum bicknellii* (Christ) Hahne, 2–3% of spores sown germinated and gametophytes formed an extremely low number of triploid sporophytes (less than 0.01% per germinated spore or ca. 6% of 155 sporophytes produced from ca. 3.7 million spores sown), hypothetically by apogamy. These data were obtained in experimental cultures. Since apogamy can be induced in sexual or nonapomictic species under certain culture conditions (Raghavan 1989), induced apogamy does not necessarily indicate that the species reproduce apogamously in nature, and therefore, both experimental and field studies are necessary to determine apomixis in natural populations of "sterile" hybrids. Data from cytology, sporogenesis, spore germination and gametophyte culture obtained in this study, combined with field observations in the Fern Garden, show that *Cornopteris christenseniana* performs apomixis. As described below, it is incomplete or incipient compared to obligate apomixis of species that originated from sterile hybrids of sexual species (Gastony and Windham 1989; Darnaedi et al. 1990; Schneller et al. 1998) and that of *Diplazium*, a predominantly apomictic genus (Takamiya et al. 1999). No apomictic species is known in *Cornopteris*, in contrast to other genera with both obligate

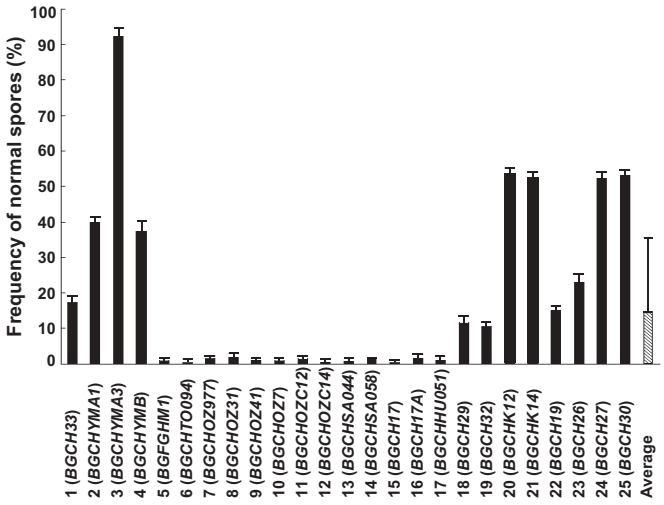

Fig. 6. Frequency of normal spores in 25 mature plants of *Cornopteris christenseniana* (numbered with given voucher numbers in parentheses; Table 1). For each plant, spores were examined in around five sporangia on each of nearly 100 pinnules. *Bars* Mean +1 SD

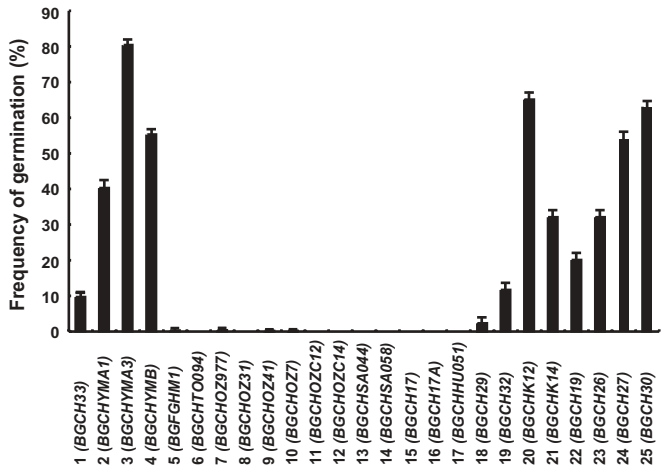

Fig. 7. Frequency of spore germination in 25 mature plants of *Cornopteris christenseniana* [numbers and voucher numbers (Table 1) as in Fig. 6]. Three random samples, each of about 200 normal spores, were examined. *Bars* Mean + 1 SD

and incipient apomictic species (e.g., *Polystichum*) (Vida and Reichstein 1975; Pintér 1995). Apomictic reproduction in the Fern Garden reasonably infers that incipient apomixis also operates in natural populations of *C. christenseniana*. Founders, when established, usually multiply vegetatively by extensive growth and branching of creeping rhizomes to colonize secondary forests – in many places *Cryptomeria japonica* forests (data not shown). This may explain, at least in part, the wide distribution of *C. christenseniana*.

Cornopteris christenseniana produces normal spores in various proportions [1–56 (92)% – figure in brackets refers to plant BGCHYMA3] in plants from different localities (Fig. 6). The rate is lower than that of natural apomicts (57–85%; Lovis 1977). In cultures, 0–80% of the normal spores

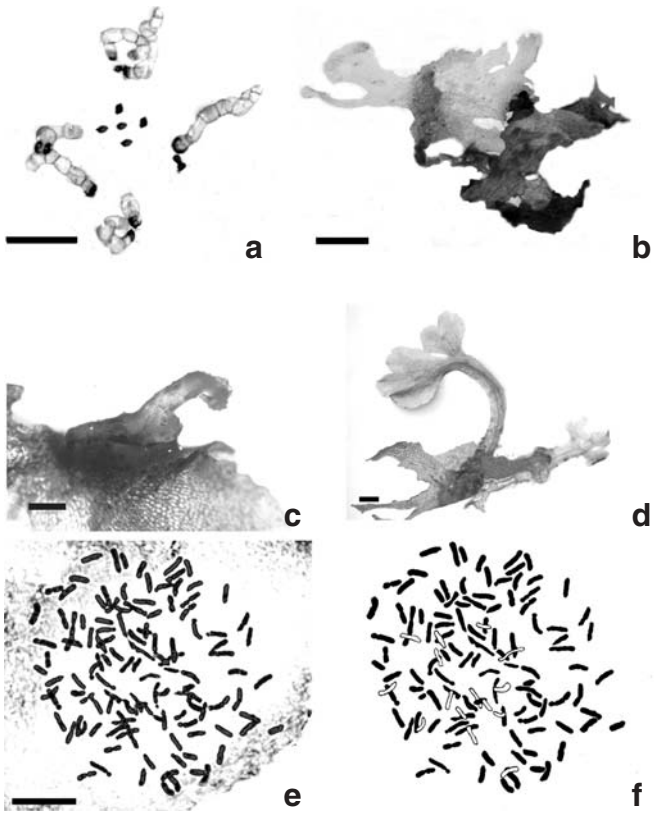

Fig. 8a–f. Young gametophytes, apogamous sporophytes and chromosomes of *Cornopteris christenseniana*. **a** Protonemata and young gametophytes (BGCHK12). Some spores did not germinate. **b** Developed gametophytes with no archegonia and antheridia formed (BGCH30). **c** Young apogamous sporophyte on gametophyte (BGCHYMB). **d** Older apogamous sporophyte with pinnate first and second leaves on gametophyte (BGCHYMA3). **e** Somatic chromosomes ($2n = 120$) of apogamous sporophyte produced from gametophyte (BGCHYMA3). **f** Explanatory drawing of **e**. Bars 100 μm in **a**; 1 mm in **b**–**d**; 10 μm in **e**

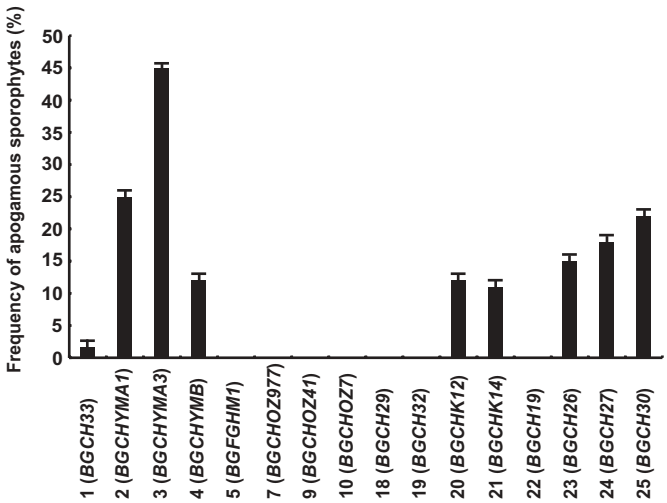

Fig. 9. Frequency of apogamous sporophytes on gametophytes from 16 mature plants of *Cornopteris christenseniana* [numbers and voucher numbers (Table 1) as in Fig. 6]. For each plant, five samples were examined. *Bars* Mean +1 SD

Table 2. Frequencies of normal spores, spore germination, apogamy, and apomixis in selected plants of *Cornopteris christenseniana*

Plant[a]	Voucher[a]	Frequency (%; mean ± SD)			
		Normal spore	Spore germination	Apogamy	Apomixis[b]
1	BGCH33	27 ± 2.21	9.6 ± 1.42	1.6 ± 1.2	0.04
2	BGCHYMA1	40 ± 1.68	40 ± 2.23	25 ± 2.2	4.0
3	BGCHYMA3	92 ± 3.45	80 ± 1.6	45 ± 2.1	33
4	BGCHYMB	38 ± 3.74	55 ± 1.52	12 ± 1.2	2.5
5	BGHM1	1.2 ± 1.25	0.2 ± 0.7	0	0
7	BGCHOZ977	2.1 ± 1.26	0.3 ± 0.3	0	0
9	BGCHOZ41	1.1 ± 1.24	0.2 ± 0.5	0	0
10	BGCHOZ7	1.1 ± 1.21	0.2 ± 0.5	0	0
18	BGCH29	14 ± 2.45	2.3 ± 1.6	0	0
19	BGCH32	12 ± 1.74	11 ± 2.1	0	0
20	BGCHK12	56 ± 2.21	65 ± 2.4	12 ± 1.3	4.4
21	BGCHK14	54 ± 2.22	32 ± 2.2	11 ± 1.2	1.9
22	BGCH19	15 ± 1.35	20 ± 2.2	0	0
23	BGCH26	34 ± 3.14	32 ± 2.6	15 ± 1.2	2
24	BGCH27	54 ± 2.32	54 ± 2.2	18 ± 1.2	5.2
25	BGCH30	55 ± 2.41	63 ± 1.6	22 ± 1.2	7.6

[a] Plant numbers and vouchers are the same as those of Table 1, Figs. 6, 7, and 9
[b] The frequency of apomixis or apomictic sporophyte per spore is the product of those of normal spore, spore gemination, and apogamy

can germinate and the frequency of spore germination is in rough proportion to that of normal spores per plant (Fig. 7). Since the frequency of spore germination was scored for normal spores selected, the germination frequency per entire spore population, whether normal or aborted, is lower [0–36 (74)%; see Table 2]. Further, gametophytes produce sporophytes apogamously at various rates in rough proportion to that of spore germination (Fig. 9). The frequency (0–36%) of spore germination and the frequency (0–45%) of apogamy are lower than that (6–76% spore germination; 56–97% apogamy) of apogamous species and hybrids of *Diplazium* (Takamiya et al. 1999). Exceptionally, plant 3 (BGCHYMA3) in Fig. 6 has comparable frequencies (74% spore germination and 45% apogamy). The frequency of apomixis (apogamous sporophyte per spore), involving sporogenesis, spore germination, gametophyte development, and apogamy in gametophytes, ranges between 0% and 8 (33)%, depending on the plant (Table 2). Plants producing a larger number of normal spores exhibit higher apomixis ability, even though it is incipient. The frequency of apomixis in most plants examined is also lower than that of *Diplazium* (7–55%; data from Takamiya et al. 1999). However, caution should be applied in evaluating these differences because of different experimental conditions. It is also noted that the apogamous species and hybrids of *Diplazium*, like many other apomictic species, show the Döpp-Manton system of sporogenesis (Takamiya et al. 1999), but in *C. christenseniana*, viable spores are produced via pathways different from those typical of apomictic ferns.

The proportional frequencies of viable spores and apogamy in *Cornopteris christenseniana*, which does not seem to possess genetic control of apomixis, suggest that viable spores have the potential for apogamy expressed at the subsequent gametophyte stage. Polyploidy (triploid) of spores and consequently that of gametophytes, or increase in gene dosage (Raghavan 1989), may be one factor of this spore-apogamy relationship (Kato 1992). The suggested relationship in this incipient apomict is similar to the hypothesis that unreduced sporogenesis has a pleiotropic effect on apogamy in apomictic species (Grimanelli et al. 1998; Savidan 2000) of the hypotheses regarding the genetics or evolution of apomixis. If sporadic viable unreduced spores have such a pleiotropic effect, apomixis may be found in various "sterile" hybrids between different species and in multiple clones of a "sterile" hybrid. The various frequencies of viable spores and apogamy may infer the possibility that *C. christenseniana* includes multiple crosses of *C. crenulatoserrulata* and *C. decurrenti-alata*. However, the question of whether the origin of *C. christenseniana* is multiple or not should be tested by further study. It is also uncertain whether plants with lower frequency of apomixis appeared more recently than those with a higher frequency, or if plants show various frequencies independently of the time of origin.

The cytotype with 120 somatic chromosomes ($2n$) and aneuploids with 121, 122 and 126 chromosomes in the young and mature plants of *Cornopteris christenseniana* examined were morphologically indistinguishable. One of the young plants is an aneuploid with 121 chromosomes, while all others are a cytotype with 120 chromosomes. It is suggested that the young plants may be derived from the two different aneuploids. Aneuploidy is closely related with polyploidy (Grant 1981), and aneuploids are reported in various polyploid pteridophytes (Lovis 1977). Aneuploids are reported several Japanese species (Takei 1974, 1978; Nakato et al. 1983; Nakato 1989, 1998a, 1998b; Takamiya et al. 1994). In *C. christenseniana*, the cytotype with 120 chromosomes is the most common and widely distributed and the other aneuploids occur along with the common cytotype in various populations, suggesting spontaneous origin. However, it is uncertain how this apparent

aneuploid increase occurs during the propagation of *C. christenseniana*.

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