Biosystematic Studies on the genus
Hemerocallis (Liliaceae)

I.   Introgressive Hybridization between *H. citrina*
v. *vespertina**** and *H. fulva sensu lato*

By

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Introduction

The occurrence of a natural hybrid swarm between *Hemerocallis citrina* v. *vespertina* (Hara) M. Hotta and two varieties of *H. fulva, i. e.,* v. *longituba* (Miq.) Maxim. and v. *disticha* (Donn) M. Hotta, was first reported by Nakao and Yamashita (1956) from Matsunoki, located at the distance of 4 km east of Takayama City, Gifu Prefecture, Central Honshu.

Based on the results of their investigation, it was concluded that this particular hybrid swarm found in a valley near Takayama represents an initial stage of introgression of genes of *H. citrina* v. *vespertina* into the population of *H. fulva* (Nakao and Yamashita, *l. c.*).   Another example of introgressive hybridization in *Hemerocallis, i. e.,* between *H. middendorffii* and *H. yezoensis* was reported by Kawano (1961) from Otanoshike near Kushiro City in Hokkaido.   In both cases, the ecological disturbance of the habitats was deemed to be the major causes in breaking down the isolation barrier previously existing between these plants.

In the present study, a natural hybrid population of *Hemerocallis* in the Matsunoki district was re-investigated from several new standpoints in order to know more precisely about the biological status of this particular population.   In addition to the analyses of gross morphology and ecology, the karyotypes and meiotic behaviours of the chromosomes were studied.   The pollen fertility, the

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*** This plant was called *H. citrina* by Nakao and Yamashita (*l. c.*), but it should be referred to *H. citrina* v. *vespertina* in the strict sense.   Although there are considerable confusions in the taxonomic treatment of the species in the genus *Hemerocallis,* we have tentatively followed the nomenclatorial treatment by Matsuoka and Hotta (1966) in the present paper.

setting rate and germination rate of the seeds were also examined. Furthermore, the electrophoretic banding patterns of acid–soluble protein extracted from the rhizomes of both parental and hybrid plants were likewise analyzed and compared with one another.

Materials and Methods

The samplings were made in several local sympatric populations of four *Hemerocallis* taxa, *i. e., Hemerocallis citrina* v. *vespertina, H. fulva* v. *kwanso,* v. *disticha,* and v. *longituba* which are distributed in a small valley of Matsunoki in the vicinity of Takayama City, Gifu Prefecture, and putative hybrid clones were found here and there among those of the typical taxa in the populations.

Although the plants of *H. fulva* v. *kwanso* with double flowers are also found in this area, this variety is triploid with 2n=33 chromosomes and entirely sterile. Usually, pistils are completely reduced and lacking, and only vestigial stamens are sometimes found. Although there remains a slight possibility of gene flow from this plant to other *Hemerocallis* taxa growing side by side at the same site, we have obtained no convincing evidence of gene flow so far, and thus, this variety was excluded from the present analysis.

Gross Morphology

Examination of the external characters were made on plants taken directly from their native habitats. The characters studied were the height of the 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. Observations were also made on the shape and size of the mature capsules, and the fertility and germination rate of the seeds. After their examination, the hybrid index method by Anderson (1949) was used to make an analysis of the composition of the populations.

For the analysis of the population constitution, the following six characters were further chosen : the color and scent of the flowers, the height of the scapes, the width of the leaves, the type of the inflorescences, and the time of flowering. Among these characters chosen, the color and scent of the flowers, the type of the inflorescences, and the time of flowering are of diagnostic value for discriminating between *H. fulva sensu lato* and *H. citrina* v. *vespertina* (Matsuoka and Hotta, 1966). Each character was assigned an arbitrary index value 0, 1, 2, 3, or 4, to dessignate, respectively, the total range of variation of the character in each category, *i. e.,* both the parental taxa and the hybrids. The total index value of a plant was obtained by adding up the index values of all its characters. Each index value and

Table 1. Variation range and index value given to each taxon (For details, see text)

Taxa Character		*H. fulva* v. *longituba*	v. *disticha*		*H. citrina* v. *vespertina*
flower color	red	reddish orange	orange yellow	lemon yellow with red or orange tint	fresh lemon yellow
	0	1	2	3	4
scape hieght		−94	−94		95−
		0	0		1
leaf width		−1.2	−1.2		1.3−
		0	0		1
inflorescence type		dichotomous	trichotomous or irregularly branched		branched
		0	1		2
fragrance of flower		none	none		present
		0	0		1
time of flowering		day-blooming	day-blooming		night-blooming
		0	0		1
Total index value	0	1	3	9	10

its variation range is found in Table 1, which exhibits 0 as the typical total index value for *H. fulva* v. *longituba,* 3 as typical for v. *disticha,* 10 as typical for *H. citrina* v. *vespertina,* and 4–9 for an intermediate hybrid.

Cytology

Studies were made on the karyotypes, meiotic behaviours of the chromosomes, and the pollen fertility of these parental plants as well as putative hybrid clones. 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 and stained with 1 % iron–arum acetic carmine.

Electrophoretic Patterns of Acid–Soluble Proteins

The fresh rhizomes of *Hemerocallis* were homogenized in 0.5 N H_2SO_4, and centrifuged at 8,000g for one hr. Supernatant was dialysed for 24 hrs against 0.25 N H_2SO_4 buffer. Protein was precipitated by adding the equal amount of aceton 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-Mercaptoethanol, and again centrifuged at 100,000g for one hr. The extracts were electrophoresed at pH 6.8 with above-mentioned gel buffer with 1.5% $(NH_4)_2S_2O_8$ and NNN'N'-Te-tramethyl-ethylenediamine on 10% acrylamide gels. A 0.2 ml sample was applied to each column and electrophoresed for one hr at 2mA per column, and subsequently for 3.5 hr at 5mA. 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
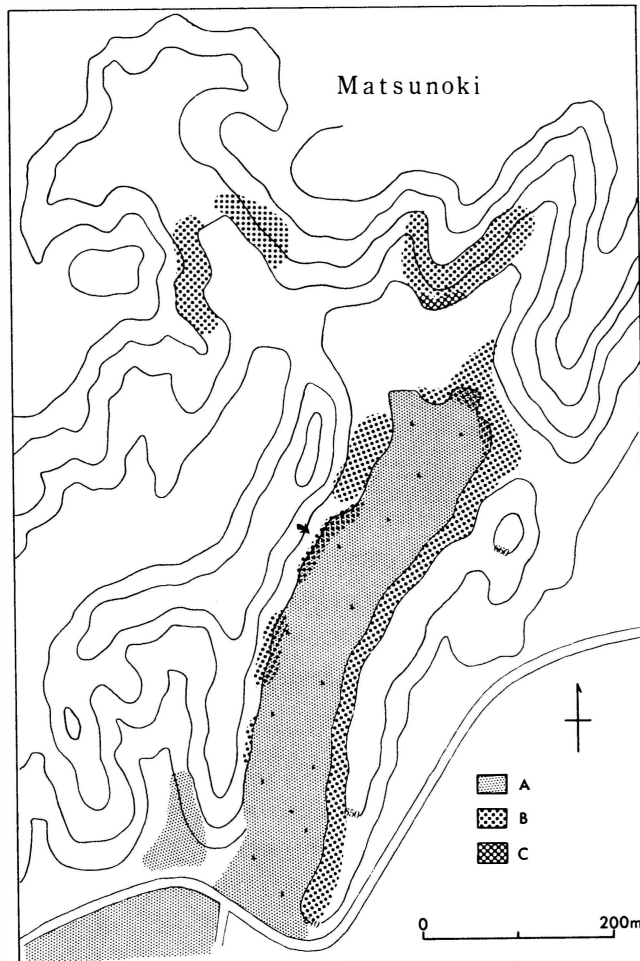
I. Ecology and Composition of the Population

Fig. 1. The habitat of a hybrid swarm between *Hemerocallis citrina* v. *vespertina*, and *H. fulva* v. *disticha* and v. *longituba* in Matsunoki near Takayama City, Gifu Prefecture; A: The main habitat of *H. fulva* v. *longituba* and v. *disticha*; B: That of *H. citrina* v. *vespertina*; C: That of *H. fulva* v. *kwanso*. Arrow indicates the site where a number of putative hybrids were discovered and collected.

In Figs. 1 and 2, the topography and ecological backgrounds in the area of Matsunoki, and also the distribution of *Hemerocallis* plants in this territory are exhibited.   At the site on the east-facing slope, indicated by an arrow in Fig. 1, a
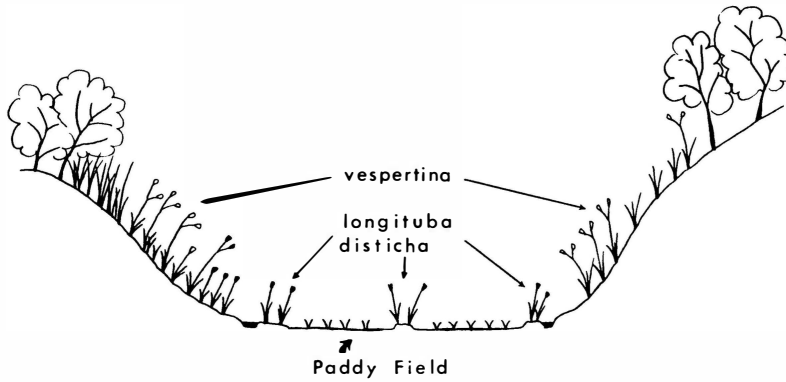

Fig. 2. The profile of the habitat of *Hemerocallis* in Matsunoki,
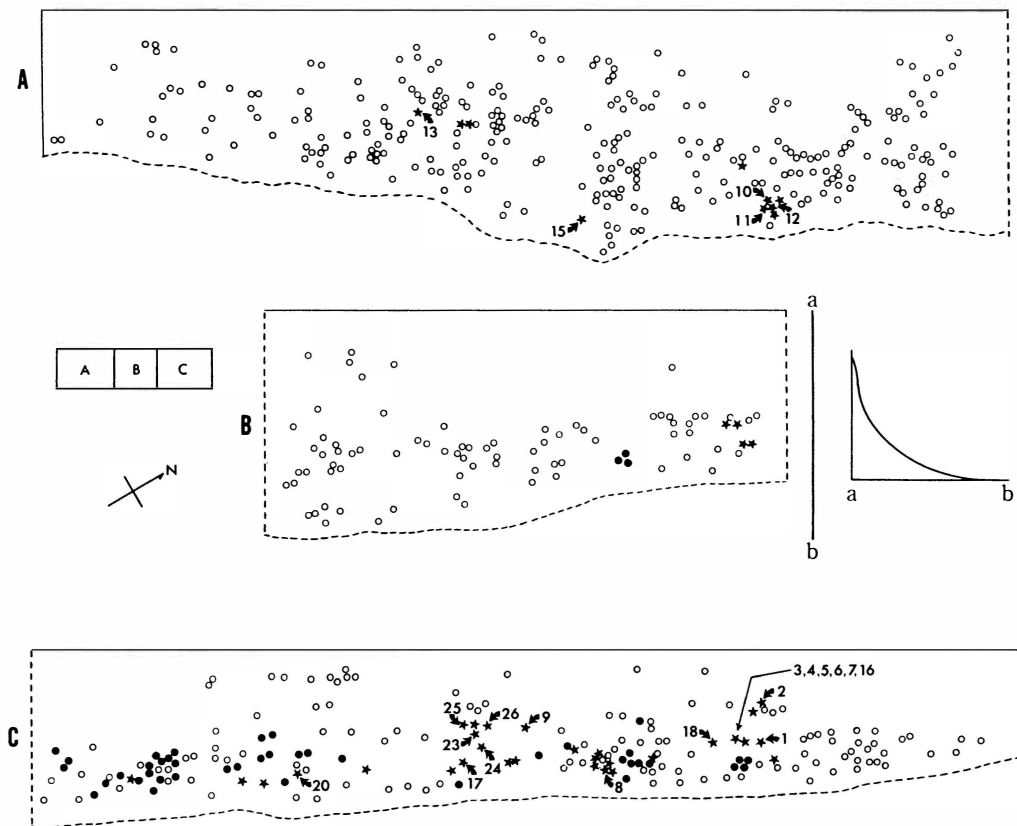illustrated in Fig. 1.

Fig. 3. The distribution of *Hemerocallis* clones at the site indicated in Fig. 1.   Open circles specify those of *H. citrina* v. *vespertina*; filled circles *H. fulva* v. *disticha* and v. *longituba,* and filled stars putative hybrids.

number of putative hybrids were discovered and collected. A more detailed distribution of the clones of respective taxa and their intermediate forms in this particular site is illustrated in Fig. 3.

Populations of *Hemerocallis* are scattered over the grassy slopes developed at the margin of the secondary forests and also at the margin of the paddy field in the valley. Ecologically, most of such grassy places in this area is strongly disturbed by some artifical means. In general, the clones of *H. citrina* v. *vespertina* chiefly spread on somewhat dry, grassy slopes of the valley (Figs. 1–B, and 2), where the following plants are associated with *Hemerocallis* plants: *Miscanthus sinensis, Thalictrum thunbergii, Artemisia princeps, Sanguisorba officinalis, Vicia unijuga, Astilbe thunbergii, Patrinia scabiosaefolia, P. villosa, Geranium thunbergii, Pteridium aquilinum, Lysimachia chlethroides, Rosa polyantha, Vicia cracca,* etc. Judging from the frequent associations of such weedy species as *Erigeron canadensis, Pueraria lobata, Prunella vulgaris,* etc. with *Hemerocallis* on such grassy slopes, occassional artificial disturbances of the habitats are very evident.

Whereas, the clones of *H. fulva* v. *disticha* and v. *longituba* spread more moist sites along the paddy field (Figs. 1–A and 2), where more frequent artifical disturbances of the habitats are evident by mowing grasses, etc. Here, premature flowering scapes of *Hemerocallis* are often cut off, and this would no doubt affect considerably the breeding behaviour and reproduction of *Hemerocallis* plants, especially those growing on the paddy field margin. Although both v. *longituba* and v. *disticha* are often growing side by side, usually v. *longituba* with markedly reddish flowers prefers the more moist sites by the paddy field, but orange flowered v. *disticha* occupies somewhat an intermediate zone between *H. citrina* v. *vespertina* and *H. fulva* v. *longituba* habitats.

According to the scoring method adopted in this study, 108 plants of a total of 396 plants examined proved to be hybrids or hybrid derivatives (Fig. 4). The total index values of such intermediate forms range from 0 to 10, but they are readily discernible as natural hybrids as judged from characteristic character combinations in the flower color, the types of the inflorescences, etc. Eighty–five of the 135 clones of *H. citrina* v. *vespertina* scored the maximum total index value of 10, but the remaining 50 plants failed to score the absolute maximum number since the scape height, leaf width, and the type of the inflorescences are somewhat variable and had smaller measures than those designated as typical for *H. citrina* v. *vespertina* by this method of scoring. With regard to the characters chosen, the plants with such minor deviations from the ideal total index value could still be accounted for as a result of intraspecific variation within the population. The same may be said for the variability of *H. fulva* v. *longituba* and v. *disticha,* but local variations in this species are much more complex than those of *H. citrina* v.

vespertina.   Future critical studies are much needed to elucidate the geographical variations within this exceedingly polymorphic and complex species, i. e., *H. fulva sensu lato.*
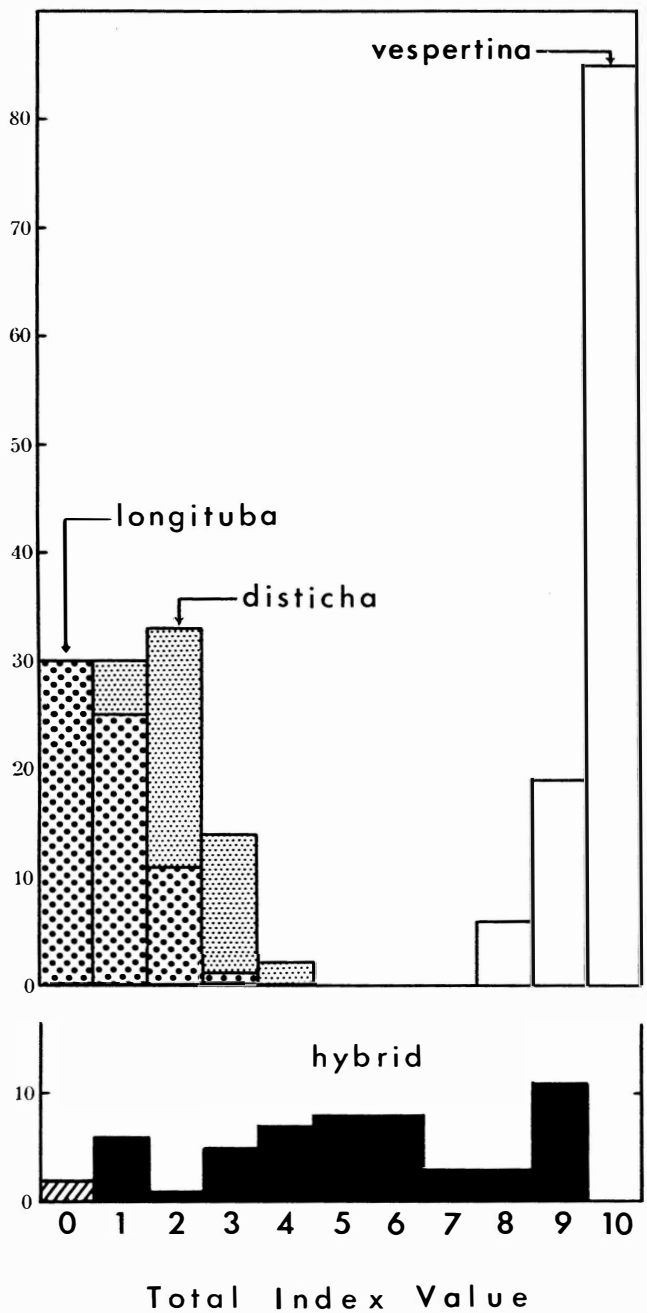

Fig. 4. Frequency distribution of total index values of *H. citrina* v. *vespertina, H. fulva* v. *disticha,* v. *longituba,* and their hybrids in Matsunoki.

II. Comparison of Characters

(1) Scape Height (Fig. 5)

Generally, the flowering scapes of *H. citrina* v. *vespertina* are much higher than that of *H. fulva* v. *longituba* and v. *disticha,* and range from 74 to 185 cm in height. *H. fulva* v. *longituba* has the lowest flowering scapes with an average of 58.8 cm in height (see Table 2).   Most of the hybrid plants is intermediate in this character between the three parental taxa.
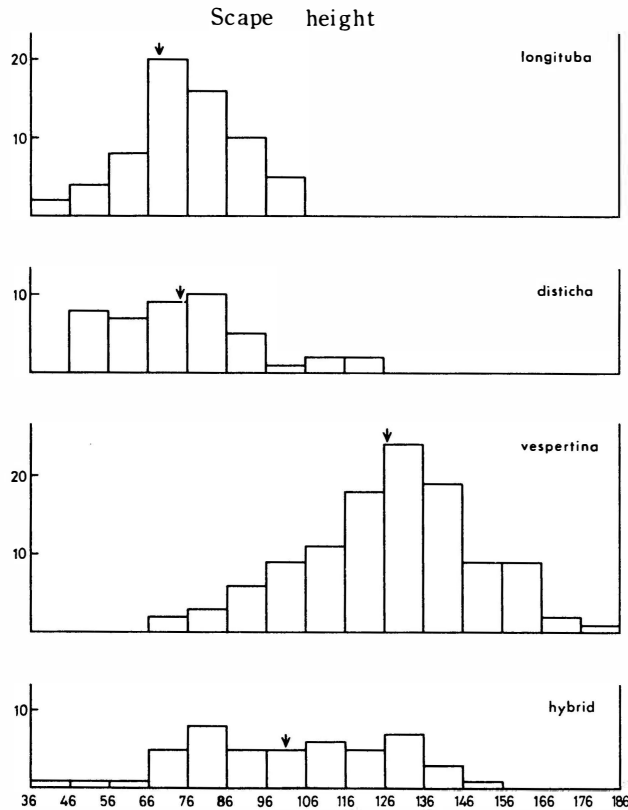
Scape   height

Fig. 5. Variations in the scape height of three parental taxa and hybrids. Arrows indicate mean values.

(2) Inflorescence (Fig. 6)

The type and length of the inflorescences are characteristic features in the genus *Hemerocallis.*   Generally, *H. citrina* v. *vespertina* possesses long and well branched inflorescences, 2.5–40.0 cm long, whereas those of *H. fulva* v. *disticha* and v. *longituba* are usually dichotomous, 1.5–18.4 cm and 0.7–14.5 cm long, respectively. The hybrid plants have intermediate inflorescences between parental plants in shape and size, ranging 1.0–24.0 cm long, but the inflorescences of hybrid plants are extremely variable, often trichotomous, irregularly branched, or non-branched and pseudo-umbelliform, etc.

Table 2. The mean value of the external characters in natural populations of *Hemerocallis citrina* v. *vespertina, H. fulva* v. *disticha,* and v. *longituba,* and their hybrids

Taxa / Characters	*H. citrina* v. *vespertina*	Hybrids	*H. fulva* v. *disticha*	*H. fulva* v. *longituba*
Scape height (cm)	74 − 185	39 − 147	46 − 120	39 − 100
Mean	126.8 ± 22.3	101.1 ± 25.9	74.6 ± 18.6	68.8 ± 13.3
Inflorescence length (cm)	2.5 − 40.0	1.0 − 24.0	1.5 − 18.4	0.7 − 14.5
Mean	15.1 ± 7.4	10.2 ± 6.7	3.7 ± 2.4	5.6 ± 3.3
No. of flowers per scape	3 − 38	3 − 19	3 − 23	1 − 12
Mean	13.5 ± 6.4	10.4 ± 5.2	7.9 ± 3.4	6.5 ± 2.4
Bract length (cm)	1.0 − 7.0	1.0 − 5.0	0.7 − 4.0	0.5 − 2.3
Mean	2.3 ± 1.3	2.2 ± 0.9	1.4 ± 0.7	1.2 ± 0.5
Leaf length (cm)	20 − 117	10 − 110	9 − 65	10 − 80
Mean	89.0 ± 23.0	62.5 ± 31.8	16.5 ± 11.7	43.9 ± 24.7
Leaf width (cm)	0.6 − 3.6	0.5 − 2.0	0.5 − 1.6	0.7 − 1.7
Mean	1.85 ± 0.37	1.43 ± 0.38	1.00 ± 0.30	1.02 ± 0.25
External perianth length (cm)	5.2 − 9.0	5.0 − 9.9	5.5 − 9.8	5.5 − 9.1
Mean	7.1 ± 0.8	7.6 ± 1.1	7.2 ± 1.1	7.4 ± 0.9
External perianth width (cm)	0.8 − 1.8	0.8 − 2.0	1.1 − 2.3	1.0 − 2.0
Mean	1.27 ± 0.22	1.43 ± 0.30	1.56 ± 0.32	1.35 ± 0.19
Internal perianth length (cm)	5.0 − 10.1	5.4 − 10.0	5.6 − 10.3	6.0 − 9.0
Mean	7.3 ± 0.9	7.1 ± 1.2	7.4 ± 0.7	7.4 ± 0.7
Internal perianth width (cm)	1.5 − 3.0	1.4 − 2.9	1.5 − 2.9	1.6 − 2.9
Mean	2.18 ± 0.35	2.11 ± 0.30	2.10 ± 0.30	2.08 ± 0.31

The number of flowers per flowering scape varies to a large extent in all taxa and hybrids examined, but normally *H. citrina* v. *vespertina* with long, well-branched inflorescences bear the most numerous flowers per scape, 3 to 38 (mean value: 13.5 ± 6.4); whereas *H. fulva* v. *longituba* bears contrastingly few flowers, 1 to 12 (mean value: 4.7 ± 2.7). *H. fulva* v. *disticha* has 3 to 23 flowers per scape (mean value: 8.5 ± 4.6). The hybrid individuals possess somewhat intermediate flower numbers, 3 to 19 (mean value: 10.4 ± 5.2). The representative types observed in the inflorescences are illustrated in Fig. 7.

(3) Flower and Flowering Time

The difference in the flower color and other flower characteristics between parental plants is most remarkable, *H. citrina* v. *vespertina* possessing a distinctly lemon yellow flowers with fragrance, whereas those of *H. fulva* v. *longitube* and v. *disticha* are fresh reddish or pale reddish and orange in color, respectively, both

Inflorescence length
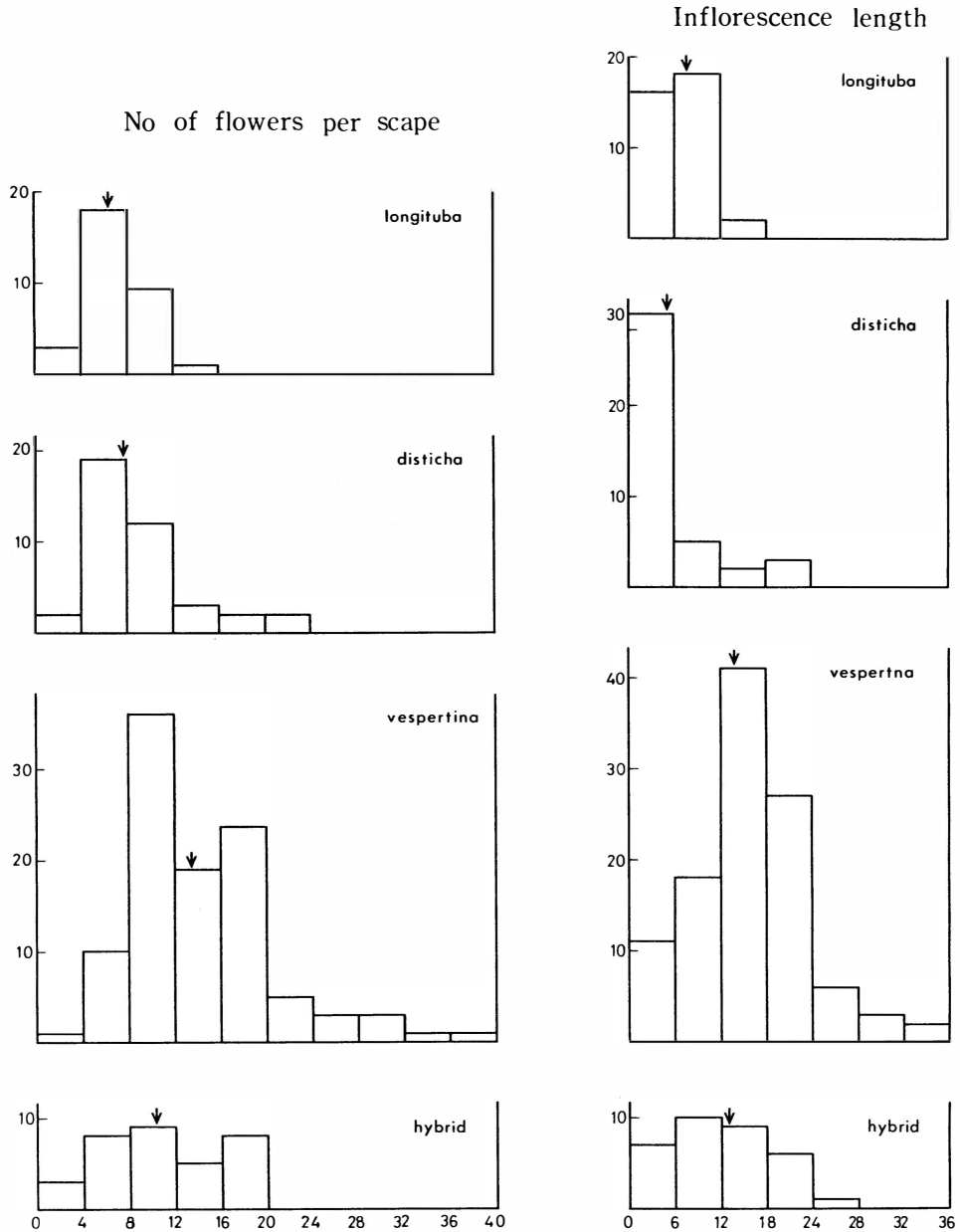
No of flowers per scape

Fig. 6. Variations in the flower number per scape and the inflorescence length of three parental taxa and hybrids. Arrows indicate mean values.

being without any scent. However, the color of the flowers in hybrid plants is exceedingly variable, sometimes pale lemon yellow with marked red or orange tint, orange yellow or reddish orange, etc. The hereditary mechanism governing the flower color is not understood at present. No clear correlation appears to exist between the flower colors and other morphological characters.

The flowering periods of *H. citrina* v. *vespertina* and *H. fulva* v. *longituba* and

v. *disticha* range from the end of June to August. Although the flowering periods of these two species are seasonally overlapping, there occurs an allochronic isolation between them, for as is called "day-lily", flowering of each flower in *Hemerocallis* plants lasts for 10 to 12 hours only, and usually, *H. fulva* v. *longituba* and v. *disticha* begin to bloom at about 6 o'clock in the morning and wither at about 7–8 o'clock in the evening, whereas flowering in *H. citrina* v. *vespertina* begins in the evening at about 6 to 7 o'clock and ceases at dawn. As stated above, *H. fulva sensu lato* is day–blooming, but *H. citrina* v. *vespertina* is nocturnal, and thus, usually on fair days no diurnal overlapping in flowering between the two species is encountered even at a sympatric site. On cloudy days, however, a slight overlap of 2–3 hours in the flowering of these two species is occassionally met with, since the flowers of day–blooming *H. fulva* v. *disticha* and v. *longituba* wither one to two hours later than usual on fine days ; on the other hands, under the unusual dim condition in the evening on cloudy or rainy days, *H. citrina* v. *vespertina* begins to flower somewhat earlier than usual. This tendency is especially evident on the east-facing slope, and this may be a prime cause in the break–down of the allochronic isolating barrier between these two species at such a sympatric site.
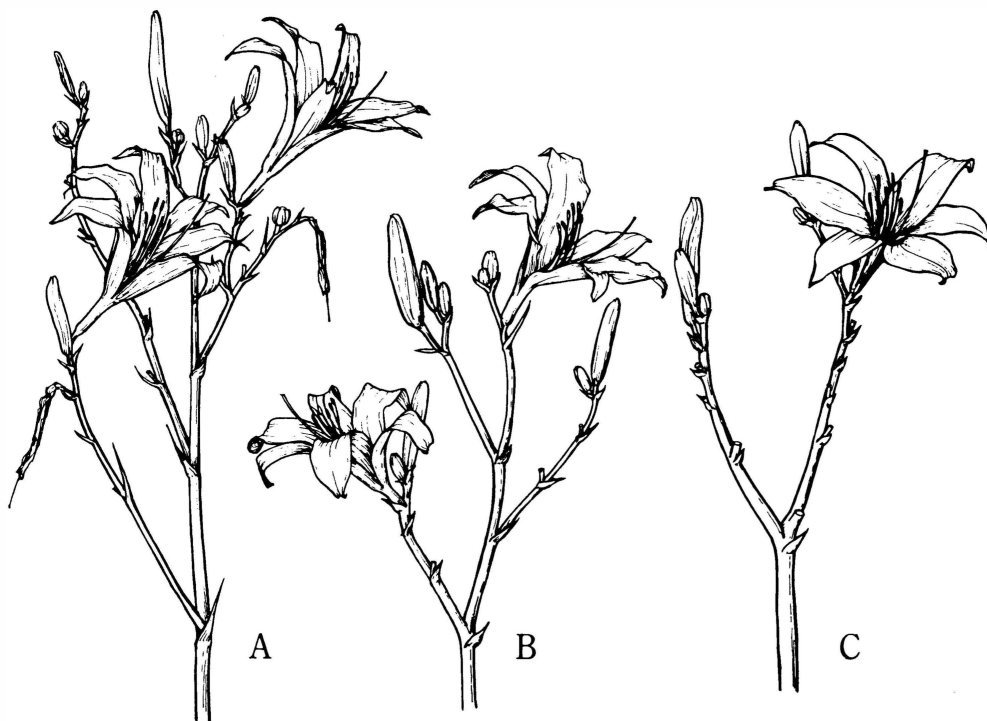

Fig. 7. Illustrating the type of the inflorescences found in typical *H. citrina* v. *vespertina* (A), an intermediate form (B), and typical *H. fulva* v. *disticha* or v. *longituba* (C).
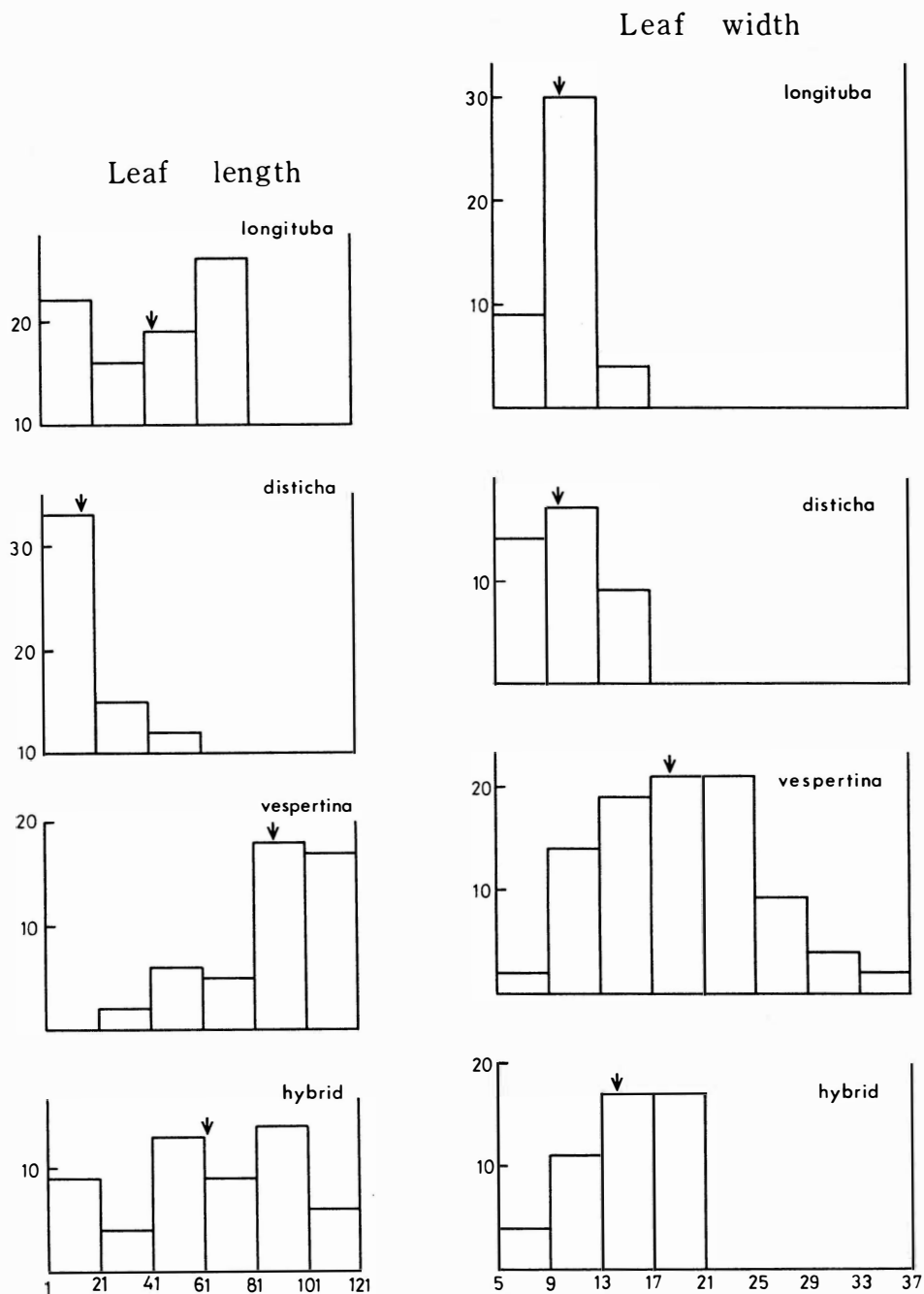
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Leaf width

Leaf length

Fig. 8. Variations in the length and width of the leaves of three
parental taxa and hybrids. Arrows indicate mean values.

Most of the hybrid plants are either day- or night-blooming, but some clones exhibited a very irregular blooming time, i. e., flowering initiating late in the evening and lasting more than 12 hours. An exceptional case was encountered in a clone of M-3, in which blooming of each flower lasts more than 15 hours, and this

fact indicates the physiology of flowering process is somewhat upset in this particular clone. The detailed character combinations of each hybrid individual is illustrated in the scatter diagram of Fig. 11.

The variation ranges in the remaining flower characters, i. e., the length and width of the external and internal perianths, and the length of the flower tube enclosing the ovary, are exhibited in Table 2. These flower characters showed almost the same range of variation in all the parental and hybrid plants examined, and no conspicuous distinction was detected between them.
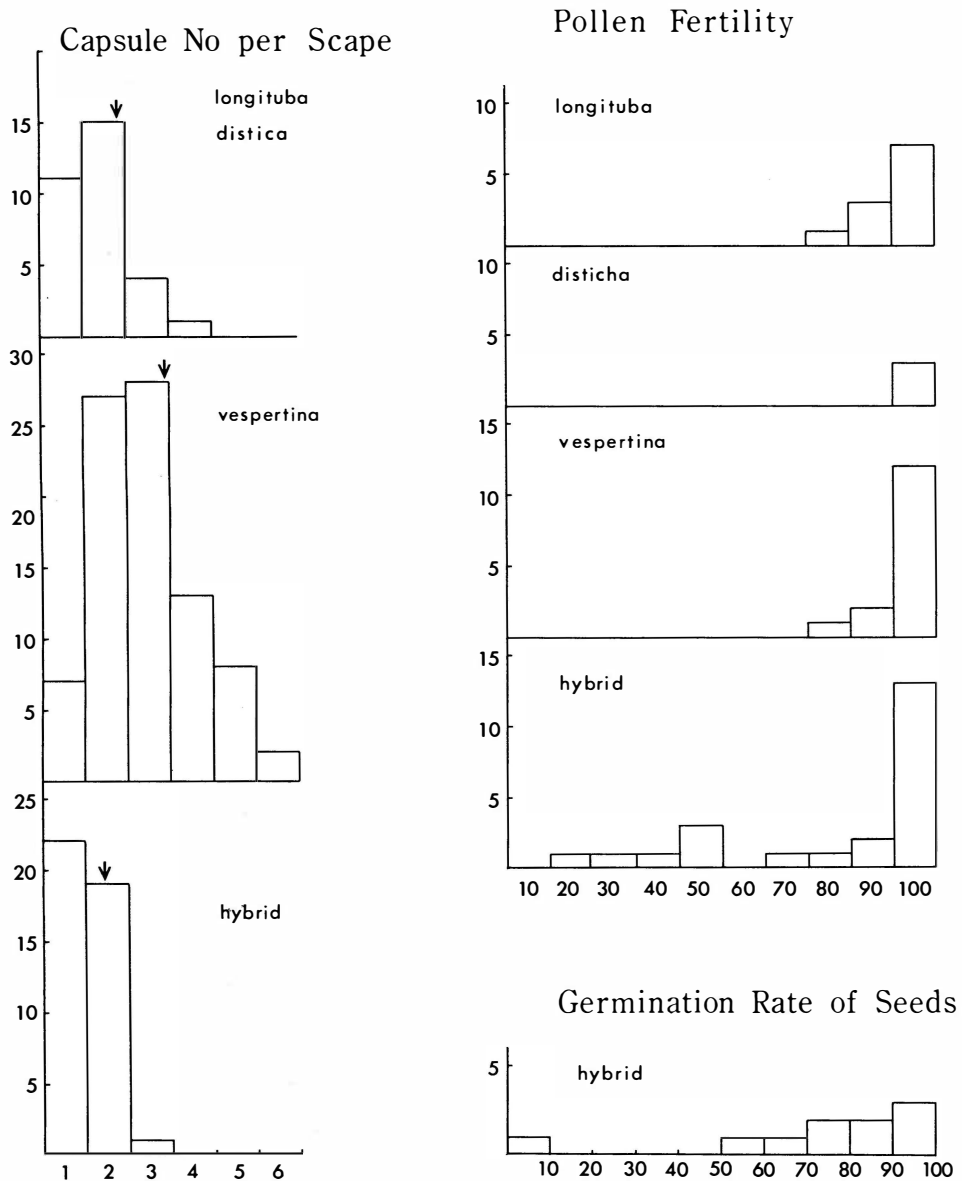

Fig. 9. Variations in the number of capsules per scape, pollen fertility and the germination rate of the seeds of three parental taxa and hybrids.

(4) Leaf and Bract (Fig. 8)

Conspicuous discontinuities in the variation range of the leaf length and width are observed between the three taxa of *Hemerocallis* (cf. Table 2; Fig. 8). *H. citrina* v. *vespertina* possesses the longest and broadest leaves, 20–117 cm long (mean value: 89.0±23.0 cm long) and 0.6–3.6 cm (mean value: 1.85±0.37 cm wide), whereas the leaves of *H. fulva* v. *disticha* are rather short in length, ranging 9–65 cm (mean value: 16.5±11.7 cm long), but those of v. *longituba* are 10–80 cm long (mean value: 43.9±24.7 cm long). The leaves of hybrids are intermediate, but longer than those of v. *disticha* and v. *longituba,* and range from 10 to 109 cm long (mean value: 62.5±31.8 cm long), and 0.5–2.0 cm wide (mean value: 1.4±0.4 cm wide).

The bract is a variable character. In particular, *H. citrina* v. *vespertina* bears long, leafy bracts at each node of the inflorescences, whereas those of *H. fulva* v. *disticha* and v. *longituba* are small and often inconspicuous. The hybrids have intermediate bracts in length and shape.
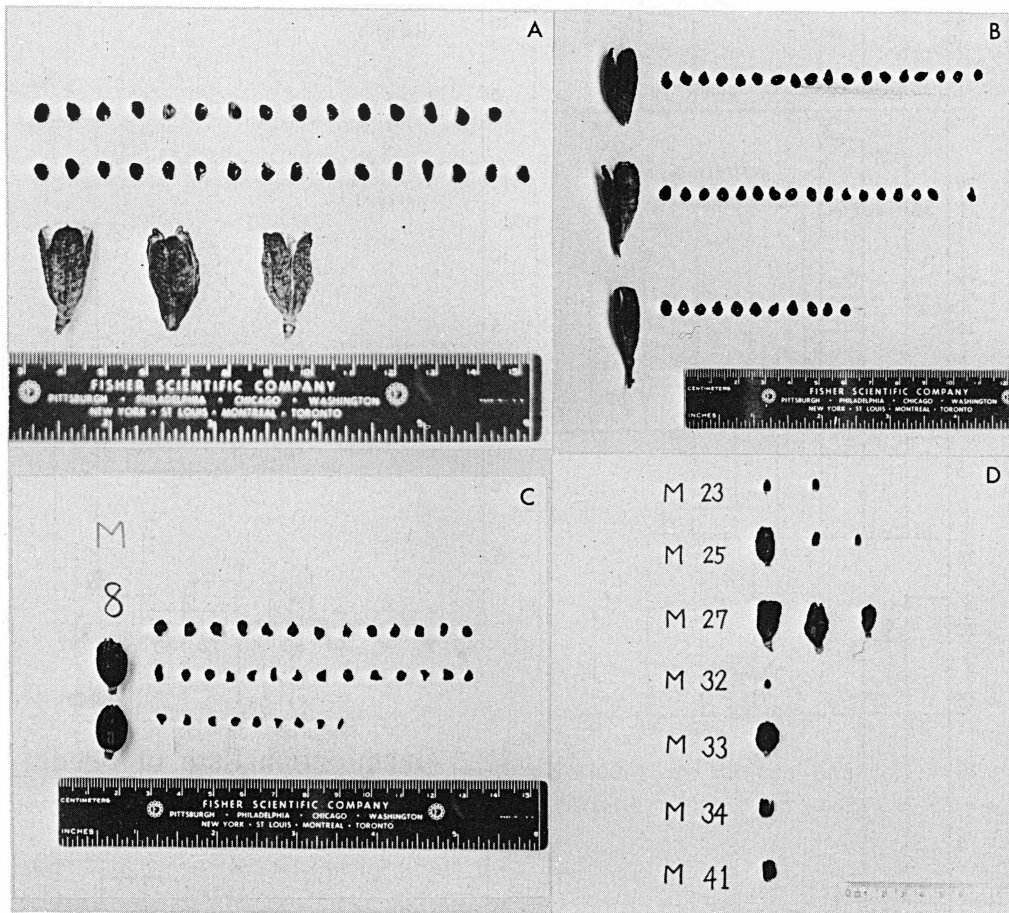

Fig. 10. Capsules of *Hemerocallis* at maturity. A: Those of *H. fulva* v. *disticha*; B: Those of *H. citrina* v. *vespertina*; C and D: Those of hybrids from Matsunoki.

(5) Capsule and Seed (Figs. 9 and 10)

The three–valved capsules of *Hemerocallis* are three–loculicidal, with black spherical seeds inside each locule. *H. citrina* v. *vespertina* has ovoid–campanulate to narrowly cone–shaped capsules which have 15 to 21 axile placental positions for seeds in each locule, whereas those of *H. fulva* v. *disticha* and v. *longituba* are ovoid-campanulate in shape, possessing 8 to 9 axile placental positions in each locule.

The capsules of the hybrids are much smaller in size, and irregular in shape, containing few seeds only. The number of the capsules borne per scape in respective taxa is illustrated in Fig. 9–A. The germination test of the seeds was undertaken. All the seeds of parental plants tested germinated well. The germination rate of the seeds in hibrid plants and various artificial crosses made is summarized and shown in Table 3.

(6) Breeding System and Pollinators

The breeding system of *Hemerocallis* is not understood well at present, but, judging from the fact that the positions of stigma and anthers are somewhat distantly located when bloomed, and as mentioned earlier, the seed setting rate is not very high even in the parental species placed in open pollination, outbreeding is perhaps the predominant system in the genus *Hemerocallis*.

Table 3. The germination rate of the seeds of hybrid plants and artificial crosses made experimentally

Strains & crosses made	Germination rate
M–4 · 5 · 6 · 16	79.1
M–31	93.9
M–1 · 2	0
M–40	100
M–20	72.7
M–4.5	57.3
M–6	100
M–7	88.2
M–25	64.8
M–10 X *M–40*	88.0
disticha X *vespertina* (a)	62.5
disticha X *vespertina* (b)	100
longituba X *vespertina*	90.4
M–25 X *M–40*	56.2
M–26 X *vespertina*	85.7
M–1.2 X *vespertina*	72.2

Many insects such as bees and butterflies were observed busily visiting *Hemerocallis* flowers during the day–time, but large butterflies such as *Papilio machaon hippocrates, Papilio bianor dehaani,* etc. seem to be most effective pollinators as their wings readily touch either long–protruding stigma or anther when they visit the flowers. For nocturnal *H. citrina* v. *vespertina* with fragrant lemon yellow flowers, hawk moths no doubt represent most effective pollinators.

(7) *Character Combinations in Hybrids* (Fig. 11)

Detailed character combinations of the hybrid plants are provided in Fig. 11. As can be seen from the diagram, the hybrids appear to be not only F_1 individuals,
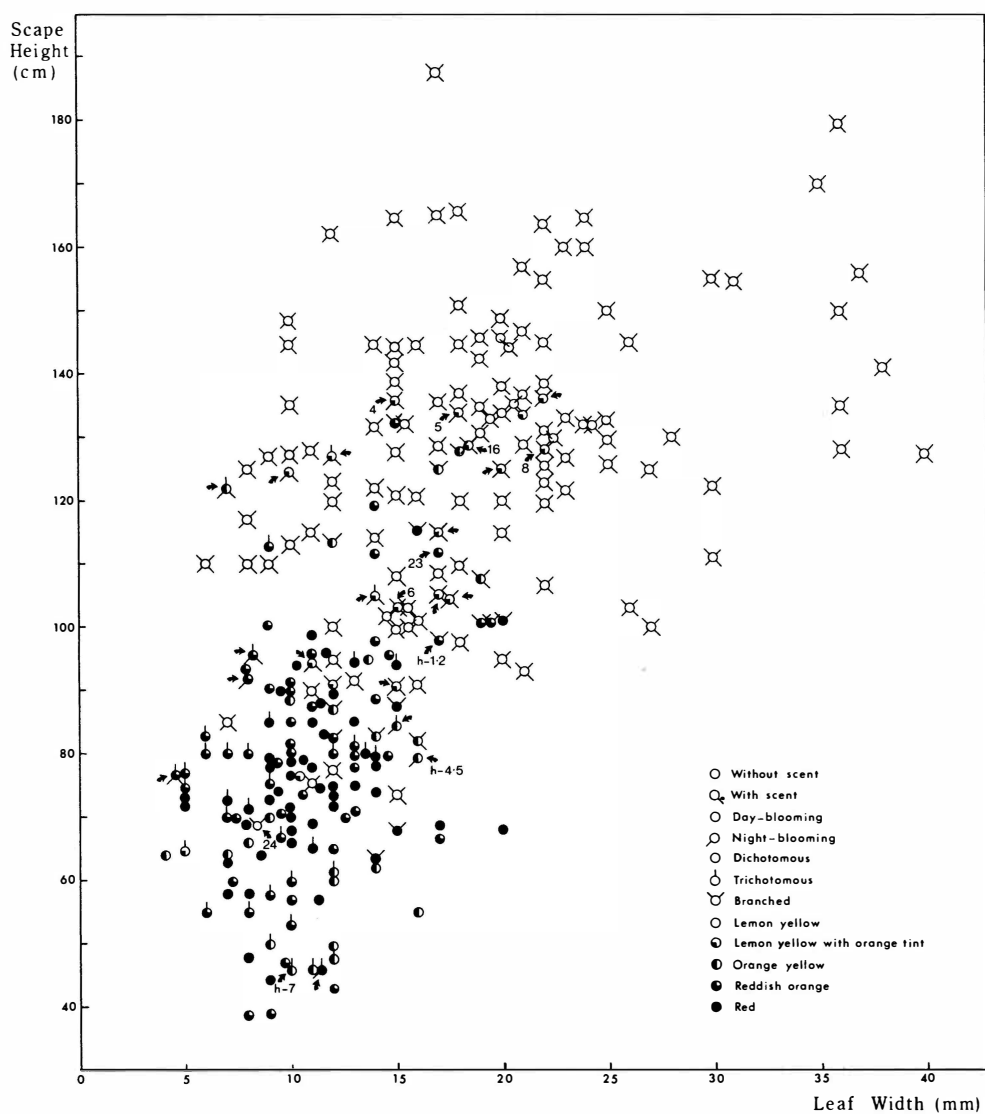

Fig. 11. Scatter diagram illustrating the variations in six different characters and the character combination of each individual collected from Matsunoki. Arrows indicate natural hybrids.

but also F_2 or backcross individuals are included in the population.   The gene flows from *H. fulva* v. *disticha* and v. *longituba* into *H. citrina* v. *vespertina,* or on the contrary, from *H citrina* v. *vespertina* into *H. fulva* v. *disticha* and v. *longituba* are very evident from the results of the present analysis.

The characteristics of both parental taxa and representative hybrid individuals are described in the following :

H. citrina v. *vespertina :* Night–blooming ; scapes tall with long, well–branched inflorescences ; flowers fresh lemon yellow with strong fragrance.

H. fulva v. *disticha :* Day–blooming ; scapes low with dichotomous inflorescences ; flowers well–open when bloomed, orange in color without any fragrance.

H. fulva v. *longituba :* Day–blooming ; scapes low with dichotomous inflorescences ; flowers fresh red in color without any fragrance.

M–1 · 2 : Day–blooming ; inflorescences branched, bearing orange–yellow flowers with reddish tint and without fragrance ; fertile and numerous capsules borne.

M–3–30 : Day–blooming ; inflorescences dichotomous or somewhat branched, bearing pale–reddish orange flowers without fragrance.

M–4 · 5 · 6 · 16 : Night–blooming ; inflorescences markedly branched, bearing reddish orange–lemon yellow flowers with fragrance.

M–7 : Day–blooming ; inflorescences irregularly dichotomous, bearing dark–red flowers without fragrance.

M–8 : Day–blooming ; inflorescences branched, bearing orange–yellow flowers with fragrance.

M–24 : Day–blooming ; inflorescences short, 3 cm long, but dichotomous, bearing lemon yellow flowers without fragrance.

M–25 : Day–blooming ; inflorescences short, but dichotomous ; flowers orange–lemon yellow with red tint and with weak fragrance.

M–31 : Day–blooming ; inflorescences trichotomously branched ; flowers with fragrance and orange–lemon yellow with reddish color on external perianths only.

M–38 : Night–blooming ; flowers small, lemon yellow with fragrance.

M–39 : Night–blooming ; scapes low, bearing open lemon yellow flowers with fragrance.

M–40 : Day–blooming ; scapes very dwarf, bearing open lemon yellow flowers without fragrance.

M–B–10 : Day–blooming ; inflorescences dichotomous, bearing only two red flowers without fragrance ; perianths broad and similar to those of v. *kwanso.*

M–vesp : Night–blooming ; similar to typical *vespertina,* pollen fertility low, 60–70% only.

III. Cytological Observations

(1) Karyotypes of Somatic Chromosomes

The $n = 11$ chromosomes of *Hemerocallis* were classified into seven types, which have been described by Takenaka (1952). The morphology of the 11 pairs is described as follows (Fig. 12).

Chromosome *L,* the longest of the complement, has a constriction situated in a submedian position. Chromosome *M* has a constriction situated close to the center.
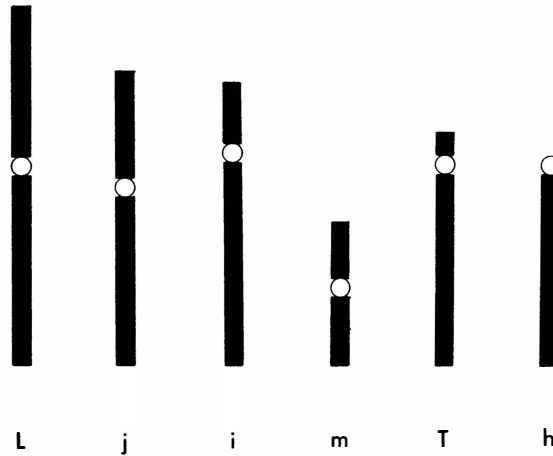

L   j   i   m   T   h

Fig. 12. Six chromosomal types found in the chromosome
complement of the genus *Hemerocallis*.

H. fulva   v. longituba

H. fulva   v. disticha

H. citrina   v. vespertina

Fig. 13–A. Chromosome complements of *H. fulva* v. *longituba,*
v. *disticha,* and *H. citrina* v. *vespertina.*

Chromosome *j*, a little shorter than *L*, has a constriction located submedially. Chromosome *i*, of about median length, has a constriction situated in a subterminal position. Chromosome *m*, one of the shortest, has a constriction close to the center. Chromosome *T*, a little shorter, or of almost the same length as *j*, has 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 extroardinary small or almost invisible arm.

(a) *Karyotypes in H. citrina v. vespertina*

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

$2n = 22 = 4L + 4j + 6i + 2m + 4T + 2h$ (Fig. 13-A).

(b) *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:

$2n = 22 = 4L + 6j + 6i + 2m + 2T + 2h$ (Fig. 13-A).

(c) *Karyotypes in H. fulva v. disticha*

The $2n = 22$ somatic chromosomes were observed in all plants examined. This plant proved to have the same basic karyotype as v. *longituba*. The basic karyotype may be expressed as follows:

$2n = 22 = 4L + 6j + 6i + 2m + 2T + 2h$ (Fig. 13-A).

Table 4. Karyotypes of hybrids examined

Chromosomal types Strains	L	j	i	m	T	h
M-1 · 2 ($2n = 22$)	4	6	4*	2	4	2
M-4 · 5 ($2n = 22$)	4	6	6	2	2	2
M-20 ($2n = 22$)	4	6	4*	2	4	2
M-21 ($2n = 22$)	4	6	6	2	2	2
M-23 ($2n = 22$)	4	6	6	2	2	2
M-24 ($2n = 22$)	4	6	6	2	2	2
M-25 ($2n = 22$)	4	6	6	2	2	2
M-31 ($2n = 22$)	4	6	4*	2	4	2
M-32 ($2n = 22$)	4	6	6	2	2	2
M-38 ($2n = 22$)	4	6	4*	2	4	2
M-40 ($2n = 22$)	4	6	4*	2	4	2
M-41 ($2n = 22$)	4	6	6	2	2	2

*Although two somatic chromosomes were tentatively referred to the *j*-type in these sample plants, they may belong to the *i*-type. The distinction between *j* and *i* is occassionally not very clear, and future critical studies are much needed.

Hybrid

h–1·2

h–4·5

4·5·6·16

20

21

22

24

Fig. 13-B. and C. Chromosome complements of various
natural hybrids (See also Table 4).

(d) Karyotypes in Hybrids

The thirteen hybrid plants were karyologically examined. The karyotypes of the hybrids are illustrated in Fig. 13–B, and Table 4, respectively. It is noteworthy that the hybrids, *M–1 · 2, M–20, M–31, M–38* and *M–40* possessed two pairs of *T*–chromosomes which are characteristic for *H. citrina* v. *vespertina;* on the other hand, it is incomprehensive why only two pairs of *i*–chromosomes are found in the chromosome complements of these hybrid plants.

25

31

32

38

40

41

(2)   *Meiotic Behaviours of Chromosomes*

The chromosome pairing and behaviours at pachytene and MI of several selected parental and hybrid plants were examined. At pachytene and MI of the hybrid plants, often observed were loops, irregular pairings, delayed terminalization
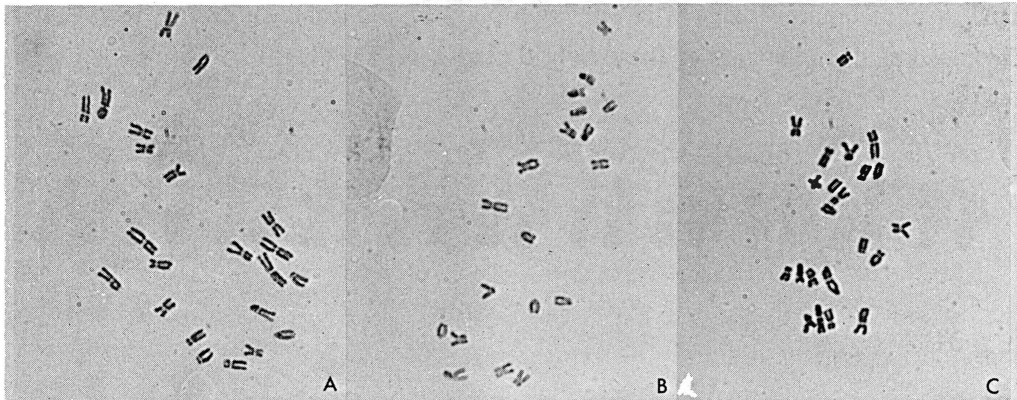

Fig. 14. A : Somatic chromosomes of a hybrid, No. 4 • 5 • 6 • 16 ; B. Those of No. 32 ; and C : Those of *H. citrina* v. *vespertina.*
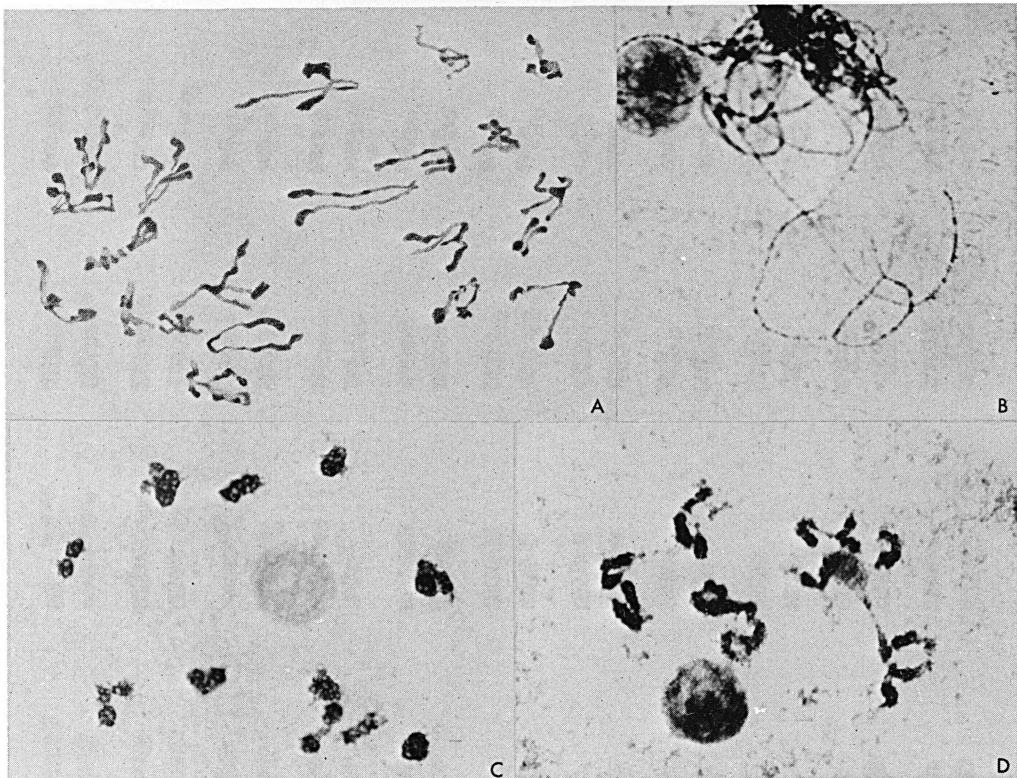

Fig. 15. Chromosome behaviours at pachytene and MI. A : Anaphase of MI in a hybrid M–10. Note the delayed terminalization of chiasmata ; B. A loop indicating the heterogeneity found at pachytene of M–32 ; C. Pairing at MI of M–1 • 2. 11 bivalents were observed ; D : Pairing at MI of M–1 • 2.

of chiasmata and laggards, indicating the heterogeneity.   As the data are rather limited, however, no concluding statements can be made at present as to the status of plants examined.   Further detailed studies are much needed.
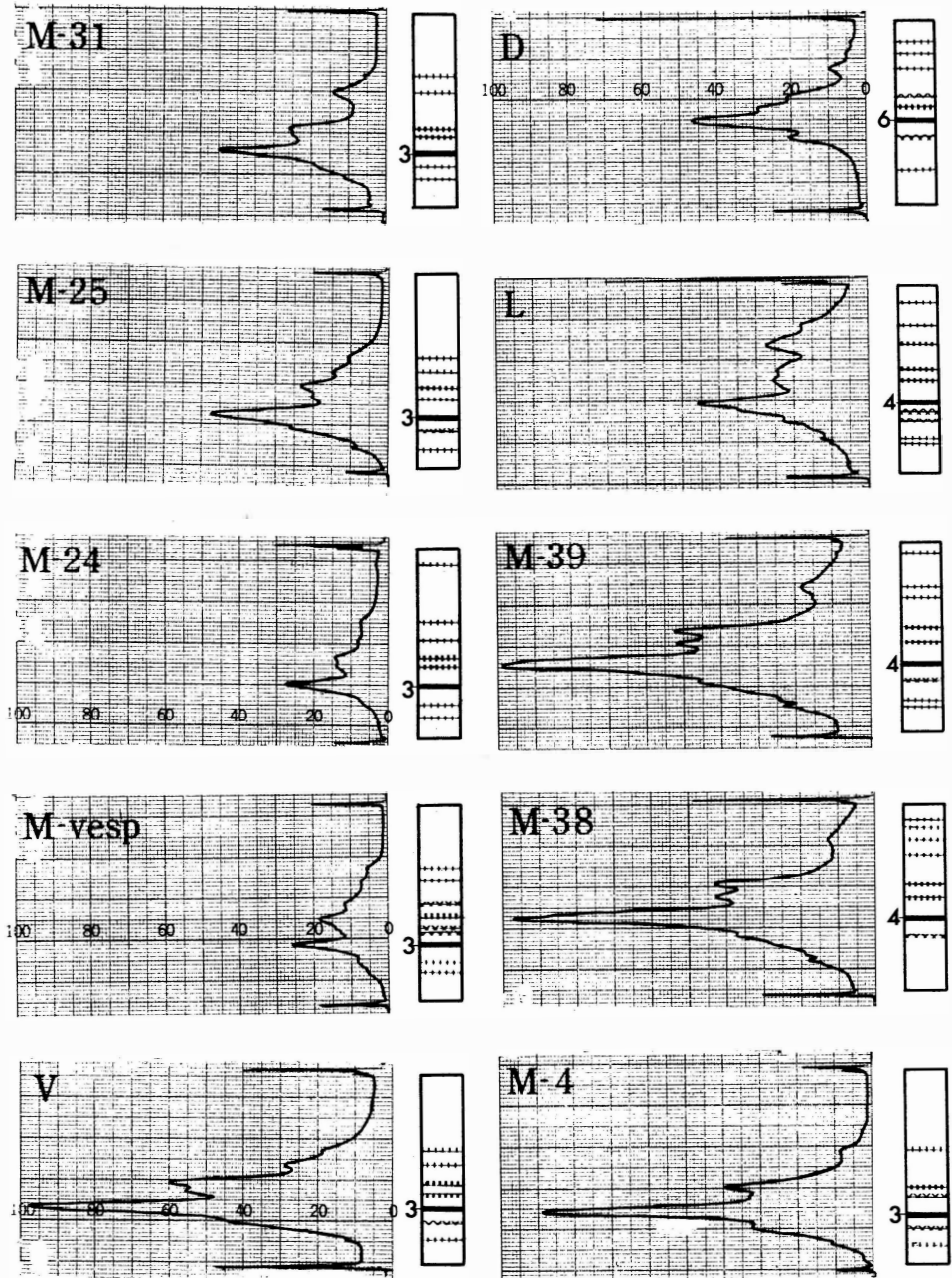

Fig. 16. Electrophoretic banding patterns and their densitometer curves of three parental taxa and hybrids; V : *H. citrina* v. *vespertina;* D : *H. fulva* v. *disticha;* L : *H. fulva* v. *longituba;* others : natural hybrids.

(3) Pollen Fertility

The variations in the pollen fertility of both parental and hybrid plants were investigated. The fertility was usually very high in the parental taxa, but varies to a considerable extent in hybrid plants, ranging 10 to 100%. This fact coincides with meiotic irregularities of hybrid plants observed.
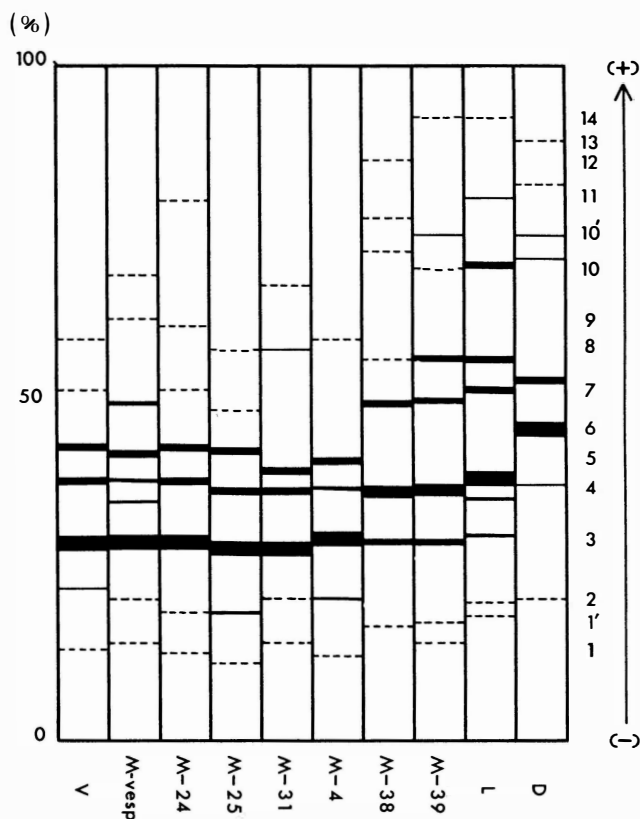

Eig. 17. A profile of electrophoretic patterns of *Hemerocallis* plants from Matsunoki. The diagram reproduced from Fig. 16.

IV. Electrophoretic Patterns of Acid–Soluble Protein

H. citrina v. *vespertina* has a uniform pattern comprising seven bands, of which No. 3 is most conspicuous and charcateristic for this taxon. It is noteworthy that the protein profile of *H. fulva* v. *longituba* is different from that of v. *disticha* which is regarded to be very closely related to the former variety. V. *longituba* possesses eleven bands, of which No. 4 exhibited the maximum absorbance in the optical density curve taken with a recording densitometer in all samples of v. *longituba;* whereas, eight bands were seen in the protein profile of v. *disticha,* and the maximum absorbance was observed at the position of No. 6. The bands of No. 1

and No. 5 which are characteristic for *H. citrina* v. *vespertina* is lacking in both *H. fulva* v. *longituba* and v. *disticha;* on the contrary, the bands of No. 10, 10', 11, 13 or 14 of v. *longituba* and v. *disticha* are not seen in *H. citrina* v. *vespertina.*

The protein profiles of hybrid plants examined are variable, comprising various combinations of the bands. It is very interesting to know that the plants M–29 and M–30 which bear night–blooming, lemon yellow flowers and are clearly indistinguishable from typical v. *vespertina* with respect to gross morphology has the band of No. 4 characteristic for v. *longituba*. This fact may suggest that not only F_1, but also F_2, or backcrosses and subsequent generations have been produced in this *Hemerocallis* population. For further details, see Figs. 16 and 17, and the morphological descriptions of the hybrid plants.

Discussion

Based on the results of morphological as well as ecological analyses, Nakao and Yamashita (1956) concluded that a hybrid population of *Hemerocallis* in Matsunoki represents an initial stage of introgression of genes of *H. citrina* v. *vespertina* into the population of *H. fulva sensu lato*. According to their analyses, the intermediate types predominate in the population and of a total number of 2193 clones examined, approximately two third of these plants fall within the variation range of the hybrids.

However, according to the results of our present analyses, the hybridization seems to have been taking place to a more limited degree than estimated previously. Numerous clones as referred to natural hybrids between *H. citrina* v. *vespertina,* and *H. fulva* v. *disticha* and v. *longituba* by Nakao and Yamashita (*l. c.*) might belong to a form of *H. fulva sensu lato*. Our present supposition is based upon the results of comparative investigations as regards variations in gross morphology, karyotypes, as well as electrophoretic banding patterns of acid–soluble protein of the plants of *Hemerocallis* from Matsunoki. A substantial evidence supporting the above conclusion was also obtained from the survey of the distribution and ecology of *Hemerocallis* plants in the habitat in Matsunoki and other neighbouring regions (Figs. 1, 2, and 3) (Kawano and Noguchi, unpublished).

Also, the evidence gathered in this study strongly indicates that the gene flow is taking place not only from the population of *H. citrina* v. *vespertina* to that of *H. fulva* v. *disticha* or v. *longituba,* brt also reversely from *H. fulva* v. *disticha* and v. *longituba* to *H. citrina* v. *vespertina.* The character combinations of individuals investigated and illustrated in the scatter diagram (Fig. 11), their karyotypes (Fig. 13–B), and also protein banding patterns (Figs. 16 and 17) clearly indicate the situation and constitution of the *Hemerocallis* population in Matsunoki.

However, as was pointed out by Nakao and Yamashita (*l. c.*), it is no doubt that a hybrid swarm in Matsunoki represents an incipient stage of introgression. Also, the relationship and the degree of intermixture between the two major parental populations are most effectively controlled by the difference of only a few hours in blooming time, and that such a difference may most effectively prohibits the pollination of one species by another. But, as mentioned earlier in this paper, an allochronic isolation existing between them may have often been broken down when there occurred an overlap of two to three hours in flowering time.

As far as is known to date, *H. citrina* v. *vespertina* is distributed on somewhat dry grasslands developed over ca. 500 m above sea level in elevation in central Honshu to Kyushu; whereas *H. fulva sensu lato* represents a typical lowland species, and thus usually there occurs a spatial isolation between these two species. It is not known at present when these two species have come into contact with in the area of Matsunoki, but it may be presumed that the disturbance over the hilly site by the human occupation has created a large area of open habitats and it has acceralated the migration of *H. citrina* v. *vespertina* into somewhat a lowland site, and as a result, they have come into contact with one another. Future studies on this particular population in Matsunoki and other adjacent regions will unravel further biological aspects and status of *Hemerocallis* plants. Just as a natural hybrid population between *H. middendorffii* and *H. yezoensis* found near Otanoshike, in the vicinity of Kushiro City in Hokkaido, this particular hybrid swarm in Matsunoki does not deserve any taxonomic recognition (cf. Kawano, 1961).

Summary

1. A natural hybrid population of *Hemerocallis*, i. e., between *H. citrina* v. *vespertina* and *H. fulva* v. *disticha* and v. *longituba,* which occurs in Matsunoki near Takayama City in Gifu Prefecture, were re-investigated from morphological, ecological as well as karyological standpoints. Also, the electrophoretic banding patterns of acid-soluble protein extracted from the rhizomes of *Hemerocallis* were analyzed and compared with one another.

2. This particular population of *Hemerocallis* in Matsunoki proved to be in an incipient stage of introgression, as was suggested by Nakao and Yamashita (1956). The introgression of genes of *H. fulva* v. *longituba* or v. *disticha* into *H. citrina* v. *vespertina* or *vice versa* is very evident from the results of the present analysis, but the hybridization seems to be rather limited to a restricted zone where the two major parental species populations are sympatric. There, an allochronic isolation of only a few hours was partially broken down, and as a result, interbreeding between both species populations have taken place.

3. It is also evident that not only F_1 plants, but also F_2 or backcross progenies are comprised in this hybrid swarm of *Hemerocallis* in Matsunoki.

4. The data on electrophoresis of acid–soluble protein supplied a valuable and substantial evidence confirming the introgressive status of hybridization in *Hemerocallis*.

Acknowledgements

References

Anderson, E. 1949. *Introgressive Hybridization*. John & Wiley, N. Y.

Kawano, S. 1961. On the natural hybrid population of *Hemerocallis*. Canad. J. Bot. **39** : 667–681.

Matsuoka, M. and Hotta, M. 1966. Classification of *Hemerocallis* in Japan and its vicinity. Acta Phytotax. & Geobot. **22** : 25–43 (in Japanese).

Nakao, S. and Yamashita, K. 1956. Variation in some plant populations. *In :* Komai, T. and Sakai, K., Syudan Idengaku (Population Genetics) (in Japanese).

Takenaka, Y. 1952. Karyotypes and sterility in *Hemerocallis*. Coordinating Comm. Research Genet. **3** : 71–90.