Biosystematic Studies on the Genus *Hemerocallis* (Liliaceae)

II. Variation in Gross Morphology of the *H. fulva* Complex with Special Reference to the Identity of *H. fulva* v. *longituba* and v. *disticha*

By

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Introduction

Hemerocallis fulva sensu lato is known to be an exceedingly polymorphic species. In a recent taxonomic revision of the genus *Hemerocallis* in Japan and its neighbouring regions, Matsuoka and Hotta (1966) distinguished primarily by gross morphology, the following seven infraspecific taxa in the *H. fulva* complex: *H. fulva* L. v. *fulva* : v. *Kwanso* Regel ; v. *sempervirens* (Araki) H. Hotta : v. *littorea* (Makino) M. Hotta ; v. *longituba* (Miq.) Maxim. ; v. *disticha* (Donn) M. Hotta ; and v. *pauciflora* M. Hotta & Matsuoka. However, there are still considerable discrepancies in the taxonomic concept and treatment of this complex group. It has not been resolved, for example, whether or not several native Japanese taxa now referred to the *H. fulva* complex, such as v. *disticha,* v. *longituba,* etc. in the sense of Matsuoka and Hotta (1. c.) should be referred to the same taxonomic group as the continental taxon, *H. fulva* v. *fulva.* This latter group also includes the double-flowered taxon, v. *Kwanso* which is widely distributed over the Japanese Islands excepting northern Hokkaido (cf. Ohwi, 1953, 1965 ; Kitamura et al., 1967).

This paper particularly attempts to draw attention to the taxonomical identity of two native Japanese taxa, *H. fulva* v. *longituba* and v. *disticha.* In the present study, the variabilities of the plants, referred to the following four taxa of this complex, i. e., v. *fulva,* v. *Kwanso,* v. *disticha,* and v. *longituba,* are analyzed and compared, and their taxonomic status briefly discussed.

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Materials and Methods

The plants of *H. fulva* v. *disticha,* v. *longituba* and v. *Kwanso* were collected at Akitsu, Higashi-Murayama City, Tokyo, and also in the vicinity of Matsunoki, Takayama City, Gifu Prefecture. *H. fulva* v. *fulva* collected from Hong Kong was cultivated at the Toyama University Pharmaceutical Garden, and was used for comparison with Japanese plants.

Gross Morphology

Examination of the external characters were made on fresh plants taken directly from their native habitats or garden. The characters studied were the height of the flowering scapes, the shape and length of the inflorescences, the color and fragrance of the flowers, the length and width of the external and internal perianths, the length of the tubes enclosing the ovary, the length of the bracts, and the length and width of the leaves.

Cytology

Studies were made on the karyotypes, meiotic behaviours of the chromosomes, and the pollen fertility of all four taxa. The cytological preparations of somatic chromosomes were made by using modifications of the colchicine-aceto-orcein squash method (Kawano, 1965). The flower buds were fixed with Newcomer's fixative either in the fields or garden, and stained with 1% iron-arum acetic carmine.

Electrophoretic Patterns of Acid-Soluble Proteins

The fresh rhizomes of *Hemerocallis* plants were homogenized in 0.5N H_2SO_4, and centrifuged at 8,000g for one hr. Supernatant was dialysed for 24 hrs against 0.25N H_2SO_4 buffer. Protein was precipitated by adding acetone in equal quantity to the sample solution. Protein extracts were then centrifuged at 5,000g for 30 min, and the precipitate was dissolved into 0.1M-$NaH_2PO_4 \cdot 2H_2O$, 0.1M-Na_2HPO_4 buffer pH 7.0 with 0.1%-sodium laurylsulfate (SDS), and 0.1%-2-mercapto-ethanol, and again centrifuged at 100,000g for one hr. The extracts were electrophoresed at pH 6.8 with the above-mentioned gel buffer with 1.5% $(NH_4)_2S_2O_8$ and NNN'N'-tetramethyl-ethylenediamine on 10% acrylamide gels. A 0.2 ml sample was applied to each column and electrophoresed for one hr at 2 mA per column, and subsequently for 3.5 hr at 5 mA. The gels were fixed and stained with 1% amido black in 7% acetic acid. After electrophoretic destaining the gels were photographed, and optical density curves were obtained directly with a recording densitometer.

Observations

I. Comparison of Characters

(1) Scape Height

The variation in height of the scapes of four *Hemerocallis* taxa is illustrated

Table 1. Measurements of various morphological characters in four varieties of *Hemerocallis fulva.*

Characters \ Taxa	var. *fulva*	var. *Kwanso*	var. *disticha*	var. *longituba*
Scape height (cm)	106–135	56–125	16–135	26–155
Mean±s. d.	124.5±8.0	83.8±15.4	75.7±22.8	100.9±21.5
Variance (δ^2)	64.0000	238.3318	517.6535	463.5409
Standard error	2.667	2.2054	1.7766	2.2570
Inflorescence length (cm)	0.5–17	0.5–17	0.5–11	0.5–23
Mean±s. d.	9.1±1.7	8.4±2.1	2.9±1.5	7.6±3.5
Variance (δ^2)	3.0276	4.5012	2.2177	12.4892
Standard error	0.5800	0.3062	0.1446	0.3570
Number of flowers per scape	4–15	4–19	3–27	3–31
Mean±s. d.	9.9±2.8	9.1±3.1	8.7±3.1	11.2±4.6
Variance (δ^2)	5.76000	9.8722	9.9049	21.5890
Standard error	0.9330	0.4199	0.2544	0.4792
Length of flower tube enclosing ovary (cm)	1.9–3.0	1.1–3.0	1.5–3.8	1.5–4.6
Mean±s. d.	2.48±0.28	2.13±0.37	2.56±0.38	3.21±0.57
Variance (δ^2)	0.0792	0.1396	0.1425	0.3292
Standard error	0.0780	0.0499	0.0299	0.0602
Length of internal perianth (cm)	6.8–8.5	5.0–9.1	5.0–10.3	5.0–10.9
Mean±s. d.	7.86±0.37	7.38±0.69	7.29±1.01	7.22±1.03
Variance (δ^2)	0.1341	0.4807	1.0203	1.0574
Standard error	0.1016	0.1022	0.0981	0.1066
Width of internal perianth (cm)	2.6–3.7	1.8–3.7	1.0–2.9	1.0–2.9
Mean±s. d.	3.09±0.21	2.89±0.13	1.98±0.38	2.02±0.31
Variance (δ^2)	0.0424	0.0165	0.1412	0.0936
Standard error	0.0571	0.0172	0.0333	0.0319
Length of external perianth (cm)	7.0–8.4	5.0–8.4	4.5–9.9	4.5–9.4
Mean±s. d.	7.81±0.28	6.97±0.70	6.84±1.11	6.95±0.93
Variance (δ^2)	0.0778	0.4959	1.2314	0.8566
Standard error	0.0774	0.0967	0.0888	0.0965
Width of external perianth (cm)	1.3–2.4	0.9–2.0	0.5–2.8	0.5–2.0
Mean±s. d.	1.91±0.07	1.37±0.22	1.28±0.30	1.31±0.06
Variance (δ^2)	0.0653	0.0491	0.0950	0.0607
Standard error	0.0709	0.0293	0.0251	0.0256
Leaf width (cm)	1.7–3.2	1.7–4.0	0.5–2.4	0.9–2.8
Mean±s. d.	2.53±0.09	2.43±0.49	1.58±0.36	1.76±0.41
Variance (δ^2)	0.0976	0.2379	0.1302	0.1679
Standard error	0.1041	0.0690	0.0359	0.0442

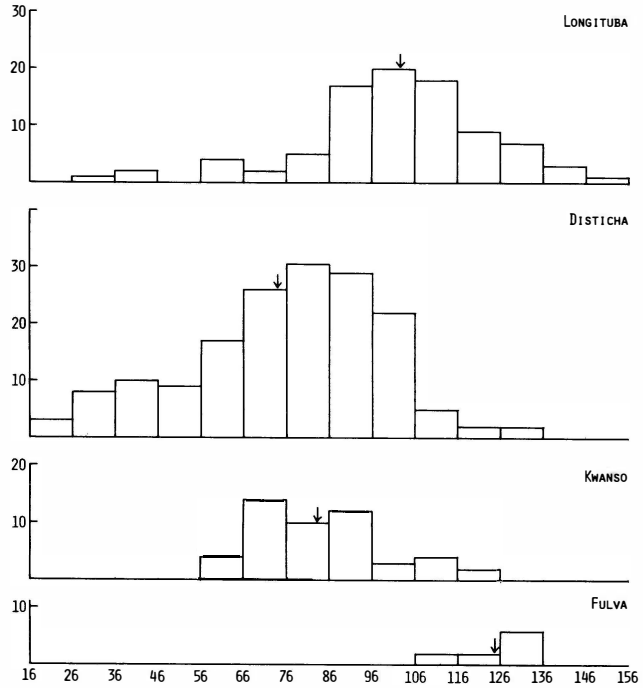

Fig. 1. Variance in scape height of the four varieties of *H. fulva.* Arrows indicate mean values.

in Fig. 1. Scape height is a trait often subjected to environmental influence. It was found that the flowering scapes of v. *fulva* and v. *longituba* are somewhat taller than those of v. *disticha* and v. *Kwanso.* It is noteworthy that there occurs a considerable difference in the variation ranges of this character between v. *longituba* and v. *disticha,* although both varieties often grow sympatrically and are presumed to be freely interbreeding. A difference found in the scape height between v. *fulva* and v. *Kwanso* needs to be reconfirmed based on more numerous materials in future studies.

(2) Inflorescence and Flower

The morphology of the inflorescences is very characteristic in the species of the genus *Hemerocallis,* and of a diagnostic value. Generally, the inflorescences of the *H. fulva* group are all essentially dichotomous ; in particular, this character was found to be very stable in v. *fulva* and v. *Kwanso,* and their inflorescences are regularly dichotomous ; whereas it is to some extent variable in v. *disticha* and v. *longituba,* and there often occur somewhat irregularly dichotomous and shortly branched, or trichotomous ones.

A more or less similar trend is to be found in the variation of flower number in these taxa, although the average flower numbers per scape is almost the same and the difference discovered between them is statistically insignificant (cf. Table 1). The number of flowers per flowering scape varies from 4 to 15 in v. *fulva*

(mean: 9.9±2.8) and 4 to 19 in v. *Kwanso* (mean: 9.1±3.1); whereas it is more variable in v. *disticha* and v. *longituba,* ranging from 3 to 27 in v. *disticha* (mean: 8.7±3.1) and 3 to 31 in v. *longituba* (mean: 11.2±4.6), respectively.

The major difference in the flower characters among these four taxa of *Hemerocallis* is seen in the size of floral parts and coloration. It is very characteristic that without exception the internal perianths of both v. *fulva* and v. *Kwanso* exhibit very clear yellow coloration at the basal to mid half, the remaining parts of perianths being dark-red or pale brownish red; whereas the

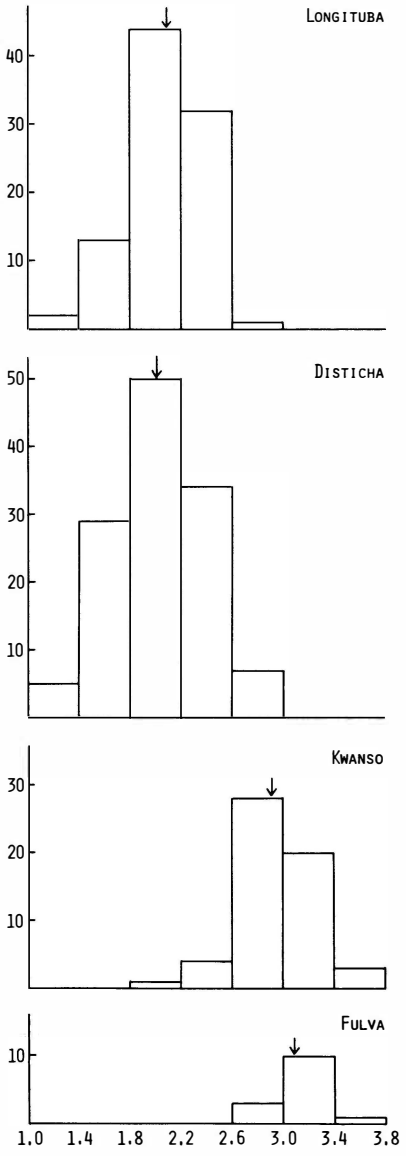

Fig. 2. Variation in the length of the internal perianths of the four varieties of *H. fulva.* Arrows indicate mean values.

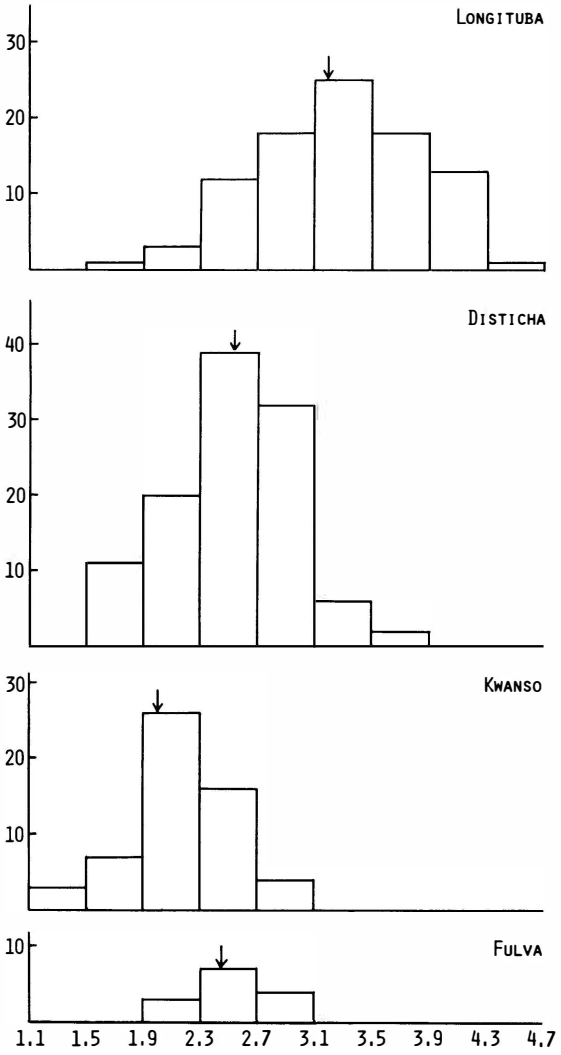

Fig. 3. Variation in the length of the flower tube enclosing the ovary. Arrows specify mean values.

color of the internal and external perianths of v. *longituba* and v. *disticha* is highly variable. Typical v. *longituba* displays a scarlet red perianth color, but v. *disticha* typically possesses orange-colored perianths. However, there are many transitional color forms beween these two typical colors, and it is not easy to distinguish these taxa by flower color alone.

Among several other quantitative characters of floral organs examined, the width of the internal perianths and the length of the flower tube enclosing the ovary showed the most distinct range of variation in these four taxa. As illustrated in Fig. 2, both v. *fulva* and v. *Kwanso* possess somewhat broader

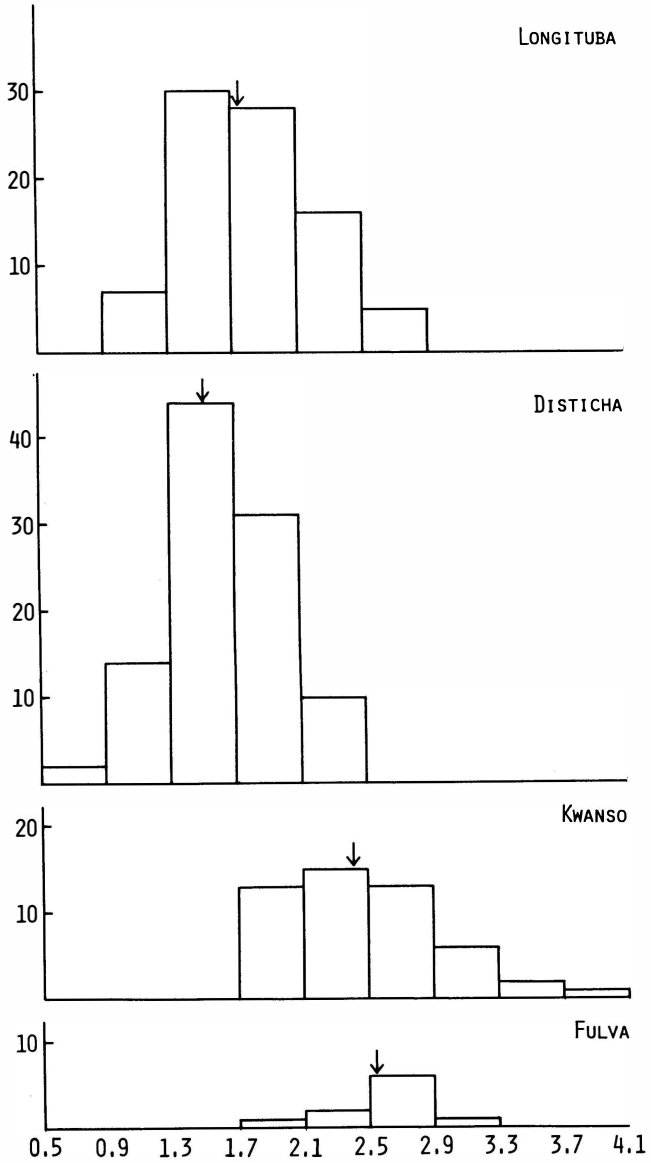

Fig. 4. Variation in leaf width of the four varieties of *H. fulva*. Arrows specify mean values.

internal perianths, ranging 2.6–3.7 cm (mean: 3.09±0.21cm) and 1.8±3.7 cm (mean: 2.89±0.13 cm) as compared with those of v. *disticha* and v. *longituba,* in both of which the internal perianths vary from 1.0 to 2.9 cm in width (mean: 1.98±0.38 cm in v. *disticha,* and 2.02 ±0.31 cm in v. *longituba).*

Fig. 3 illustrates the range of variation in the length of the flower tube enclosing the ovary. The flower tube is the longest in v. *longituba,* ranging from 1.5 to 4.6 cm in length (mean: 3.2±0.57 cm); whereas no significant difference was detected in the range of variation of this character among the other three taxa, v. *disticha,* v. *fulva,* and v. *Kwanso* (see Table 1), all of which vary in length from approximately 1.1 to 3.8 cm.

(3) Flowering Time

The clones of v. *fulva* cultivated in Toyama produce in June flowers which

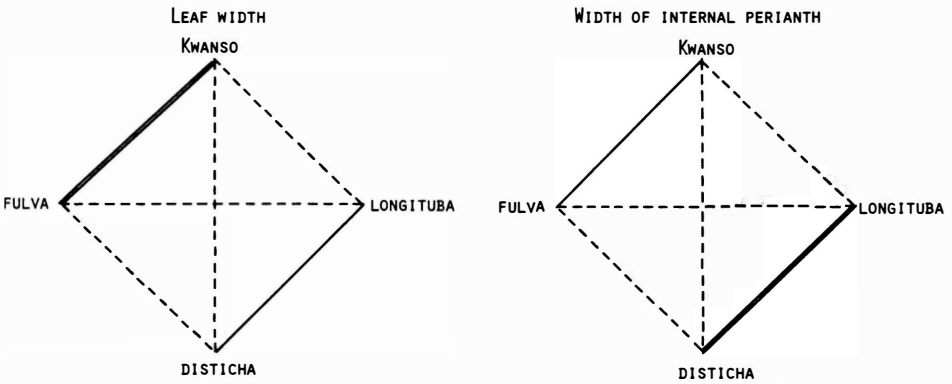

Fig. 5. Similarity found in two characters (i. e., the width of the leaves and the width of the internal perianths) among the four varieties of *H. fulva.* Broken lines indicate a significant difference*** ($t > 3$) under t-test in leaf and flower characters between the taxa; single lines insignificant** ($t \fallingdotseq 3$); and double lines strongly insignificant* ($t < 3$), thus indicating variation of the character highly similar to each other.

The width of the internal perianths

v. *fulva* — v. *Kwanso* — v. *disticha* — v. *longituba*

3.335** 0.8406*

24.2834***

24.0914***

16.7164***

16.3079***

The width of the leaves

v. *fulva* — v. *Kwanso* — v. *disticha* — v. *longituba*

0.8327* 3.2334**

10.8233**

7.9964***

8.6300***

6.7524***

usually last for a month. Matsuoka and Hotta (1966) also reported that v. *fulva* cultivated in Osaka flowered from June to July for a month. But, it is unknown when this plant blooms in the continent.

The plants of the remaining three Japanese taxa begin to bloom at the end of June or at the beginning of July and flowering usually lasts for about a month until the end of July or the beginning of August. Thus, as far as native Japanese taxa are concerned, no clear seasonal isolation barrier occurs between them (cf. Matsuoka and Hotta, 1. c.).

(4) Leaf

Fig. 4 shows the range of variation in leaf width of four *Hemerocallis* taxa. It is very clear from this histogram that v. *Kwanso* and v. *fulva* possess broader leaves, 1.7 to 4.0 cm (mean: 2.43 ± 0.49) and 1.7 to 3.2 cm (mean: 2.53 ± 0.09 cm); whereas both v. *longituba* and v. *disticha* have much narrower leaves than the former two taxa, ranging from 0.5 to 2.4 cm (mean: 1.58 ± 0.36 cm) in v. *disticha* and 0.9 to 2.8 cm (mean: 1.76 ± 0.41 cm) in v. *longituba,* respectively. No significant difference was detected in leaf length among these four taxa.

II. Cytological Observations

(1) Karyotypes of Somatic Chromosomes

The $n=11$ chromosomes of *Hemerocallis* were classified into seven types by Takenaka (1952), and all these seven chromosome types were confirmed in later cytological investigations (Kawano, 1961; Kawano & Noguchi, 1973 and unpublished). Chromosome *L,* the longest of the complement, has a constriction situated in a submedian position. Chromosome *j,* a little shorter than *L,* has a constriction located submedially. Chromosome *i,* of about medium length, has a constriction situated in a subterminal position. Chromosome *m,* one of the shortest, has a constriction close to the center. Chromosome *T,* slightly shorter, or almost the same length as *j,* possesses a constriction situated in an extremely subterminal position. Chromosome *h,* has no visible constriction. It may have an extreme subterminal or terminal centromere with an extraordinary small or almost invisible arm.

(a) Karyotypes in H. fulva v. longituba

All materials examined had $2n=22$ somatic chromosomes. The basic karyotype of this plant may be expressed as follows:

$$2n=22=4L+6j+6i+2m+2T+2h$$

(b) Karyotypes in H. fulva v. disticha

The $2n=22$ somatic chromosomes were observed in all plants examined. This plant possesses the same basic karyotype as v. *longituba,* as reported previously (Kawano and Noguchi, 1973). The basic karyotype of this variety may be expressed as follows:

$2n=22=4L+6j+6i+2m+2T+2h$

(c) Karyotypes in H. fulva v. fulva

Two clones of v. *fulva* collected from Hong Kong were cytologically examined, and both proved to be triploid with $2n=33$ somatic chromosomes. The basic karyotype of this plant was found to be identical with those of diploid plants, i. e., v. *longituba* and v. *disticha,* and thus karyotype of this variety may be expressed as follows:

$2n=33=6L+6j+9i+3m+6T+3h$

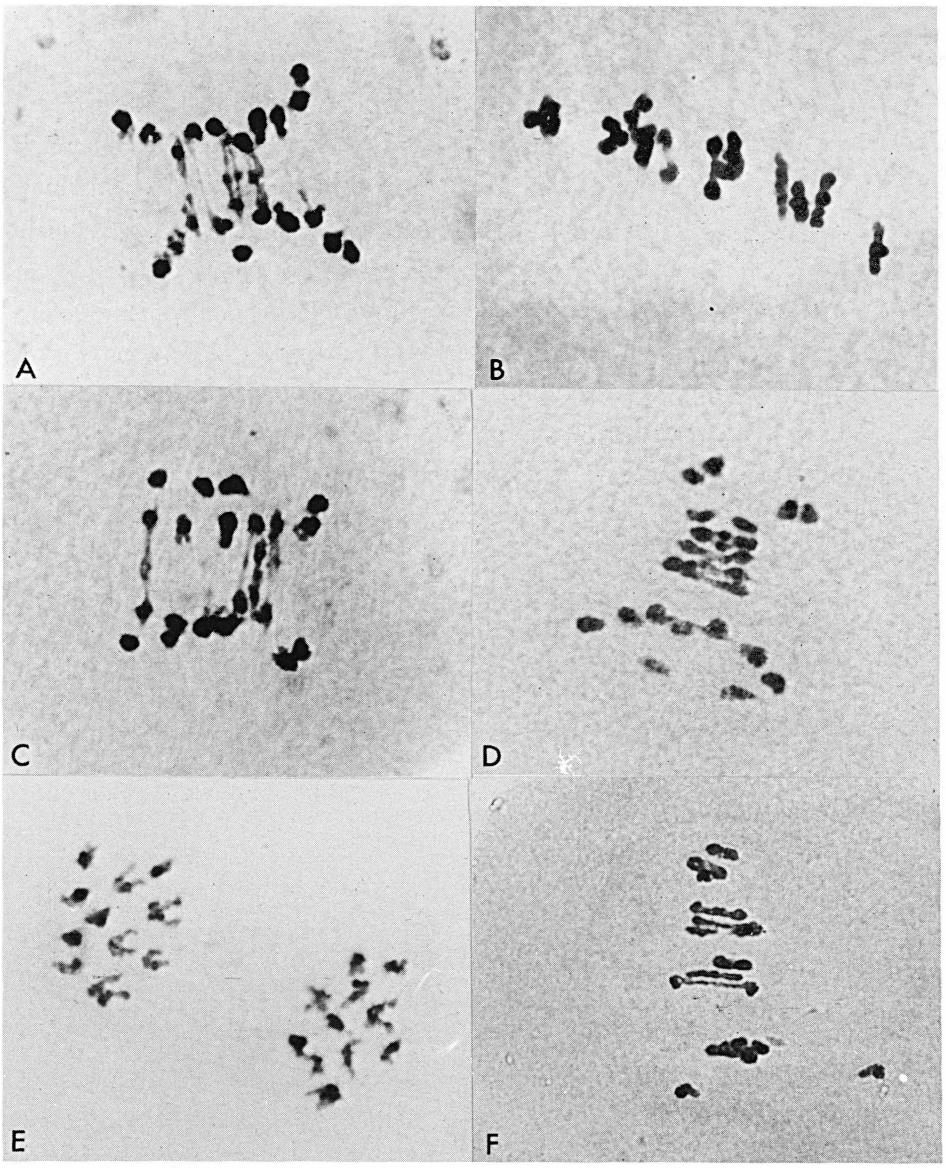

Fig. 6. Meiotic behaviours of the chromosomes in *H. fulva* v. *disticha.* All (A–F) collected from Akitsu.

(d) Karyotypes in H. fulva v. Kwanso

Several clones of v. *Kwanso* collected from Akitsu, Higashi-Murayama City, Tokyo, and Matsunoki, Takayama City, Gifu, were karyologically studied. All materials examined proved to be triploid with $2n=33$ somatic chromosome. The basic karyotype of v. *Kwanso* also turned out to be identical with those of three varieties described above.

The karyotype of v. *Kwanso* may be expressed as follows:

$$2n=33=6L+6j+9i+3m+6T+3h$$

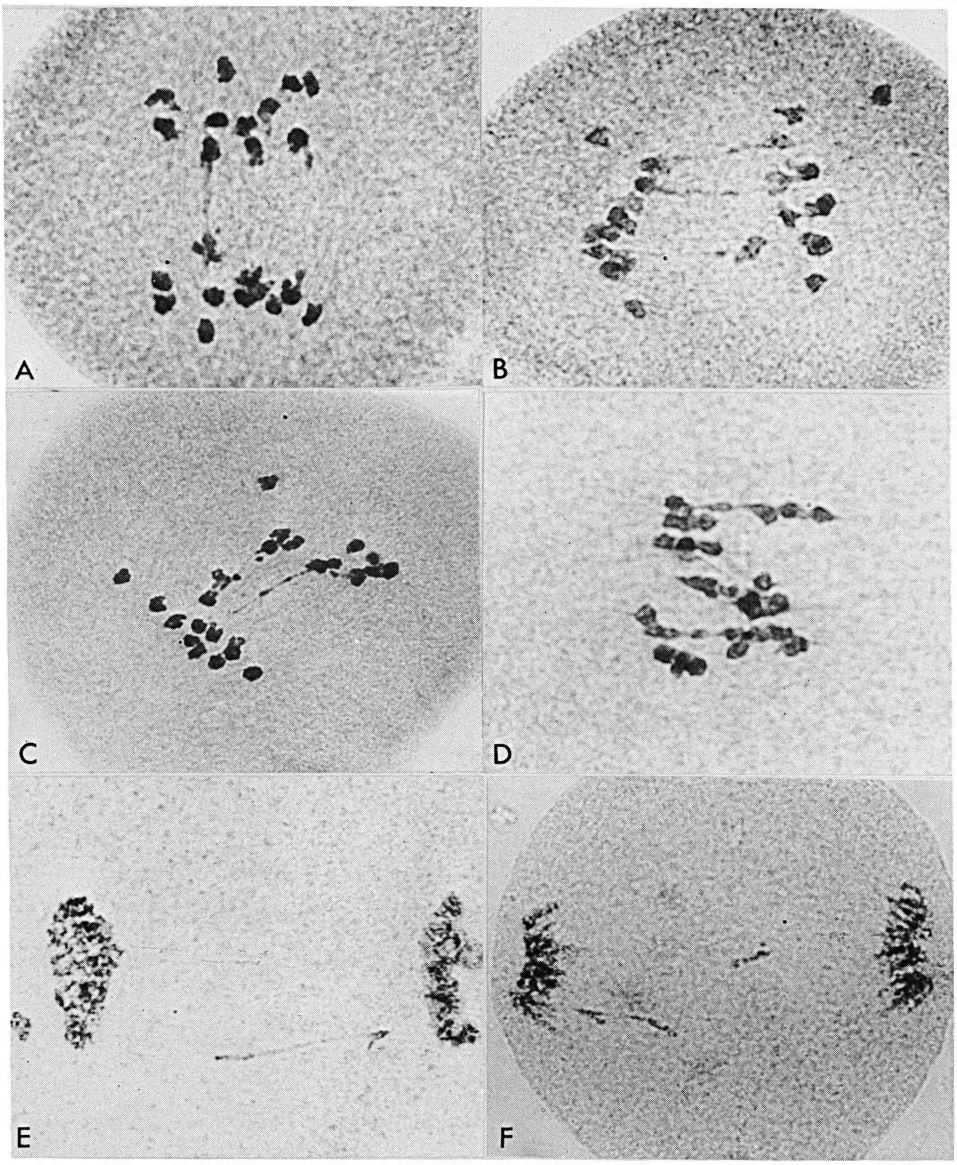

Fig. 7. Meiotic behaviours of the chromosomes in v. *longituba*. All (A–F) collected from Akitsu.

(2) Meiotic Behaviours of Chromosomes

The chromosome pairing and behaviours at pachytene and MI of the plants of all four varieties were observed.

Most of the materials of v. *disticha* and v. *longituba* examined exhibited normal pairing at MI, forming 11 regular bivalents (Fig. 6-B). However, as illustrated in Figs. 6 and 7, somewhat irregular pairings were often observed, including delayed terminalization of chiasmata, and laggards, indicating partial structural heterogeneity of the chromosomes. Such phenomena are called sticky bridges, long chromosomes (Fig. 6-A, C, D; Fig. 7-A, B, C, D), or early chromosome separation (Fig. 6-F). The meiotic irregularities mentioned above were mainly observed in materials of both v. *disticha* and v. *longituba* from Akitsu, suggesting the occurrence of partial hybridization between these two taxa at sympatric sites in Akitsu. In fact, the *Hemerocallis* population occurring in Akitsu is found to include individuals exceedingly variable in their morphology (Kawano and Noguchi, unpublished).

From the cytological examination of root tip cells, both v. *fulva* and v. *Kwanso* were confirmed to be triploid with $2n=33$ chromosomes. Observations of the meiotic behaviour and pairing of chromosomes of these two taxa also clearly displayed the triploid nature of these plants. Nonetheless, as shown in Table 2, individuals with 11 trivalents were rare, and instead, those with $1_I+1_{II}+10_{III}$, $2_I+2_{II}+9_{III}$, $3_I+3_{II}+8_{III}$, or $4_I+4_{II}+7_{III}$ were more frequently observed (cf. Figs. 8 and 9). This fact may suggest that both v. *fulva* and v. *Kwanso* are at least not simple autotriploids in their origin, and the possibilities are not to be excluded that they are allotriploids of hybrid origin from some now unknown ancestral groups. As a matter of course, however, this needs to be confirmed based on more critical future studies.

Table 3 and Figs. 10 and 11 show the pollen fertility and size variation in all

Table 2. Number of univalent chromosomes at MI of *H. fulva* v. *fulva* and v. *Kwanso*.

Taxa	No. of univalents	0	1	2	3	4
v. *fulva*	No. of cells observed	2	7	22	12	1
v. *Kwanso*	No. of cells observed	3	10	20	11	2

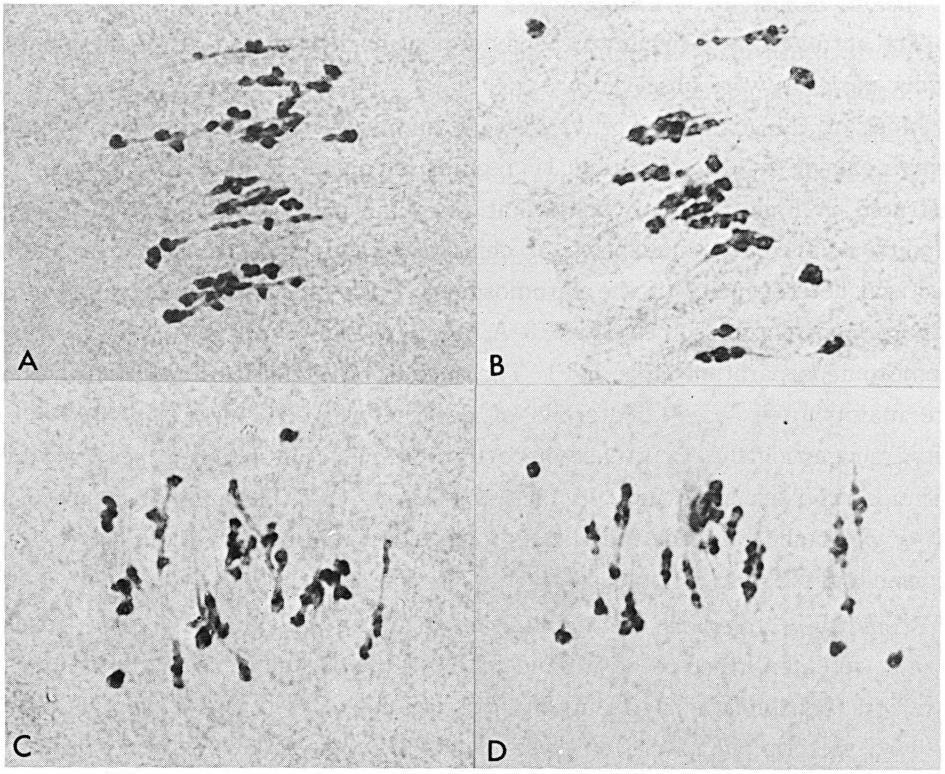

Fig. 8. Meiotic behaviours of the chromosomes in v. *Kwanso* collected from Matsunoki.

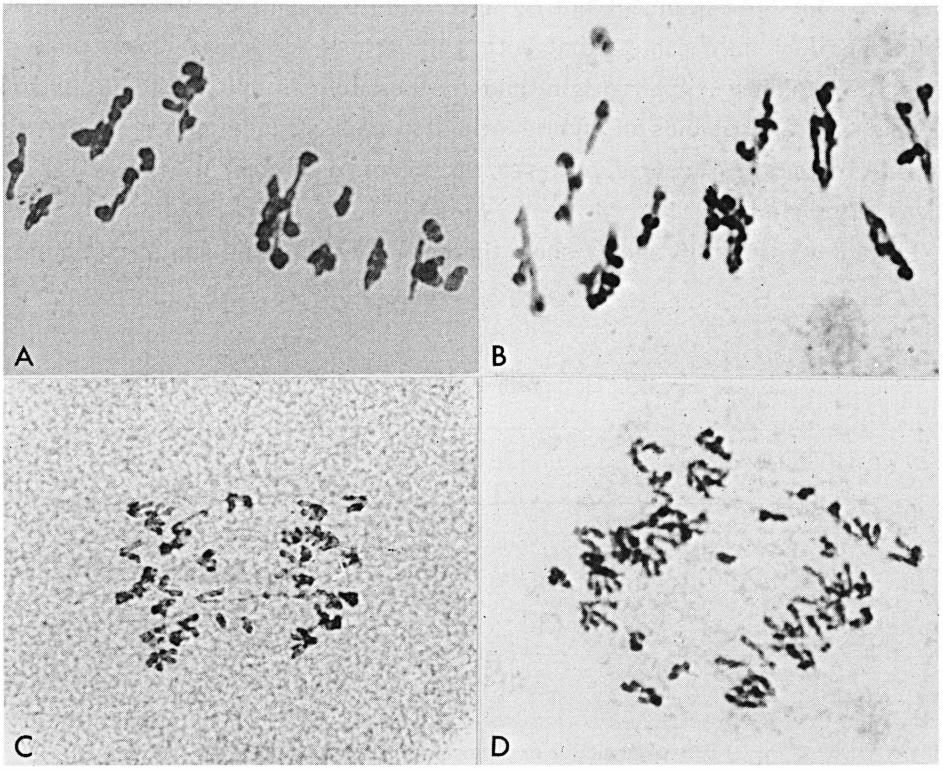

Fig. 9. Meiotic behaviours of the chromosomes in v. *fulva* collected from Hong Kong.

four *Hemerocallis* taxa in study. Over 97% of the pollen of both v. *longituba* and v. *disticha* was stainable with acetic carmine or cotton blue with the sole exception of v. *longituba* from Akitsu, in which pollen fertility attained only ca. 83%. There is no doubt that approximately 10% of the sterile pollen grains observed in v. *longituba* and v. *disticha* were produced by the meiotic irregularities just described above.

As was presumed, the pollen fertilities of triploid v. *fulva* and v. *Kwanso* were considerably lower, only 40 to 60% (cf. Table 3), which is doubtlessly due to irregular pairings of chromosomes at meiosis. Variation in pollen size is illustrated in Fig. 11, and as is shown, triploid *fulva* and *Kwanso* possess larger pollen grains of 78–104 X (86) 95–140 μ in size, whereas diploid *longituba* and *disticha* have somewhat smaller pollen grains, 65–83 X 87–124 μ in size.

III. Electrophoretic Patterns of Acid-Soluble Proteins

Figs. 12 and 13 illustrate the protein profiles of four *Hemerocallis* taxa. It was reported previously (Kawano and Noguchi, 1973) that the protein profile of v.

Table 3. Pollen fertility in the four varieties of *H. fulva.*

Taxa and samples \ Fertilty		Total no. of pollen grains examined	No. of fertile pollens	No. of sterile pollens	Fertility in%
v. *disticha*		6327	5792	535	91.5
v. *longituba*	No. 1	8651	8040	611	92.9
	No. 2	5383	4906	481	91.1
	No. 3	5785	5303	482	91.7
	No. 4	7873	7255	618	92.1
	No. 5	6580	5453	1127	82.9
v. *Kwanso*	No. 1	3734	1511	2223	40.5
	No. 2	6207	3706	2501	59.7
	No. 3	8650	5339	3311	61.7
	No. 4	9140	5250	3890	57.4
	No. 5	10483	6350	4133	60.6
v. *fulva*	No. 1	7755	5051	2704	65.1
	No. 2	10308	6030	4278	58.5
	No. 3	10116	4909	5207	48.5
	No. 4	10444	6752	3692	64.6
	No. 5	9086	5801	3285	63.8
	No. 6	9885	5956	3929	60.3
	No. 7	9307	6580	2727	70.7

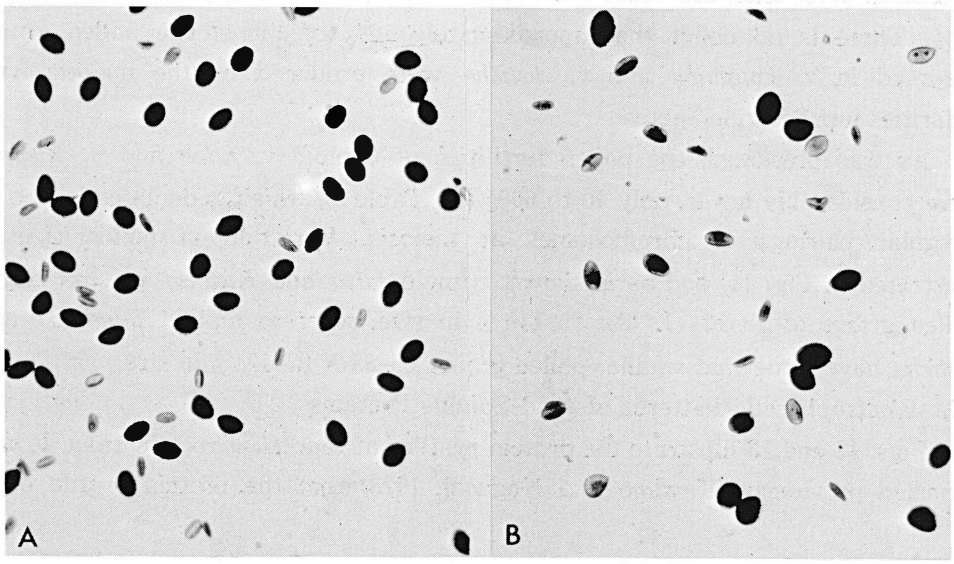

Fig. 10. Pollen grains of *H. fulva* v. *longituba* (A) and v. *Kwanso* (B). Note a number of sterile pollens.

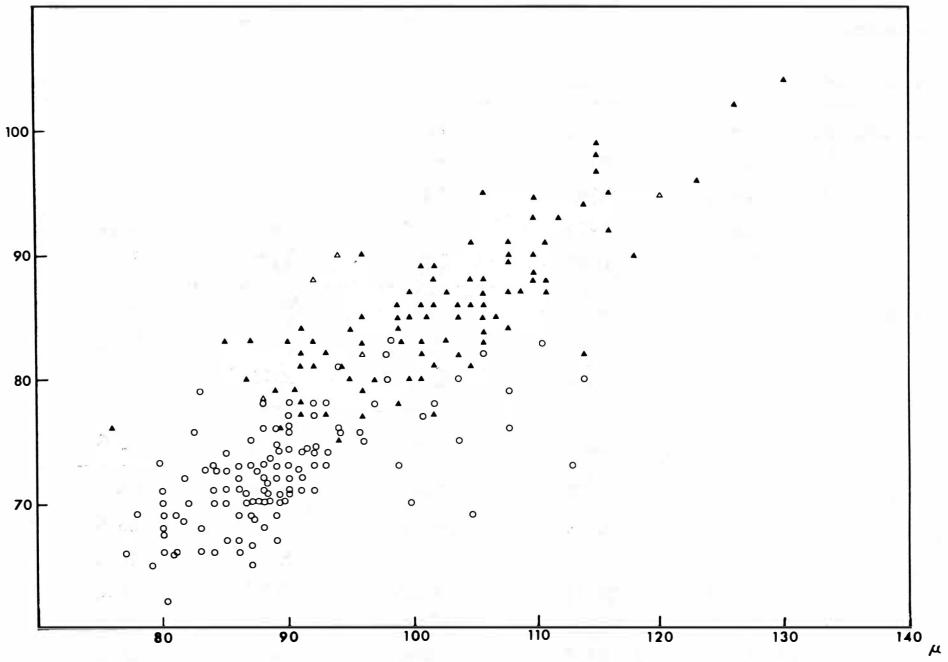

Fig. 11. Variation in pollen size of the four varieties of *H. fulva*. Open circles show the pollen grains of both v. *longituba* (2x) and v. *disticha* (2x); filled triangles those of v. *Kwanso* (3x); and open triangles those of v. *fulva* (3x).

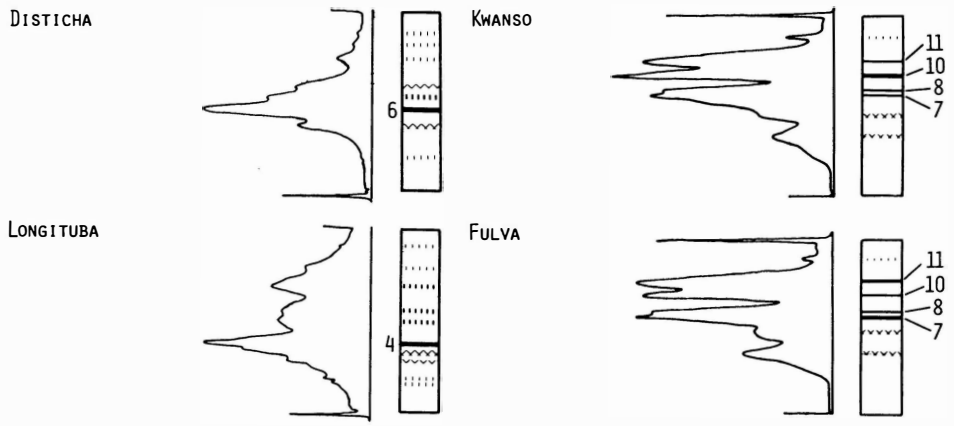

Fig. 12. Electrophoretic banding patterns and their densitometer curves of the four varieties of *H. fulva*.

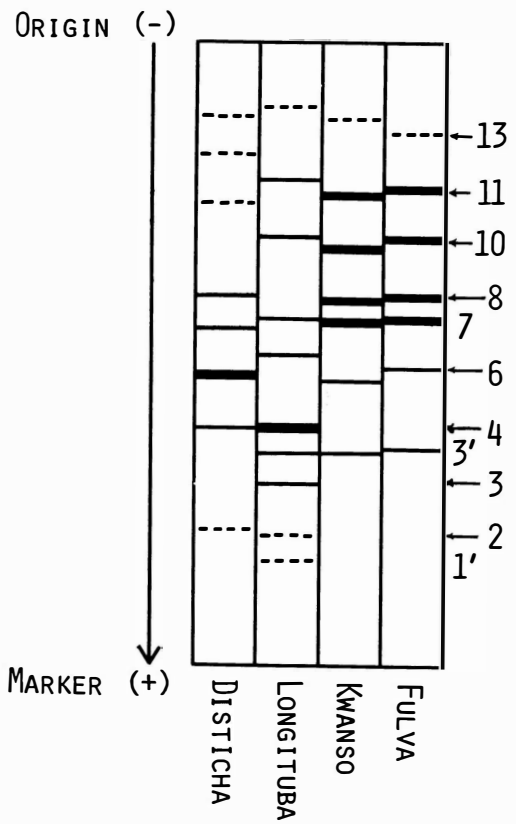

Fig. 13. Profiles of electrophoretic banding patterns reproduced from Fig. 12.

longituba is markedly different from that of v. *disticha.* V. *longituba* possesses ten bands (plus one very faint band), of which No. 4 exhibited the maximum absorbance in the optical density curve taken with a recording densitometer. Eight bands were seen in the protein profile of v. *disticha,* its maximum absorbance being found at the position of No. 6.

It is noteworthy that the protein profiles of v. *fulva* and v. *Kwanso* are quite similar, but are considerably different from either that of v. *longituba* or of v. *disticha* (see Figs. 12 and 13). Very conspicuous absorbances in the densitometer curves were observed at the positions of No. 7, 8, 10, and 11 in both v. *fulva* and v. *Kwanso.* It is interesting to note that the bands of No. 3 and No. 5 which are very characteristic of another *Hemerocallis* species, *H. citrina* v. *vespertina* (cf. Kawano and Noguchi, 1973) are lacking in both v. *fulva* and v. *Kwanso,* and also in v. *districha,* although v. *longituba* exhibits a weak absorbance at the position of No. 3.

Discussion

The evidence gathered in the present investigation as to variation in gross morphology, cytology, and protein banding patterns in electrophoresis suggests that two native Japanese taxa of *Hemerocallis,* i. e., *H. fulva* v. *longituba* and v. *disticha* which have been regarded as conspecific with *H. fulva* v. *fulva sensu stricto* (Matsuoka and Hotta, 1966) no doubt represent a different biological entity from the continental taxon *H. fulva* v. *fulva,* and double-flowered v. *Kwanso.* As shown here, they showed a marked difference in the variation of several gross morphological characters and in their protein banding patterns, whereas v. *Kwanso* displayed a remarkable similarity to continental taxon v. *fulva* in these characters.

Two varieties, v. *fulva* and v. *Kwanso,* are characterized by having broad leaves attaining 4 cm in width, and also broad internal perianths, ranging from 2.6 to 3.7 cm in width, which show a marked yellow coloration at the basal part. In addition, both are triploid with $2n=33$ somatic chromosomes, exhibiting very similar chromosomal pairing patterns and irregularities at meiosis. Therefore, both varieties are completely sterile, and no seeds are usually formed. This cytological feature suggests that both are at least not simple autotriploids in origin. The major difference detected between v. *fulva* and v. *Kwanso* is that the former has a much stouter growth habit with tall and thick flowering scapes and has somewhat larger, fresh pinkish red flowers; whereas v. *Kwanso* is more dwarfish and always possesses double-flowers in which pistils are completely reduced and only vestigial stamens are often present attached to the perianth fringe.

Usually, v. *longituba* is readily distinguished from v. *disticha* by its scarlet red

flowers, the typical plant of the latter usually possessing orange-colored flowers. It is interesting to note that there occurs a difference in the length of the flower tube enclosing the ovary between these two taxa, i. e., v. *longituba* as its epithet means has somewhat slender, long flower tubes ranging from (1.5)2.0 to 4.6cm in length, whereas those of v. *disticha* are much shorter and thick, ranging 1.5–3.8cm long. Although a conspicuous difference was observed in the protein banding patterns in electrophoresis between them, further critical analyses are needed to confirm this difference, since these two varieties are often sympatric, growing side by side, and assumed to be freely interbreeding with each other. The occasional occurrences of extremely variable individuals at sympatric sites, manifesting all possible transitional forms in variation between the typical two forms, doubtlessly indicate that interbreeding takes place among these plants to considerable extent, and there is no strong sterility barrier between them. However, sticky bridges or early chromosome separations observed at MI indicate at least their partial structural heterogeneities in chromosomes, and that both varieties do not constitute a genetically uniform and homogeneous population system.

In conclusion, what is to be stated based on the data available at present is that it should be more natural to regard the two native Japanese taxa, *H. fulva* v. *longituba* and v. *disticha* in the sense of Matsuoka and Hotta (1966) to be not conspecific with the continental taxon, *H. fulva* v. *fulva,* and its variety, v. *Kwanso.* However, the biological status of v. *longituba* (or *H. longituba*) and v. *disticha* is still not clear at present, and we must await future studies in order to determine more acurrately their taxonomic status and to make nomenclatorial transfers and changes.

Summary

1. Variation in gross morphology of the four *Hemerocallis* taxa referred to the *H. fulva* complex, i. e., v. *fulva,* v. *Kwanso,* v. *longituba,* and v. *disticha,* was analyzed and compared. As a result, it was found that two native Japanese taxa, v. *longituba* and v. *disticha* differ significantly from the continental taxon, *H. fulva* v. *fulva,* and its double-flowered variety, v. *Kwanso,* in leaf width and several floral characters.
2. Substantial evidence showing the phylogenetic relationships assumed based on gross morphological variation in these four taxa was obtained in the analysis by electrophoresis of the protein banding patterns. A remarkable similarity in the banding patterns was found between v. *fulva* and v. *Kwanso,* but their patterns differed significantly from those of either v. *longituba* or v. *disticha.* A difference detected in the banding patterns between v. *longituba* and v.

disticha needs to be reconfirmed, for they often grow sympatrically and interbreed freely.

3. Somatic chromosomes, and meiotic pairings and behaviours of all four taxa were also examined. Both v. *fulva* and v. *Kwanso* proved to be triploid with $2n=33$ chromosomes, and $2n=22$ chromosomes were also counted from v. *longituba* and v. *disticha,* which coincides with previous results reported by various authors. All the four taxa, however, turned out to possess essentially the same basic karyotypes, i. e., $n=11=2L+3j+3i+1m+1T+1h$. Meiotic pairings and behaviours of chromosomes in both triploid plants suggest that they are of at least not a simple autotriploid in origin. In is noteworthy that sticky bridges and early chromosome separations were observed in MI of the plants referrable to either v. *longituba* or v. *disticha* from Akitsu, which suggest their partial structural heterogeneities.
4. All the evidence gathered in the present study, though still limited, suggests that both v. *longituba* and v. *disticha,* though regarded as varieties of *H. fulva* by Matsuoka and Hotta (1966), represent a distinct biological entity, and should be regarded as a different species.

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