

Kunio MITUI\*: **Correlation between the chromosome numbers and morphological characters in the genus *Lepisorus***

三井邦男\*: ノキシノブ属の染色体数と形態学的諸形質との関連性について

*Lepisorus* is a very unique genus in the family Polypodiaceae (sensu stricto) in view of the chromosome numbers. Although the most genera of the Polypodiaceae have one or two basic chromosome numbers ( $x=36, 37$ ), various basic numbers have been reported in the genus *Lepisorus* ( $x=24, 25, 26, 35, 36, 37, 47$ ). Up to this time, the chromosome numbers of 5 species and 1 variety are reported in Japan, and it seems that this genus can be divided into two groups from the view point of the basic chromosome numbers. One is species complex with the numbers of  $x=25$  or  $26$  and the other  $x=35$ . On the other hand, two distinct groups have been recognized basing on some morphological characters (as the rhizome scales or paraphyses) in this genus. For example, Tagawa (1959) classified 6 species and 1 variety into two groups basing on the morphology of scales in Japanese *Lepisorus*; one is the group with transparent scales and the other has the opaque scales. Furthermore, it also has been reported that some morphological characters (for instance the shapes or sizes of scale cells) vary with the change of chromosome numbers, such as polyploidy and aneuploidy (Mitui, 1968). The present paper is concerning with the correlation between the chromosome numbers and some taxonomically important characters (e. g. rhizome scales, paraphyses, the size and shape of stomata and the season of spore maturation). I wish to express my sincere appreciation to Prof. Hiroshi Ito of Tokyo Kyoiku University for his kind suggestion. Thanks are also due to Prof. Satoru Kurata of Faculty of Agriculture, University of Tokyo, for his kindness in providing me with the important specimens.

**Materials and methods**

The gametic chromosome numbers were determined from meiosis in spore mother cells with the usual aceto-carmine squash method. The somatic

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chromosome numbers were counted in the root tips with the following procedure. Root tips were immersed for two hours in 0.01% colchicine at 16°C and fixed in 1:3 acetic alcohol for 20 minutes, and hydrolyzed for 1 minute in 1N Hydrochloric acid solution at 60°C and stained with acetocarmine. Rhizome scales were collected from the specimens which were authenticated cytologically and the herbarium specimens in Botanical Institute, Faculty of Science, Tokyo Kyoiku University and Faculty of Agriculture, University of Tokyo. The scales were mounted on the glass with Canada balsam, and their shapes, sizes and colors were observed. The leaf blades with mature sporangia were immersed in 1% NaOH for the observation of stomata size and shape.

### Observations

1. *Chromosome numbers* Table 1 shows the chromosome numbers of Japanese *Lepisorus*. As shown in table 1, two cytotypes were found in *L. uchiyamae*. One has the chromosomes of  $n=70$  (Fig. 1, D), and 64 normal spores per sporangium were counted, and the other has  $2n=95$  (Fig. 1, H) and shows irregular meiosis. The former cytotype ( $n=70$ ) was found in Ashizurimisaki Kochi Pref., and Takisawa in Shizuoka Pref. The latter ( $2n=95$ ) form was collected from Matsuzaki, Izu Peninsula, Shizuoka Pref., and it shows approximately 30 bivalents and 35 univalents in meiosis (Fig. 1, B) and their spores show almost sterile shapes. From these cytological evidences, it seems that the cytotype with  $2n=95$  should be a hybrid between *L. uchiyamae* with  $n=70$  and such a species with  $n=25$  as the diploid type of *L. thunbergianus* which is found commonly in Izu Peninsula. *L. mikawanus* is known only from the type locality, which is broken recently, and I could not observe the chromosome number of this species. However, the specimens of *L. thunbergianus* which were collected near the type locality of *L. mikawanus*, in Shinshiro City Aichi Pref., were tetraploids with  $n=50$ . I reported  $n=26$  for *L. thunbergianus* var. *angustus* and in the present study, it was made clear that this species had two cytotypes, diploid ( $n=26$ ) and tetraploid ( $n=52$ ) (Fig. 1, E) as well as *L. thunbergianus*. From the table 1 it is clear that the Japanese *Lepisorus* can be divided into two groups in the view of the basic chromosome numbers. The first group has the basic number of  $x=25$  or 26 and the other  $x=35$ . Further, it may be said that each group has the similar cytological divergence from the evi-

Table 1. Chromosome number of Japanese *Lepisorus*.

Species	Chromosome number	Locality	Worker
<i>L. hachijoensis</i>	n=25*	Hachijo-Isl., Tokyo Pref., MCN 648	
<i>L. oligolepidus</i>	n=c. 50*	Toyamagawa, Nagano Pref., MCN 650	
<i>L. onoei</i>	n=25	Kawamata, Saitama Pref., MCN 268, Ozaku, Tochigi Pref., MCN 490	Mitui 1965 Mitui 1968
<i>L. thunbergianus</i>	n=25	Hashimoto, Kanagawa Pref., MCN 401, Taura, Kanagawa Pref., MCN 402	Kurita 1967 Mitui 1966 Mitui 1968
	n=75/2	Kawai, Tokyo Pref., MCN 500	Mitui 1968
	n=50	Hanno, Saitama Pref., MCN 400, Kawai, Tokyo Pref., MCN 499	Mitui 1965 Mitui 1968
		Shinshiro, Aichi Pref., MCN 645	
	2n=50*	Oshima Isl., Tokyo Pref., MCN 693	
<i>L. thunbergianus</i> var. <i>angustus</i>	n=26	Kawamata, Saitama Pref., MCN 614	Mitui 1968
	n=52*	Toyamagawa, Nagano Pref., MCN 646	
<i>L. tosaensis</i>	n=75*	Danto, Kumamoto, Pref., MCN 649	
<i>L. annuifrons</i>	n=35	Toyamagawa, Nagano Pref., MCN 653	Mitui 1970
<i>L. clathratus</i>	n=c. 70 2n=c. 140	Mt. Toyoguchi, Nagano Pref., MCN 637	Mitui 1970
<i>L. ussuriensis</i> var. <i>distans</i>	n=35	Mameyakizawa, Saitama Pref., MCN 611	Mitui 1968
<i>L. uchiyamae</i>	n=70*	Takisawa, Shizuoka Pref., MCN 710	
	2n=95*	Matsuzaki, Shizuoka Pref., MCN 654	

\* An asterisk indicates the first report of chromosome number.

dence of equal incidence of polyploidy in each group.

2. *Scales* The scales on the rhizome of this genus consist of single cell layer and the young ones have one glandular cells at their tips. The

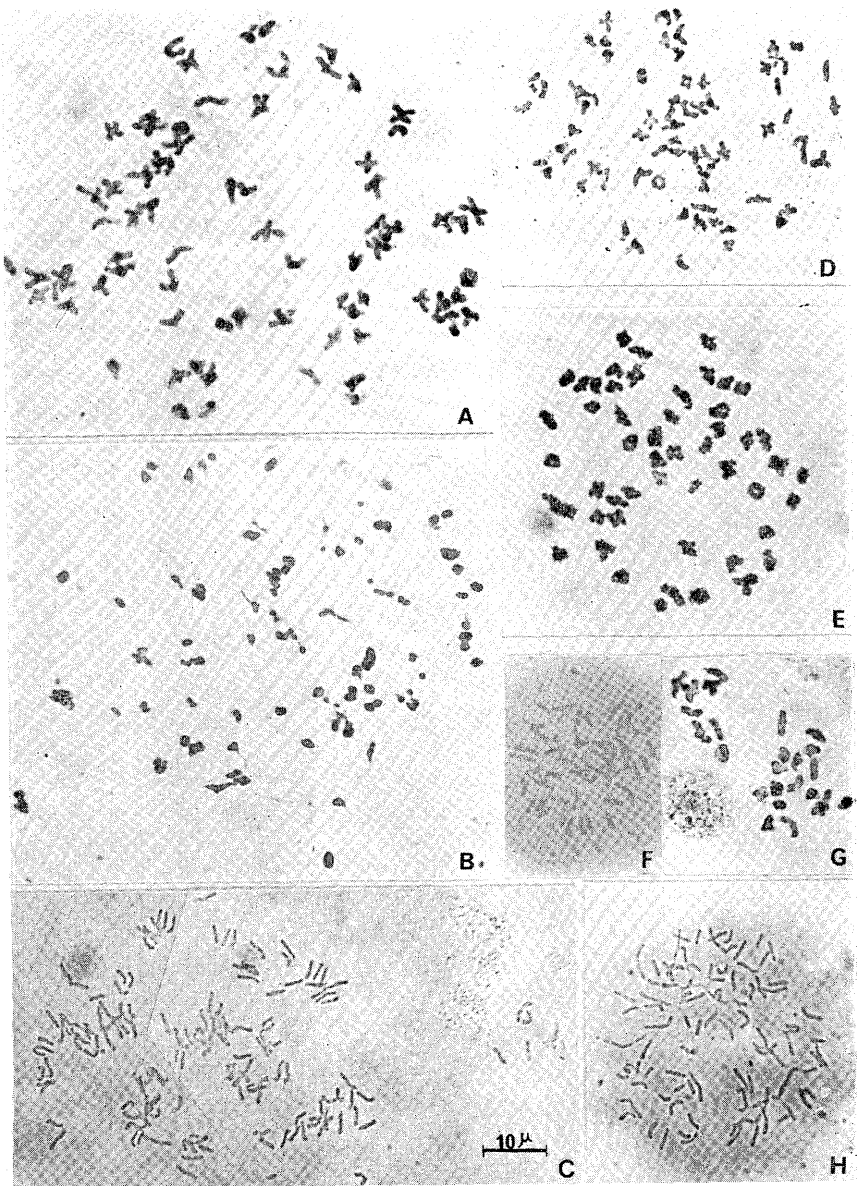


Fig. 1. Chromosomes of *Lepisorus*. A. Meiosis in *L. tosaensis* ( $n=75$ ). B. Meiosis in *L. uchiyamae* (hybrid). C. Mitotic metaphase in root-tip cell in *L. clathratus* ( $2n=c. 140$ ). D. Meiosis in *L. uchiyamae* ( $n=70$ ). E. Meiosis in the tetraploid of *L. thunbergianus* var. *angustus* ( $n=52$ ). F. Mitotic metaphase in root-tip cell in *L. thunbergianus* ( $2n=50$ ). G. Meiosis in *L. hachijoensis* ( $n=25$ ). H. Mitotic metaphase in root-tip cell in *L. uchiyamae* (hybrid,  $2n=95$ ).

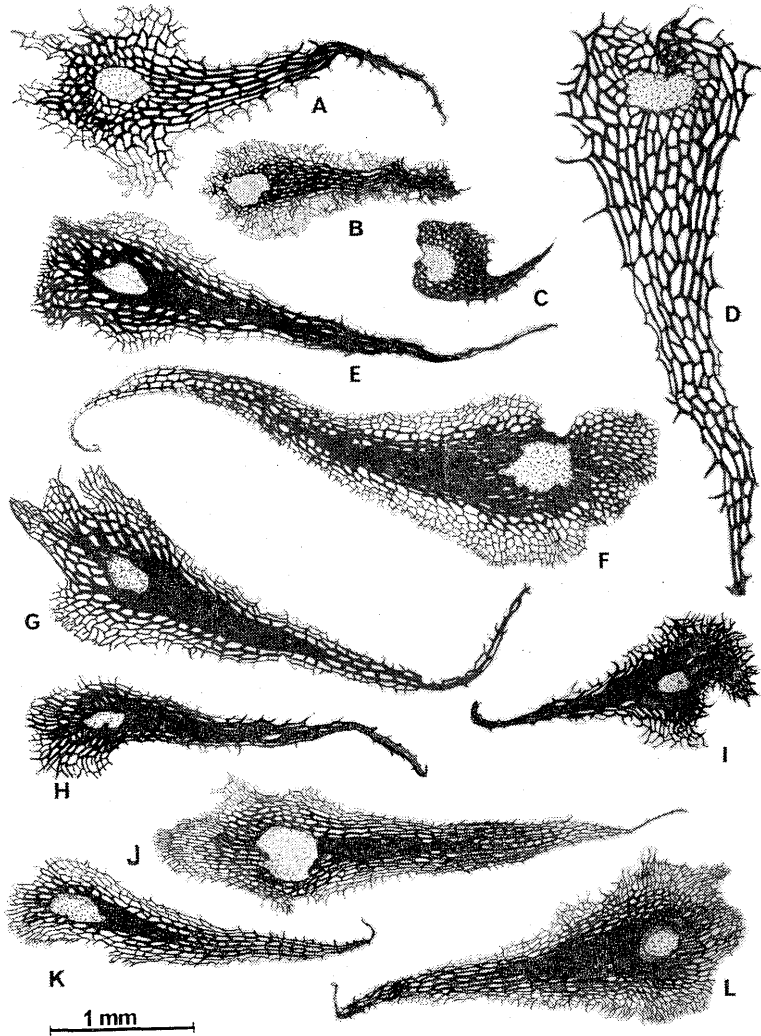


Fig. 2. Scales of *Lepisorus*. A. *L. uchiyamae* (tetraploid). B. *L. annuifrons*. C. *L. ussuriensis* var. *distans*. D. *L. clathratus*. E. *L. thunbergianus* (tetraploid). F. *L. thunbergianus* (diploid). G. *L. mikawanus*. H. *L. oligolepidus*. I. *L. tosacnsis*. J. *L. hachijoensis*. K. *L. onoei*. L. *L. thunbergianus* var. *angustus* (diploid).

size and shape of rhizome scales are different from each species. For example, *L. clathratus* has the largest ovate-lanceolate scales (5 mm long) (Fig. 2, D), and *L. ussuriensis* var. *distans* has the smallest deltoid scales (0.5 mm long) in this genus (Fig. 2, C). Usually the anticlinal walls of scales are more or less thickened and these walls are brown with tannin (Hutoh, 1969). However, there are some differences of the thickening in the surface walls of the scales in this genus. For instance, the surface walls of *L. thunbergianus* are much thickened but those of *L. clathratus* are never thickened. The degree of the cell-wall thickening is different among the species. Generally, the rhizome scales of this genus may be divided into two types from the standpoint of cell-wall thickening. The first has thickened anticlinal cell-walls with blackish brown but the outer walls never thickened; thus the scales are always transparent and clearly clathrate (transparent group) (Fig. 2, A-D). The second type is the scale in which the cell-wall thickening is formed in their vertical walls and in the surface walls along the longitudinal axis. Consequently the scales of this type have the bicolor, the blackish brown midrib and the transparent margin (opaque group) (Fig. 2, E-L).

*Opaque group*: Six species belong to this group, and all of this group have the chromosome numbers of  $x=25$  or  $26$ . There is certain correlation between increasing of chromosome numbers and cell-wall thickening. In intraspecific polyploids of *L. thunbergianus* and var. *angustus*, the scales of polyploids have much thickened walls than those of diploids. In this regard, the same tendency is observed among species in this group. *L. thunbergianus* var. *angustus* ( $n=26$ ) has more thickened walls than those of *L. onoei* ( $n=25$ ) and *L. hachijoensis* ( $n=25$ ) which have similar cell-wall thickenings. Therefore, the area along the longitudinal axis is blackish brown in the former but it is light brown in the latter. Furthermore, the most thickened walls are observed in the scales of species with the high level commensurate of chromosome numbers, as *L. tosaensis* ( $n=75$ ) and the tetraploids of *L. thunbergianus* and var. *angustus*. In this group *L. tosaensis* seems to be different from other species in the point of the arrangement of cells in the scales. The cells of other species are arranged regularly along the longitudinal axis of scales, but the cell arrangement of *L. tosaensis* is irregular. The polyploids of *L. thunbergianus* and var. *angustus*

never show such irregularity of cell arrangement. Thus, it seems that the irregular cell arrangement of scales is not polyploid character but the specific character of *L. tosaensis*. Furthermore it may be said that the scale with the irregular arrangement is more primitive than those with regular arrangement, because irregular cell-arrangement is observed only in *L. tosaensis* having the highest incidence of polyploidy in this genus. The degree and type of cell-wall thickening in *L. mikawanus* are closely related to those of the tetraploid of *L. thunbergianus* (Fig. 2, E, G).

*Transparent group*: Four species belong to this group and they have the basic number of  $x=35$ . In the opaque group the scales of all species are closely similar each other, but two types of scales are observed in the transparent group. The first is the small scales with much thickened anticlinal walls. This type of scales was found in *L. annuifrons* and *L. ussuriensis* var. *distans* (Fig. 2, B, C). In spite of low incidence of polyploidy these species have the scales consisting of thicker anticlinal walls, but there are some differences between these two scales. The anticlinal walls are thick along the longitudinal area in the scales of *L. annuifrons*, but in *L. ussuriensis* var. *distans* the thickenings occur all over the scales uniformly. In both species it is sometimes observed that there are some opaque cells originated from the thickening of anticlinal walls, but not thickening of surface walls. The second type is the larger and clearly clathrate scales which is observed in *L. clathratus* and *L. uchiyamae* (Fig. 2, A, D). These species have the scales with anticlinal walls thickened faintly uniformly, notwithstanding the high level commensurate of chromosome number. As mentioned in observation 1, *L. uchiyamae* has two cytotypes, tetraploid with number of  $n=70$  and hybrid with  $2n=95$ , and there are some differences in each scales. They have similar size and shape of scales but the tetraploid scales have no thickened surface walls; on the other hand, the scales of hybrids have one or more thickened surface walls which are considered to be originated from one parent species with thick surface walls in the scales.

As mentioned previously the thickening of surface walls are observed only in the scales of the species with the basic number of  $x=25$  or  $26$  and are not found in the species with number of  $x=35$ . In the former group the polyploidy seems to induce the more thickening of cell wall but in the latter the polyploidy have no role of inducing of cell-wall thickening. Thus,

it may be said that the thickening of cell walls has no direct correlation to the polyploidy and the polyploidy increase merely the thickening of walls in some species.

3. *Paraphyses* Hutoh (1969) described the paraphyses of this genus (except *L. mikawanus*, *L. hachijoensis* and *L. oligolepidus*) (Fig. 3) and reported that the paraphyses were originated from the rhizome scales; that is, the linear lanceolate rhizome scales decrease gradually their sizes and become circular paraphyses. Therefore, the ovate or lanceolate paraphyses are considered to be more primitive than the circular ones. He observed several characters of paraphyses and discussed the evolution of them in this genus. Judging from his description the paraphyses of this genus can be classified into two groups as shown in rhizome scales. One is the paraphyses of the species with the basic number of  $x=25$  or 26 and the other is that of  $x=35$ . In the former group all species have closely related circular paraphyses but in the latter group, each paraphyses of four species is different from one another, especially, *L. clathratus* has the most specific lanceolate paraphyses. The primitive characters of paraphyses (determined by Hutoh) are found mostly in the species having the basic number of  $x=35$ , especially polyploid species. In the species with  $x=25$ , *L. tosaensis* ( $n=75$ ) seems to have the primitive characters in the paraphyses. *L. mikawanus* has the same paraphyses as *L. thunbergianus*, and the paraphyses of *L. oligolepidus* are similar to those of species with  $x=25$ . There is no clear difference between the paraphyses of two cytotypes of *L. uchiyamae*.

4. *Epidermal cells and stomata* Fig. 4 shows the epidermal cells and stomata in 10 species, and it may be said that relatively the epidermal cell-walls are smooth in  $x=25$  group and they are rugged in  $x=35$  group. However, this difference is not so clear as shown in rhizome scales and paraphyses. Fig. 5 shows the length of stomata in these species, and from this figure, it is clear that the sizes of stomata have no connection with the cytological groups, but it is closely related to the incidence of polyploidy of each species. In both cytological groups, the diploid stomata are smaller than those of polyploids. The largest stomata are observed in *L. clathratus* ( $n=70$ ) and *L. tosaensis* ( $n=75$ ).

5. *The season of spore formation* The season of spore formation varies with different habitats of species but as far as my cytological observations



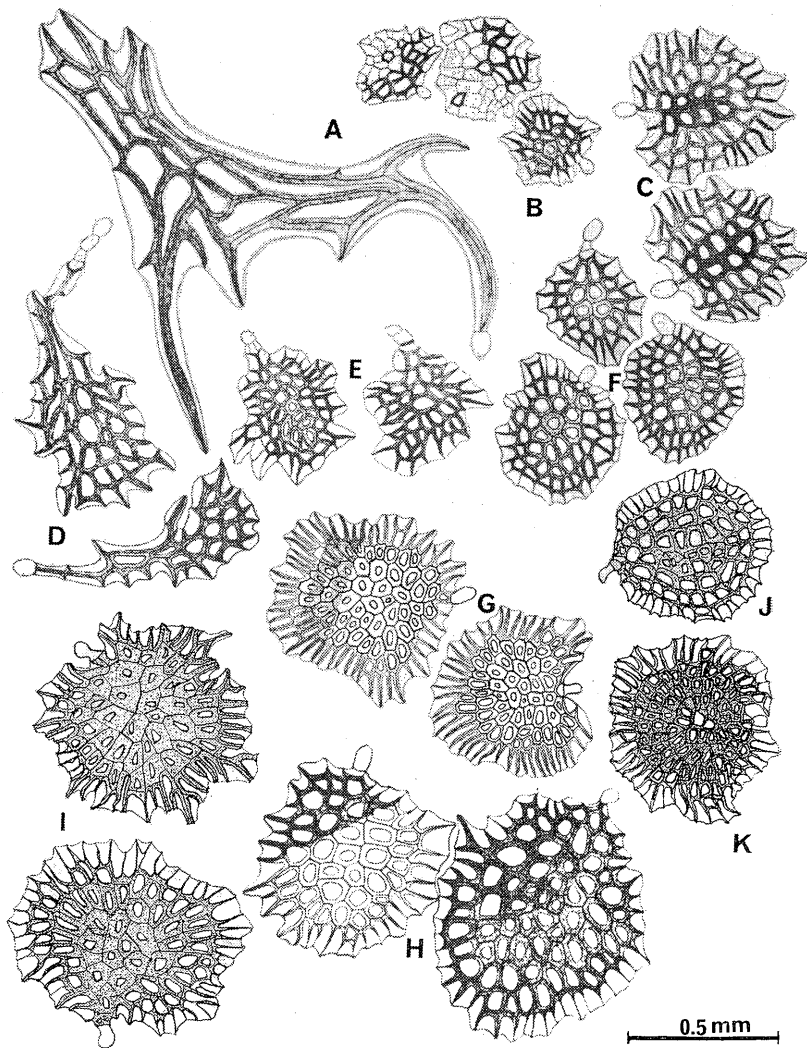


Fig. 3. Paraphyses of *Lepisorus*. A. *L. clathratus*. B. *L. ussuriensis* var. *distans*. C. *L. tosaensis*. D. *L. uchiyamae*. E. *L. annuiifrons*. F. *L. onoei*. G. *L. thunbergianus* var. *angustus*. H. *L. thunbergianus*. I. *L. mikawanus*. J. *L. hachijoensis*. K. *L. oligolepidus*. (A-H: after Hutoh, 1969).

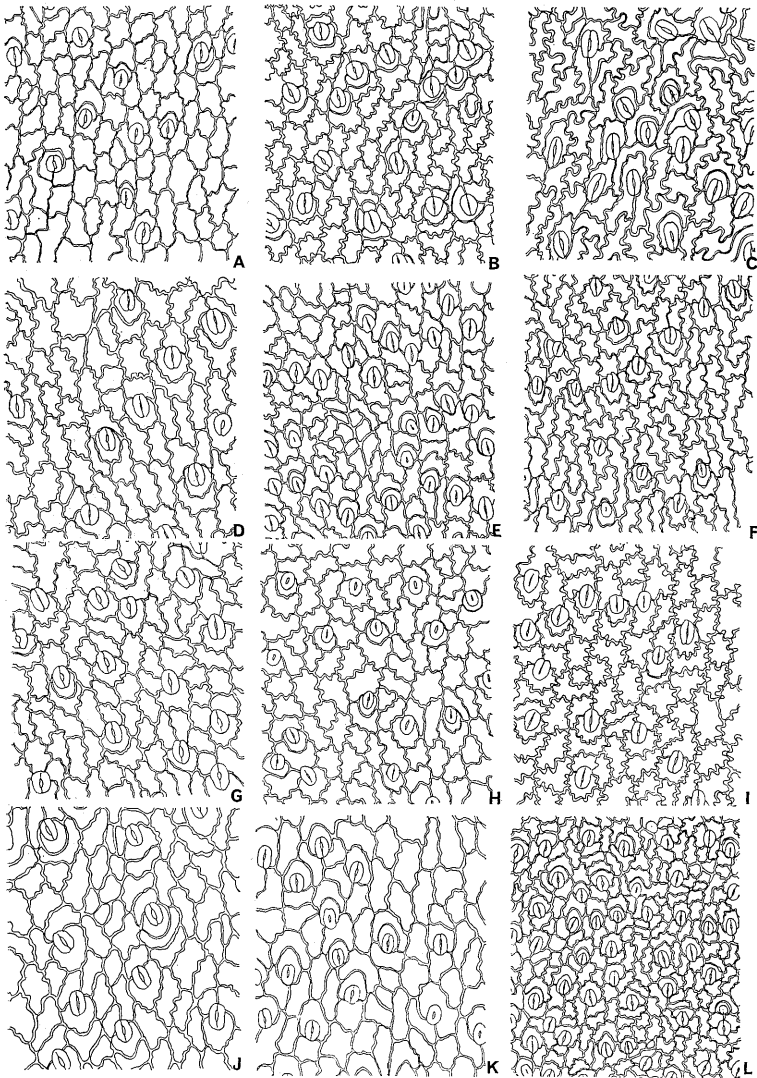


Fig. 4. Epidermal cells of *Lepisorus* ( $\times 200$ ). A. *L. thunbergianus* (2x). B. *L. thunbergianus* var. *angustus* (4x). C. *L. clathratus*. D. *L. thunbergianus* (tetraploid collected from Shinshiro City). E. *L. thunbergianus* var. *angustus* (2x). F. *L. annuifrons*. G. *L. oligolepidus*. H. *L. onoei*. I. *L. uchiyamae* (4x). J. *L. tosaensis*. K. *L. hachijoensis*. L. *L. ussuriensis* var. *distans*.

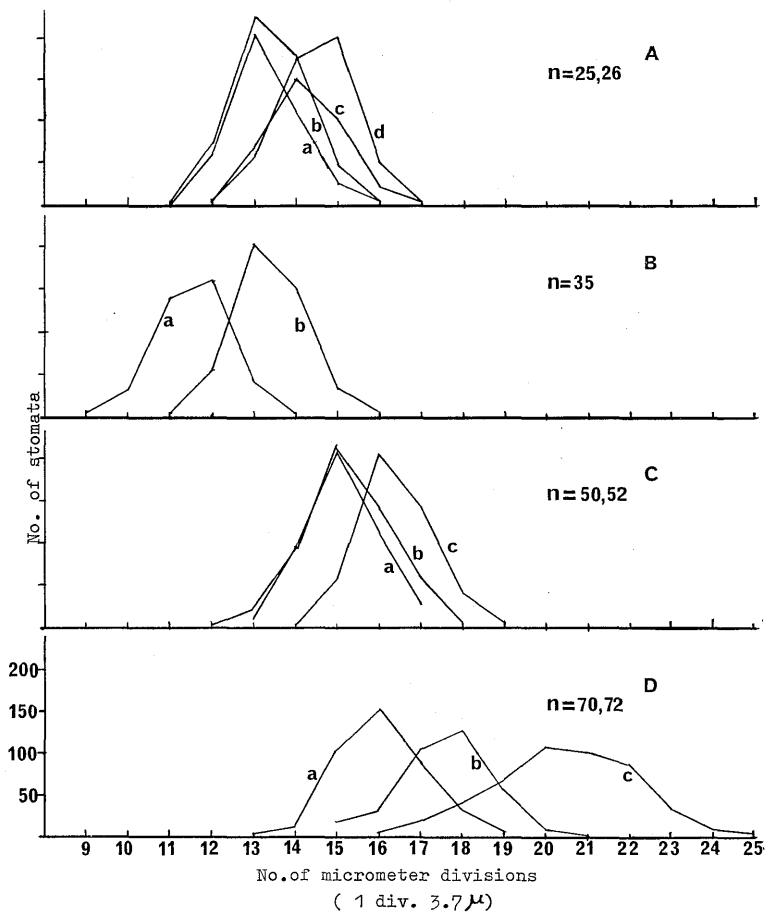


Fig. 5. Length of stomata in *Lepisorus*. A. a. *L. onoei*, b. *L. thunbergianus* var. *angustus* (2x), c. *L. thunbergianus* (2x), d. *L. hachijoensis*. B. a. *L. ussuriensis* var. *distans*, b. *L. annuifrons*. C. a. *L. oligolepidus*, b. *L. thunbergianus* var. *angustus* (4x), c. *L. thunbergianus* (4x). D. a. *L. uchiyamae* (4x), b. *L. tosaensis*, c. *L. clathratus*.

concerned, their seasons are shown as Fig. 6 in Japan. From this figure, it is clear that two cytological groups have each different season. For example, the spore formation of *L. thunbergianus* begins in November at the earliest and at latest it seems to finish by May. On the contrary, *L. annuifrons* seems to make spores between May and September. In spite of different groups, *L. clathratus*, *L. ussuriensis* var. *distans* and *L. thunber-*

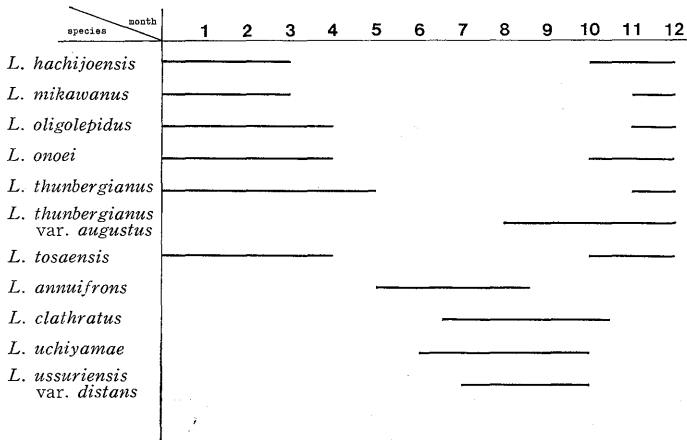


Fig. 6. Season of spore formation.

*gianus* var. *angustus* have similar season of spore formation. However, this similarity seems to be caused by their similar habitats as montane zone.

### Discussion

In addition to the five points observed in the above, some morphological characters are used for the classifications of the genus, for example the color of stipe, the width of leaf, the length of rhizome, the position of sori and the texture of leaf. These characters are sometimes useful for identification of species, but these are very variable and are considered to have no phylogenetic value in this genus. As mentioned above, Japanese species of this genus may be divided into two cytological groups basing on the basic chromosome numbers of  $x=25$  or  $26$  and  $x=35$ . Each group shows the qualitative differences in rhizome scales and paraphyses which are used in the classification of this genus. The species of  $x=25$  group are closely related each other in such characters as mentioned above. In this group, *L. tosaensis* ( $n=75$ ) may be different from other species judging from their rhizome scales and paraphyses, and this species seems to be primitive. In the other group ( $x=35$ ), *L. annuifrons* and *L. ussuriensis* var. *distans* are separated from another two species in the cytological and morphological character. However, the affinity between former two species seems to be faint. Especially, *L. ussuriensis* var. *distans* has the most specific characters

in rhizome scales and paraphyses. From these results, it may be said that this genus consists of two phylogenetic elements. The first is the species which have the basic chromosome numbers of  $x=25$  or  $26$ , opaque scales and circular paraphyses. The second is the species based on  $x=35$  and they have the transparent scales and non-circular paraphyses. The former is considered the species group in which rapid speciation is occurring, and is youthful genetically. On the contrary, the latter seems to be the group that consists of species differentiated in remote phylogenetic age. Therefore, some primitive characters are found among the species of this group. Furthermore, it may be said that polyploidy is one process of maintaining old characters for a long time, because the polyploids have some primitive characters in this genus. As far as this study concerned, *L. mikawanus* seems to be one type of tetraploids of *L. thunbergianus* and this species will be the first example in ferns that autotetraploid is recognized to be a species. Finally, *L. thunbergianus* var. *angustus* can be separated specifically from typical variety as Ching originally described it as *L. angustus*.

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日本産のノキシノブ属の染色体数と形態学的形質との関連性について論じた。染色体数から、この属は大きく  $x=25, 26$  の群と  $x=35$  の 2 群に分けられる。一方従来この属の分類の重要な形質とされている鱗片、側糸からもこの属は 2 群に分けられる。今回細胞学的な群と形態学的な群とが一致することが明らかにされた。即ち  $x=25, 26$  の群に含まれる各種は不透明な鱗片と円形の側糸を持ち、他方  $x=35$  の群の各種は透明な鱗片と披針形や広卵形の側糸を持っている。この他孢子形成の時期や表皮細胞の形にも両群の相違が観察された。 $x=25, 26$  の群に含まれる 6 種は先に述べた形質が非常に類似していて強い近縁性を示すが  $x=35$  の群の 4 種では各形質がそれぞれの種

で非常に特殊化を示し、これら4種の類縁性の薄いことを示している。以上のことから日本産のノキシノブ属は系統的に異なる2群より成り立っているように思われる。そして  $x=25, 26$  の群は盛んに種分化の起りつつある群であり、他方  $x=35$  の各種はかなり特殊化のすすんだものであると考えられる。鱗片、側糸の原始的と思われる形質の多くは  $x=35$  の種類、特にコウラボシ、トヨグチウラボシのように高倍数体の種に観察された。 $x=25, 26$  の群でも最高の染色体数を持つツクシノキシノブの鱗片、側糸に原始性が観察された。

○台湾産ミヤマハタザオについて (井上賢治) Kenji INOUE: *Arabis lyrata* L. subsp. *kamtschatica* (Fisch.) Hultén in Formosa

台湾のナズナハタザオは1908年に *A. taraxacifolia* として早田文蔵博士により報告され、後に、区別され *A. morrisonensis* として1911年に記載発表された。この種については、伊藤武夫が台湾植物図譜に、日本のミヤマハタザオに似ているが、毛の多少、葉の切込などの点を違いにあげている。今回台湾のナズナハタザオと、日本産のミヤマハタザオを比較してみたところ、ミヤマハタザオは毛を密布するものから少ないもの、葉の切込は浅裂のものまで、広い変異をみせ、台湾のナズナハタザオもその範囲に入るものであるという考えに到った。従って、北アメリカから日本までの分布域が台湾にまで広げられることになる。尚、お世話になった東京農大、植物学研究室の先生方、及び、東京大学、総合研究資料館の先生方にお礼申し上げます。

(東京農業大学植物学研究室)

***Arabis lyrata* L. subsp. *kamtschatica* (Fischer) Hultén, Fl. Aleut. Is. 202 (1937).**

*A. morrisonensis* Hay., Mat. Fl. Form. 29 (1911). **syn. nov.**

*A. taraxacifolia* (non Anders.) Hay., Fl. Mont. Form. 49 (1908).

Hab. Formosa: Mt. Morrison (S. Nagasawa Nov. 3, 1905 KYO, T. Kawakami et U. Mori Oct. 20, 1906 No. 2234 TI, Chien-chang Hsu et R. Hsu Sept. 29, 1967 No. 4018 TI, Chien-chang Hsu May 17, 1969 No. 5342 TI, T. Nakamura July 10, 1968 No. 125, S. Masuda July 9, 1968 No. 107, S. Matsumoto July 18, 1970 No. 1232). Mt. Sylvia (T. Nakamura July 22, 1968 No. 466 TI, S. Masuda July 22, 1968 No. 465 TI, K. Inoue July 21, 1968 No. 464 TI). Pref. Nan-tou, Mt. Neng-Kao (M. Tamura et H. Koyama Aug. 14, 1964 No. 245419 NSM). Pref. Ilan in route from Nan-shan (Piyanan) to Mt. Chung-yangchien, along River Matakotou (M. Tamura et H. Koyama Aug. 28, 1964 No. 245448 NSM).

Distr. N. America, Alaska, Aleutian Islands, Kamchatka, Sakhalin, Japan, Korea, and Formosa.