# 日本産オオバコ属植物数種の核型分析とトウオオバ コの分類学的位置

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# Kazuhito MATSUO\* and Junko NOGUCHI\*\*: Karyotype Analysis of Several Plantago Species in Japan, with Special Reference to the Taxonomic Status of Plantago japonica

松尾和人\*・野口順子\*\*:日本産オオバコ属植物数種の核型分析と トウオオバコの分類学的位置

#### Abstract

A karyological analysis was conducted to re-evaluate the taxonomic status of two endemic plantain species, *Plantago japonica* and *P. togashii*. *P. japonica* and *P. togashii* had the same chromosome number of 2n = 12, and showed almost identical karyotypes, suggesting their close affinity, and thus the result supports the current taxonomic conclusion made based upon critical morphological, populational as well as ecological studies by MATSUO (1989 a), that is *P. togashii* is conspecific with *P. japonica* in spite of the high variability. For comparison, the chromosome numbers and karyotype constitutions of three other indigeneous species, *P. asiatica*, *P. yakusimensis* and *P. camtschatica*, and two introduced species *P. major* and *P. lanceolata* were also investigated. Although *P. asiatica* and *P. yakusimensis* had the same tetraploid chromosome number of 2n = 24, their basic karyotype were different, i.e., in the position of the constriction of the chromosomes with satellites. The diploid chromosome number of 2n = 12 was confirmed for *P. camtschatica*, *P. lanceolata*, and *P. major* in the present study.

**Key Words**: Karyotype — Chromosome number — *Plantago togashii* — *Plantago japonica* — *Plantago* 

Several Plantago species referred to Section Plantago (sensu PILGER, 1922 and in ENGLER, 1937) are known to occur in the Japanese Islands and its neighborhood (OHWI, 1953, 1973; KITAMURA and MURATA, 1961; HARA, 1948). They are represented by four native species, P. asiatica L., P. japonica FRANCH. et SAVAT., P. togashii MIYABE et TATEWAKI, and P. yakushimensis MASAMUNE, and one introduced species. P. major L. (HARA, 1948; MATSUO, 1989a,b). However, the taxonomic status of these taxa, e.g., P. japonica and P. togashii, has long been con-During the course of a series of population biology and comparative life history studies of the genus Plantago in Japan (KAWANO and MATSUO, 1983; MATSUO, in preparation), it became necessary to redefine the status of these taxa based upon their ecological as well as geographical variations in gross morphology, and their biological features. The present study is

part of such attempts and a supplement to biosystematic revision of the genus Plantago in Japan, with special reference to the status of P.  $japonica\ sensu\ stricto$ .

Earlier karyological studies of the Japanese *Plantago* species are limited to those by MCCULLAGH (1934), OHNO (1953), and FUJIWARA (1955, 1956a,b). In the present study, we report the somatic chromosome numbers and karyotypes of six *Plantago* species in Japan, of which four taxa (*P. asiatica, P. japonica, P. camtschatica*, and *P. yakushimensis*) are indigenous and two (*P. major* and *P. lanceolata*) were introduced to the Japanese Islands.

#### Materials and Methods

The taxa, sampling sites, and their habitats surveyed in this study are shown in Table 1. The somatic chromosome numbers and karyotypes were observed in the root tip cells of juvenile

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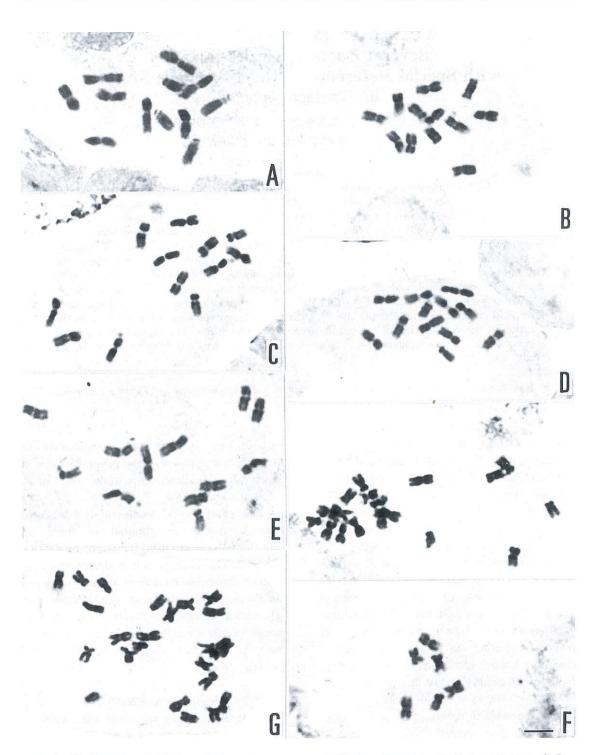


Fig. 1. Photographs of mitotic metaphase chromosomes. A, *Plantago lanceolata*; B, *P. camtschatica*; C, *P. japonica* from Sakaeura population in Hokkaido; D, *P. japonica* from Choujyagasaki population in Honshu; E, *P. major*; F, *P. asiatica*; and G, *P. yakushimensis*. The bar represents 3  $\mu$ m.

Table 1 Locality and habitats of the sampling sites observed in this study.

Species	Localities	Habitats	
Plantago lanceolata L.	Hokkaido University, Sapporo city, Hokkaido	Grassy site	
P. camtschatica CHAM.	Barou, Yubetsu-cho, Monbetsu-gun, Hokkaido	Coastal grasslands of Lake Saroma	
P. japonica FRANCH. et SAVAT.	Horonai, Okushiri Island, Hokkaido	Rocky seashore	
	Sakaeura, Tokoro-cho, Tokoro-gun, Hokkaido	Salt marsh of Lake Saroma	
	Barou, Yubetsu-cho, Monbetsu-gun, Hokkaido	Salt marsh of Lake Saroma	
	Choujyagasaki, Zushi city, Kanagawa Prefecture	Sandy beach	
P. major L.	Hokkaido University, Sapporo city, Hokkaido	Roadside	
P. asiatica L.	Heiwa, Nishi-ku, Sapporo city, Hokkaido	Grassy site by the trail in Teine hill	
P. yakushimensis MASAM.	Miyanouradake, Yakushima Island, Kagoshima Prefecture	Rocky site near the summit	

Table 2 The somatic chromosome numbers of the *Plantago* species examined in this study.

Species	Present study		Previous reports
Plantago lanceolata L.	2n=12	2n=12	GADELLA & KLIPHUIS 1966; 1970a,b; RUNEMARK 1967; TAYLOR & MULLIGAN 1968; PODLECH & DIETERLE 1969; ARYAVAND 1980; STRID & FRANZEN 1981; GONZALEZ & SILVESTRE 1980; Van Den BRAND et al. 1979; KLIPHUIS & WIEFFERING 1979; LESSANI & CHARIAT-PANAHI 1979; BROWN & JACKSON 1982; CASTROVIEJO 1982; MCCULLAGH 1934
		2n=13	RAHN 1957
		2n=24	McCullagh 1934
		2n=96	McCullagh 1934
P. camtschatica CHAM.	2n=12	2n=12	McCullagh 1934; Ono 1954; Fujiwara 1956b; Mahn 1957; Sokolovskaya 1966; Provatova & Rudyka 1981; Provatova & Sokolovskaya 1981
P. japonica FRANCH. et SAVAT.	2n=12	2n=12	Sinoto 1925; Fujiwara 1956a
forma <i>polystachya</i> MAKINO		2n=36	FUJIWARA 1956a; MCCULLAGH 1934; ONO 1953; RAHN 1957, 1966
P. major L.	2n=12	2n=12	GADELLA & KULIPHUIS 1963, 1966; MULLIGAN & CODY 1968; TAYLOR & MULLIGAN 1968; KUZMANOV & KOZUHAROV 1969; KAPOOR 1972; MULIN & SHEIKH 1971; GONZALEZ & SILUESTRE 1980; JAVURKOVA 1979; LESSANI & CHARIATA-PANAHI 1979; FUJIWARA 1956a; RAHN 1957; GARAJOVA 1959; SORSA 1962, 1963
P. asiatica L.	2n=24	2n=12 2n=24	McCullagh 1934 Subramanyam & Kamble 1966; Larsen 1966; Fujiwara 1956a; Rahn 1957
P. yakushimensis MASAM	i. 2n=24	2n=24	Fujiwara 1956a

plants germinated from the seeds which were directly collected from their habitats. The chromosome numbers and karyotypes were examined in more than five plants for each population. The method of chromosome preparation followed NOGUCHI *et al.* (1983).

#### Results

## Chromosome Numbers and Karyotype Constitutions

(1) Plantago japonica FRANCH. et SAVAT. Plants from Okushiri Island; Sakaeura and Barou, Lake Saroma, in eastern Hokkaido; and Choujyagasaki, Miura Peninsula in central Honshu were investigated. The three populations in Hokkaido are all referable to Plantago togashii MIYABE et TATEWAKI, but MATSUO now regards that P. togashii in Hokkaido is conspecific with P. japonica in Honshu. This new concept is based upon detailed comparative studies of various morphological characters and also on the ecology of these taxa (MATSUO, 1989a).

This paper is the first report on the karyology of P. japonica (= P. togashii) from Hokkaido. All individuals from Hokkaido and Honshu had the same number of chromosomes, 2n = 12, and almost identical karyotypes (Figs. 1-C,D, 2-C,D, and 3-A,B). The chromosome complement consisted of four median pairs (Fig. 2-C,D Nos. 1-8) and two submedian pairs (Nos. 9-12). A pair of median chromosomes and a pair of submedian chromosomes had satellites at the distal portions of their short arms. The diploid chromosome number of 2n = 12 has already been reported for P. japonica in previous studies by SINOTO (1925) and FUJIWARA (1956a), based on individuals collected from Miura Peninsula, Kanagawa Prefecture. Although McCullagh (1934) reported the hexaploid chromosome number of 2n = 36 from P. japonica, the identification of his material may not be correct. However, the hexaploid chromosome number was also reported from the cultivated plants of P. japonica forma polystachya, which has branched spikes (FUJIWARA, 1956a; ONO, 1953;

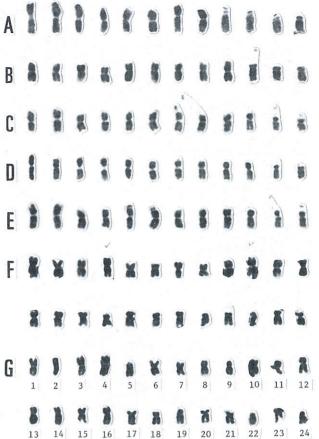


Fig. 2 Karyotypes at mitotic metaphase. A, *Plantago lanceolata*; B, *P. camtschaticum*; C, *P. japonica* from Sakaeura population in Hokkaido; D, *P. japonica* from Choujyagasaki population in Honshu; E, *P. major*; F, *P. asiatica*; G, *P. yakushimensis* The bar represents 2µm.

Table 3. Chromosome length, chromosome index, and chromosome morphology of *P. japonica* collected from Sakaeura, Hokkaido.

Complement	Chromosome length (µm)	Chromosome index*	Chromosome morphology
1	1.1+1.2=2.3	91.6	m
2	1.1+1.3=2.4	84.6	m
3	0.9 + 1.2 = 2.1	75.0	m
4	1.0+1.2=2.2	83.3	m
5	1.0+1.3=2.3	76.9	m
6	1.0+1.2=2.2	83.3	m
7	0.3+0.8+1.2=2.3	66.7	m
8	0.3+0.8+1.2=2.3	66.7	m
9	0.6+1.3=1.9	46.1	s m
10	0.6+1.2=1.8	50.0	s m
11	0.2 + 0.4 + 1.0 = 1.6	40.0	s m
12	0.2+0.4+0.8=1.4	50.0	s m

\*chromosome index is represented as 100s/1 (Levan et al., 1964).

RAHN, 1957, 1966).

#### (2) Plantago major L.

The individuals of P. major collected from the Hokkaido University campus in Sapporo, Hokkaido, possessed 2n = 12 somatic chromosomes (Fig. 1-E). The results obtained are in agreement with the previous reports on P. major (Table 2). The somatic chromosome complement of the materials examined in the study consisted of five median pairs (Fig. 2-E Nos. 1-10) and one submedian pair (Nos. 11 and 12), of which one median pair and one submedian pair had satellites at the distal portions of their short arms (Fig. 3-C, Table 5). The karyotypes of the chromosome complements of P. japonica and P. major are similar, but differ in the following features. In P. japonica, the median chromosomal pair (Nos. 7 and 8), which has a satellite at the distal portion of the short arm, is the smallest and possesses the index values of 61.5-66.7, the lowest among the median chromosome pairs (Tables 3 and 4), while in P. major the median pair (Nos. 5 and 6) is the second in size, but with more or less the same index value of 64.3 (Table 5).

#### (3) Plantago asiatica L.

Three different somatic chromosome numbers, i.e., 2n = 12, 24, and 36, are known from Plantago asiatica (Table 2). The somatic chromosome number of P. asiatica collected from the grassy site by the trail at the foot of Mt. Teine, Hokkaido, was tetraploid with 2n = 24 chromosomes (Fig. 1-F). The chromosome complement consisted of twelve median (Fig. 2-F, Nos. 1-consisted of twelve median (Fig. 2-F, Nos. 1-12) and twelve submedian chromosomes (Nos. 13-24). The median chromosomes with the chromosome index value of 70.0 had a satellite at the terminal portion of

Table 4. Chromosome length, chromosome index, and chromosome morphology of *P. japonica* collected from Choujyagasaki, Kanagawa Prefecture.

Complement	Chromosome length (µm)	Chromosome index	Chromosome morphology
1	1.3+1.6=2.9	81.3	m
2	1.1+1.5=2.6	73.3	m
3	1.1+1.3=2.4	84.6	m
4	1.0+1.3=2.3	76.9	m
5	0.9 + 1.3 = 2.2	69.2	m
6	0.9 + 1.3 = 2.2	69.2	m
7	0.2+0.8+1.3=2.3	61.5	m
8	0.2+0.8+1.3=2.3	61.5	m
9	0.7 + 1.3 = 2.1	53.8	s m
10	0.7 + 1.3 = 2.1	53.8	s m
11	0.2+0.4+1.2=1.8	33.3	s m
12	0.2 + 0.4 + 1.1 = 1.7	36.4	s m

Table 5. Chromosome length, chromosome index, and chromosome morphology of *P. major*.

Complement	Chromosome length (µm)	Chromosome index	Chromosome morphology
1	1.3+1.6=2.9	81.3	m
2	1.3 + 1.6 = 2.9	81.3	m
3	1.0+1.2=2.2	83.3	m
4	1.0+1.2=2.2	83.3	m
5	0.3+0.9+1.4=2.6	64.3	m
6	0.3+0.9+1.4=2.6	64.3	m
7	0.9 + 1.3 = 2.2	69.2	m
8	0.9 + 1.3 = 2.2	69.2	m
9	0.8+1.3=2.1	61.5	m
10	0.8 + 1.3 = 2.1	61.5	m
11	0.2+0.7+1.2=2.1	58.3	s m
12	0.2+0.7+1.2=2.1	58.3	s m

Table 6. Chromosome length, chromosome index, and chromosome morphology of *P. asiatica*.

Complement	Chromosome length (µm)	Chromosome index	Chromosome morphology
1	1.2+1.5=2.7	80.0	m
2	1.2+1.3=2.5	92.3	m
3	1.0+1.0=2.0	100.0	m
4	1.0+1.3=2.3	76.9	m
5	0.8+1.0=1.8	80.0	m
6	0.7 + 1.1 = 1.8	63.6	m
7	0.8 + 0.9 = 1.7	88.9	m
8	0.8 + 0.9 = 1.7	88.9	m
9	0.3+0.7+1.0=2.0	70.0	m
10	0.3+0.7+1.0=2.0	70.0	m
11	0.3+7.1+1.0=2.0	70.0	m
12	0.8+1.0=1.8	80.0	m
13	0.7 + 1.2 = 1.9	. 58.3	s m
14	0.7 + 1.2 = 1.9	58.3	s m
15	0.7 + 1.2 = 1.9	58.3	s m
16	0.7 + 1.0 = 1.7	70.0	s m
17	0.6+1.2=1.8	50.0	s m
18	0.6+1.2=1.8	50.0	s m
19	0.6+1.2=1.8	50.0	s m
20	0.6+1.2=1.8	50.0	s m
21	0.2+0.4+1.2=1.8	33.3	s m
22	0.5 + 1.2 = 1.7	41.7	s m
23	0.5+1.2=1.7	41.7	s m
24	0.4 + 1.0 = 1.4	40.0	s m

the short arm (Nos. 9-11), although a satellite was lacking in the 12th chromosome. This particular chromosome (No. 12) had a different index value of 80.0, indicating that it has the constriction at the somewhat different position (Fig. 2-F, Nos. 9-12, Table 6). The smallest submedian pair had a satellite at the distal portion of the short arm, although its presence was not constant (Figs. 2-F and 3-F).

The basic karyotype of P. asiatica collected from Mt. Teine in Hokkaido was very similar to those of P. major examined in this study. However, the chromosome complements of P. asiatica contained heterogeneous pairs, and thus it seems that P. asiatica may not be of simple autopolyploid origin. MCCULLAGH (1934) reported the chromosome number of 2n = 12 from P. asiatica. However, the locality of materials and the morphological characteristics were not described, so that it is difficult to judge whether or not the plant truly belongs to P. asiatica.

#### (4) Plantago yakushimensis MASAMUNE

Plantago yakushimensis collected from Isl. yakushima was tetraploid with 2n = 24 somatic chromosomes, the result agreeing with the previous report by FUJIWARA (1955) (Fig. 1-G). The chromosome complement of *P. yakushimensis* examined in this study consisted of sixteen median chromosomes (Fig. 2-G, Nos. 1-16) and eight submedian chromosomes (Nos. 17-24). One of the median chromosomes (No. 5) which had a satellite at the distal portion of the short arm was the smallest among the median chromosome pairs with the highest chromosomal index value of 89.3 (Table 7, Figs. 2-G and 3-G).

The basic karyotype of *P. yakushimensis* is different from that of *P. asiatica*. The position of the constriction of the chromosomes with satellites was different in *P. asiatica* and *P. yakushimensis* (Table 3-D,E, Tables 6 and 7).

#### (5) Plantago lanceolata L.

The somatic chromosome numbers of 2n = 12, 13,24,96 have so far been identified for *Plantago lanceolata* (Table 2). All the plants collected from the grassy site on the Hokkaido University campus had the somatic chromosome number of 2n = 12 (Fig. 1-A). The chromosome complement consisted of four pairs with median constriction (Fig. 2-A, Nos. 1-8), one pair with telocentric

Table 7. Chromosome length, chromosome index, and chromosome morphology of P. vakushimensis.

Complement	Chromosome length (µm)	Chromosome index	Chromosome morphology
1	1.0+1.2=2.2	83.3	m
2	1.0+1.2=2.2	83.3	m
3	1.0+1.2=2.2	83.3	m
4	1.0+1.2=2.2	83.3	m
5	0.2+0.8+0.9=1.9	88.9	m
6	0.8+0.9=1.7	88.9	m
7	0.8+0.9=1.7	88.9	m
8	0.8+1.0=1.8	80.0	m
9	0.8+1.0=1.8	80.0	m
10	0.8+1.2=2.0	66.7	m
11	0.8+1.2=2.0	66.7	m
12	0.8+1.2=2.0	66.7	m
13	0.8+1.3=2.1	61.5	m
14	0.8+1.3=2.1	61.5	m
15	0.8 + 1.3 = 2.1	61.5	m
16	0.8+1.3=2.1	61.5	m
17	0.7 + 1.2 = 1.9	58.3	s m
18	0.6+1.0=1.6	60.0	s m
19	0.6+1.0=1.6	60.0	s m
20	0.6+1.0=1.6	60.0	s m
21	0.4+1.1=1.5	36.4	s m
22	0.4+1.0=1.4	40.0	s m
23	0.4+1.1=1.5	36.4	s m
24	0.4+1.0=1.4	40.0	s m

Table 8. Chromosome length, chromosome index, and chromosome morphology of *P. lanceolata*.

Complement	Chromosome length (µm)	Chromosome index	Chromosome morphology
1	1.6+1.8=3.4	91.0	m
2	1.6+1.8=3.4	91.0	m
3	1.6+1.6=3.2	100.0	m
4	1.6+1.6=3.2	100.0	m
5	1.3+1.5=2.8	88.6	m
6	1.3+1.4=2.7	92.8	m
7	1.4 + 2.1 = 3.5	66.7	m
8	1.4+1.9=3.3	73.7	m
9	0.3+0.3+2.3=2.9	13.0	t
10	0.4+0.3+2.4=3.1	12.5	t
11	0.3+0.4+1.9=2.6	21.1	s t
12	0.4+0.6+1.9=2.9	31.5	s t

Table 9. Chromosome length, chromosome index, and chromosome morphology of *P. camtschatica*.

Complemen	t Chromosome length (µm)	Chromosome index	Chromosome morphology
1	1.3+1.4=2.7	92.8	m
2	1.1+1.3=2.4	84.6	m
3	1.1+1.3=2.4	84.6	m
4	0.9+1.3=2.2	69.2	m
5	1.1+1.4=2.5	78.5	m
6	1.1+1.4=2.5	78.5	m
7	1.3+1.4=2.7	92.9	m
8	1.3+1.4=2.7	92.9	m
9	0.4+9.1+1.4=2.7	64.2	s m
10	0.3+0.6+1.7=2.6	35.3	s m
11	0.1+0.5+1.4=2.0	35.7	s m
12	0.1+0.5+1.4=2.0	35.7	s m

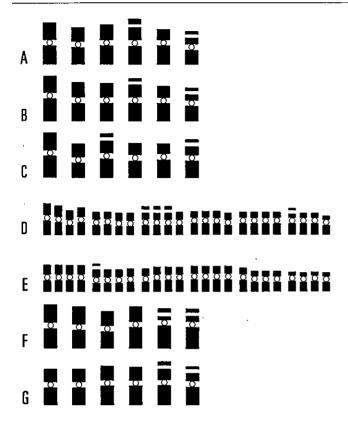


Fig. 3 Idiogram of haploid chromosomes at mitotic metaphase. A, P. japonica from Sakaeura population in Hokkaido; B, P. japonica from Choujyagasaki population in Honshu; C, P. major; D, P. asiatica; E, P. yakushimensis; F, plantago lanceolate; and G, P. camtschaticum

constriction (Nos. 9 and 10), and one small pair with subterminal constriction (Nos. 11 and 12). The telocentric and subterminal pairs are satellite chromosomes with satellites at the distal end of the short arm (Fig. 3-F, Table 8). The karyotype of the metaphase somatic chromosomes determined in this study was slightly different from that of MCCULLAGH (1934) in the position of the MCCULLAGH showed secondary constriction. that secondary constrictions are at the distal portion of the short arm of the terminal chromosome pair and of the long arm of the submedian pair, but plants examined in this study possessed secondary constrictions at the distal portion of the short arms of the telocentric and subterminal pairs.

#### (6) Plantago camtschatica CHAM.

The specimens of *Plantago camtschatica* examined in this study were diploid with somatic

chromosomes of 2n = 12 (Fig. 1-B), the results corresponding with the previous karyological studies (MCCULLAGH, 1934; ONO, 1954; FUJIWARA, 1956b; SOKOLOVSKAYA, 1966; PROVATOVA and RUDYKA, 1981; PROVATOVA and SOKOLOVSKAYA, 1981 [Table 2] ). The somatic chromosome complement of plants observed in this study consisted of four median chromosome pairs (Fig. 2-B, Nos. 1-8) and two submedian pairs (Nos. 9-12), the latter two submedian pairs having satellites at the distal end of the short arms (Fig. 3-B). The karyotype of the individuals investigated in this study was different from those reported by FUJIWARA (1956b) and McCullagh (1934), i.e., in the position of the secondary constriction.

### Taxonomic Identity of Plantago togashii

From the maritime rocky cliffs or gravel barrens, and also salt marsh communities in Honshu and Hokkaido, two endemic *Plantago* species, i.e., *P. japonica* and *P. togashii*, have been known. In the present series of studies (MATSUO, 1989a,b), the senior author finally reached the conclusion, based upon critical studies of the variations in

various gross and micro-morphological characters, that *P. togashii* is identical with *P. japonica*, in spite of the differences in their ecological distributions. The present karyological study has also provided good supporting evidence of the above taxonomic conclusion, i.e., that both plants possess the same chromosome numbers and basic karyotypes, as well documented in this paper.

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#### 摘要

著者の一人松尾は, 現在日本産オオバコ属植物に 関する広汎な種生物学的研究を行っている。この一 連の研究との関連で、北海道の海岸塩湿地に主とし て生育するイソオオバコ Plantago togashii MIYABE et TATEWAKI とよばれる種の正確な分類 学的位置の決定が必要となってきた。前報(松尾 1989 a) では、Plantago 節に属する帰化雑草のセイ ヨウオオバコ、本州の海岸に生育するトウオオバコ 並びに北海道・サハリンの海岸岩れき地や塩湿地に 生育するイソオオバコの3種の外部形態の詳細な比 較を行い、それぞれの分類群の位置づけを行った。 この研究では、本州産トウオオバコ (狭義) と北海 道産の、従来イソオオバコと呼ばれてきた植物の体 細胞分裂中期染色体における核型の比較を行い、上 記の分類群の類縁関係の解明に必要なデータを得る ことに努めた。また併せて, 比較のために日本に自 生するエゾオオバコ P. camtschatica CHAM,,オオバ コ P. asiatica L.,ヤクシマオオバコ P. yakushimensis MASAM.の3種と、外来のヘラオオバコP. lanceolata L.並びにセイヨウオオバコ P. major L. の2種の体細胞中期染色体における核型の分析も 行った。

北海道産のイソオオバコ(狭義)の核型分析はこ の研究ではじめて行われたが、本州産のトウオオバ コ(狭義)と同一の染色体数 2n = 12 をもち,中期染 色体の核型も同一であることが判明した。この事実 は、外部形態その他の形質の変異性においてこれら の両植物が同一分類群に属するという結論(松尾, 1989 a) を支持するものである。また、今回調べたそ の他の種については、セイヨウオオバコとトウオオ バコはいずれも 2n = 12 の 2 倍体であり、中期染色 体の基本核型はお互いによく似ているが、細部にお いては、異なる特徴を示した。また、オオバコは2n = 24 の 4 倍体で基本核型はセイヨウオオバコと同じ であるが、ヘテロな対を含んでおりセイヨウオオバ コとは異なる核型構成をしていることが明かとなっ た。ヤクシマオオバコも2n=24の4倍体であった が、オオバコとは付随体染色体の形態が異なってお り、核型構成上相違することが判明した。

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