Okada 1990

A natural hybrid of Monophyllaea (Gesneriaceae) in the tropical rain forests of West Sumatra.

Pl. Syst. Evol. 169: 55-63.

REFNO: 2748

KEYWORDS:

Chromosome Numbers, Cytology, Hybridization, Indonesia, Limestone, Monophyllaea, Sumatra

Pl. Syst. Evol. 169, 55–63 (1990)

Plant Systematics and Evolution

A natural hybrid of *Monophyllaea* (*Gesneriaceae*) in the tropical rain forests of West Sumatra

Hiroshi Okada

Received July 30, 1988

Key words: Angiosperms, *Gesneriaceae, Monophyllaea, M. hirtella, M. horsfieldii.* – Chromosome number, hybridization, fertility, tropical rain forest, habitat segregation. – Flora of Indonesia, Sumatra.

Abstract: A natural hybrid (2 n = 21) between the parapatric rain forest species *Monophyllaea hirtella* (2 n = 20) and *M. horsfieldii* (2 n = 22) (*Gesneriaceae*) has been observed at Sg. Lubuk Paraku, Padang, W. Sumatra. The hybrids showed intermediary characters between the parental species in the inflorescence structure, flower size and colour, indumentum, chromosome numbers, and habitat. The 29% pollen fertility of a single hybrid specimen suggests that the genetical isolation between the parental species may break down and reproduction over subsequent generations may occur.

The genus *Monophyllaea* (*Gesneriaceae*) comprises peculiar herbaceous plants occurring in the understory of tropical rain forests at limestone areas in SE. Asia. The plants have only a single leaf that sometimes reaches more than 80 cm in length and 50 cm in width. This leaf corresponds to one of the two cotyledons and does not stop growing during the whole perennial life of the plant. From the base of this leaf there arise numerous many-flowered inflorescences.

Largely based on his collections and field experience in Sarawak, Burtt (1978) provided a "preliminary" taxonomic revision of the genus, recognizing more than 30 species. Detailed morphological analyses refer to two species, *M. horsfieldii* and *M. hirticalyx* (Weber 1975, 1976 a, b). Apart from the field observations reported by Burtt (1978), information about ecological aspects is very scanty. There is also no report of hybrids in the genus. Recently, the author had the opportunity to observe two species, *M. horsfieldii* and *M. hirtella*, in their natural habit in W. Sumatra, and to take part in biometrical growth studies (Kohyama & Hotta 1986). At this occasion, hybrids between the two species were found. The present paper describes the characters of the hybrids and thus may contribute to a better knowledge of biological aspects of *Monophyllaea* as well as to a better understanding of the mechanisms of evolutionary diversification in an everwet tropical environment.

Materials and methods

Monophyllaea hirtella MIQ. and *M. horsfieldii* R. BR. occur rather commonly at the limestone areas along Sg. (River) Lubuk Paraku, Ladang Padi (0°57′ S, 100°30′ E), near Padang city, W. Sumatra, Indonesia. At the limestone area along Sg. Gadut Gadang Ketil, near Bt. (Hill) Pinang Pinang, Ulu Gadut (about 3 km northwest from the former site), both species are distributed parapatrically as well. The study sites and the distribution of *Monophyllaea* around Ulu Gadut were described in detail by HOTTA (1984, 1986 a) and KOHYAMA & HOTTA (1986). Main observations and sampling were made at plot 2 of KOHYAMA & HOTTA (1986) and adjacent areas. One inflorescence per specimen was collected.

Chromosome studies were carried out using young flower buds. The buds were fixed in the field by a fresh mixture of EtOH : chloroform : glacial acetic acid = 2 : 1 : 1 (modified Carnoy's fluid) for at least one day. Then they were transferred into 70% EtOH and preserved herein. At the laboratory, the material was transferred into 45% acetic acid. Young anthers with pollen mother cells (PMCs) were extricated and processed according to the conventional chromosome techniques.

The fertility of pollen grains was estimated using preparations stained with lacto-phenol cotton blue solution. Darkly stained, globose pollen grains were scored to be fertile. More than 500 grains were observed.

Germination tests were carried out under artificial conditions. The seeds were incubated in pots at the green house (20 – 30 °C) of Osaka University, Japan. The pots were covered by glass plates to keep high humidity.

As was pointed out by BURTT (1978), *M. horsfieldii* has a wide distribution. This taxon may contain variable types, so that the author tentatively used the scientific name, *M. horsfieldii* for one of the parental species in the present study. Voucher specimens are deposited at Kyoto University (KYO) and Andalas University (AND) (cf. HOTTA 1986 b).

Results

Characters and habitat. The appearance and the growth features of both parental species and the hybrids are described in detail by KOHYAMA & HOTTA (1986). The floral characters are summarized in detail in Fig. 3 and Table 1.

M. hirtella and *M. horsfieldii* co-existed parapatrically in the limestone areas inhabiting segregatively different habitats (Figs. 1 and 2): *M. horsfieldii* grew straggly on the ± vertical surfaces of limestone cliffs, while *M. hirtella* was found on the ground below the cliffs in limestome gravel mixed with humus soil. *M. hirtella* formed patches with high individual number (especially at plot 1 of KOHYAMA & HOTTA 1986). The hybrids were found growing in the transition zone (Fig. 2). The branched and long inflorescences, the pink flowers, and the sterile fruits were conspicuous characters for recognizing the hybrids in the field (Fig. 3 C).

Germination tests of *M. hirtella, M. horsfieldii* and the hybrid were carried out under the artificial conditions. In all cases small seedlings, less than 1 mm, were observed after one month. It seemed that there was no difference of viability of seed and seedlings among both parental species and the hybrid. In the field, almost all capsules of these taxa did not dehisce on the inflorescences. The seeds may be dispersed within the capsules by accidental break of inflorescences or the whole plants. In nature, the seeds of *M. horsfieldii* sometimes germinated already in the capsules on the inflorescences.

Chromosome observations. Karyological information relating to *Monophyllaea* is rare and only includes the counts of OEHLKERS (1923) and RATTER & PRENTICE (1967) in *M. horsfieldii.* By the present studies, the chromosome number of *M.*

Table 1. Floral characters of *Monophyllaea hirtella*, *M. horsfieldii*, and the hybrid *M. hirtella* × *M. horsfieldii*. Mean value ± SD mm (sample number). * The specimen with 29% fertility excluded

Characters 2n	*M. hirtella* 20	*M. horsfieldii* 22	Hybrid 21
Calyx length	4.8±0.3 (4)	2.6±0.2 (7)	3.0±0.3 (12)
Calyx width	4.3±0.5 (4)	3.2±0.3 (7)	3.1±0.2 (12)
Corolla length	10.7±0.8 (2)	4.9±0.4 (2)	7.6±0.3 (4)
Corolla colour	white	purple	pink
Indumentum on pedicel	densely setose	glandular	glandular or glandular+setose
Indumentum on calyx	densely setose	glandular or glabrous	glandular+setose or glabrous
Inflorescence	stout, short, branched in the upper part	thin, long, not branched	thin, long, branched in the middle part
Flower arrangement	dense	loose	loose
Fruit set	fertile	fertile	almost sterile
Pollen fertility	92±2 %(3)	94±4 %(7)	4±2 %(10)*

hirtella was established with n=10 at diakinesis of meiosis of PMCs (Fig. 4 A). Complementary chromosomes constantly formed 10 normal bivalents, one pair being attached to a nucleolus. The pollen grain fertility was estimated to be more than 90% (Fig. 3 G, Table 1).

The chromosome number of *M. horsfieldii* was counted to be 2n=22 at late prophase of mitosis of anther wall cells (Fig. 4 B). This chromosome number is in aneuploid relation to the former. The present result is different from the previous reports of *M. horsfieldii* by OEHLKERS (1923), namely n=16, and by RATTER & PRENTICE (1967), n=10. The species has a wide distribution in SE. Asia, and is variable to some degree (BURTT 1978). More detailed cytotaxonomical studies from a wide range are required for understanding the biological meaning of this difference. The pollen grains were fertile like in *M. hirtella* (Fig. 3 H, Table 1).

The chromosome number of one hybrid specimen was estimated to be 2n=21 based on the observations of pairing configurations at diakinesis of PMCs, such as $2n=21=1_{III}+8_{II}+2_{I}$ (Fig. 4 C). Pairing configurations varied among cells (Fig. 4 C–F). The most frequent component was $9_{II}+3_{I}$ (Fig. 4 D). In some PMCs, one trivalent was observed. It seemed that some unequal-sized and/or different-shaped chromosomes formed synapses. It appears possible that the paired chromosomes do not correspond to homologous chromosomes. All hybrid specimens had almost sterile pollen grains, i.e., only 1 to 6% fertility (Fig. 3 I, Table 1). Unexpectedly, a single individual showed rather high fertility of pollen grains, namely 29% (Fig. 3 J). The conflicting features that the hybrid showed high fre-

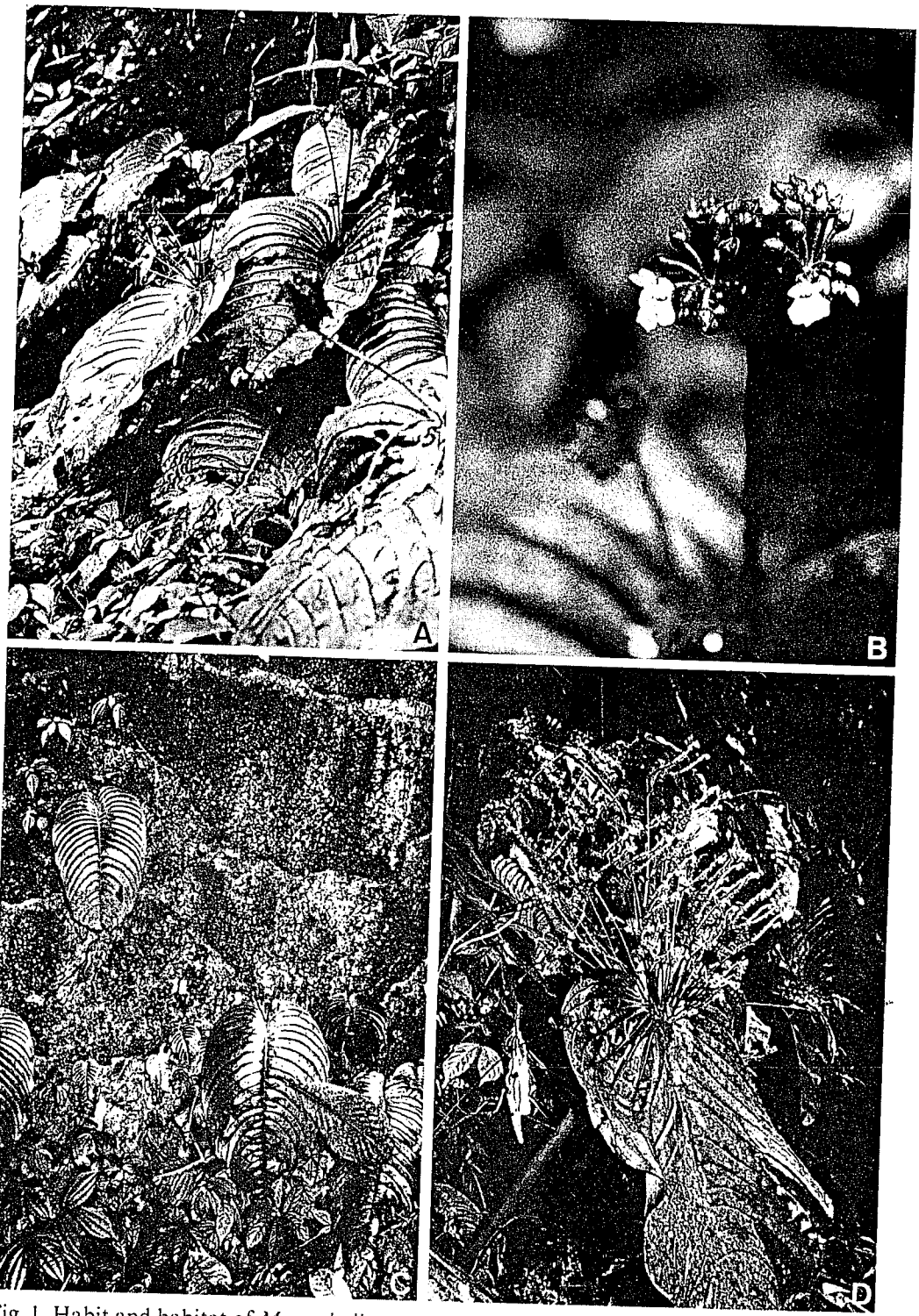

Fig. 1. Habit and habitat of *Monophyllaea*. *A Monophyllaea hirtella* (right) and hybrid (left) on humus soil ground. Hybrids grow at the boundary of the habitat of *M. hirtella*. Note shape of inflorescences. *B. M. hirtella*, pseudo-dichotomous and short inflorescence. *C M. horsfieldii* on limestone cliff. *D* Hybrid with many branched and elongate inflorescence

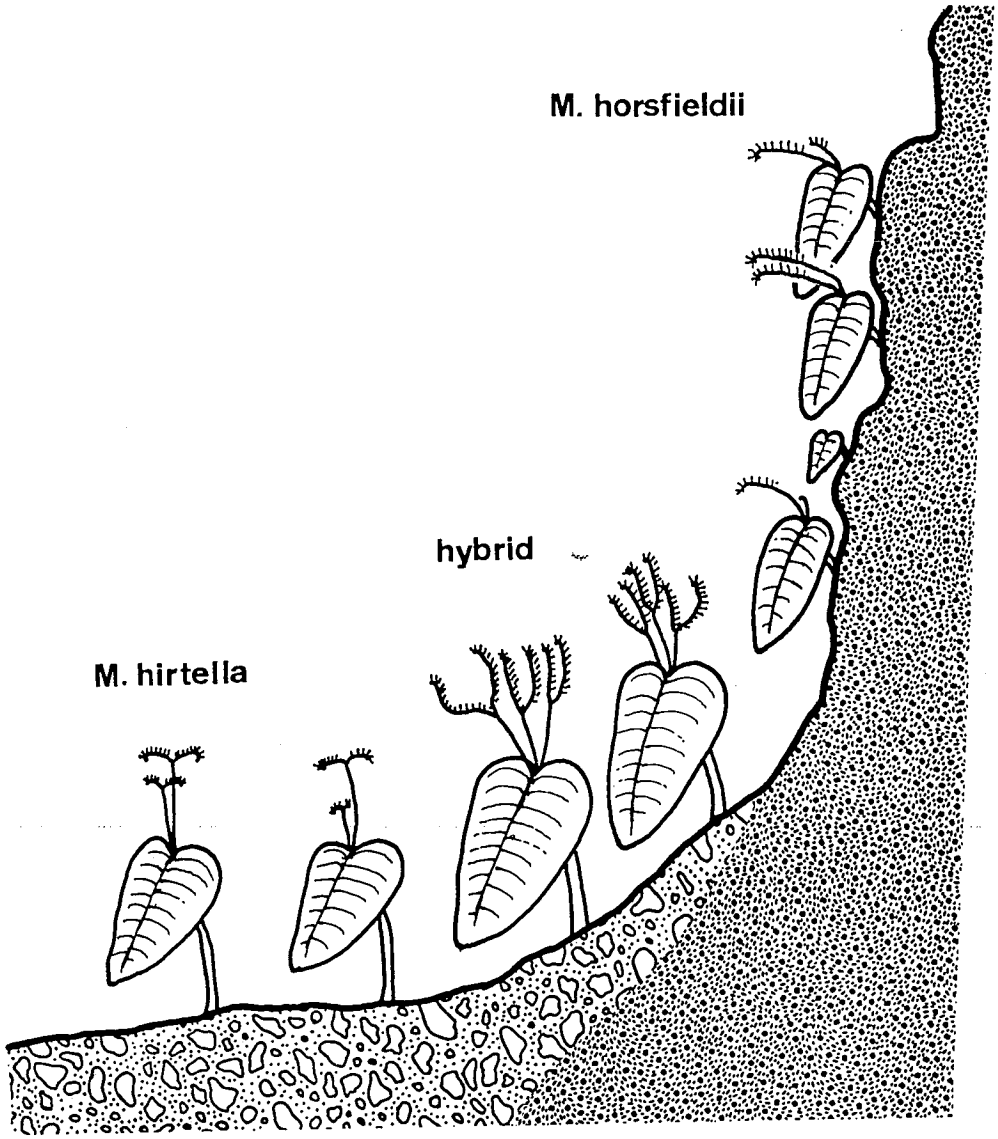

Fig. 2. Diagram of habitat. *Monophyllaea horsfieldii* grows on the vertical face of limestome cliffs, *M. hirtella* on the horizontal ground below, hybrid specimens are found in the transition zone

quency of bivalents, but had almost sterile pollen grains are comparable with the case of diploid hybrids between *Ranunculus silerifolius* and *R. chinensis* (OKADA 1984).

Discussion

The above observations prove clearly the occurrence of hybrids within the genus *Monophyllaea*. This is apparently the first report of hybridization within the genus, as BURTT (1978) does not mention the appearance of hybrids at habitats with two

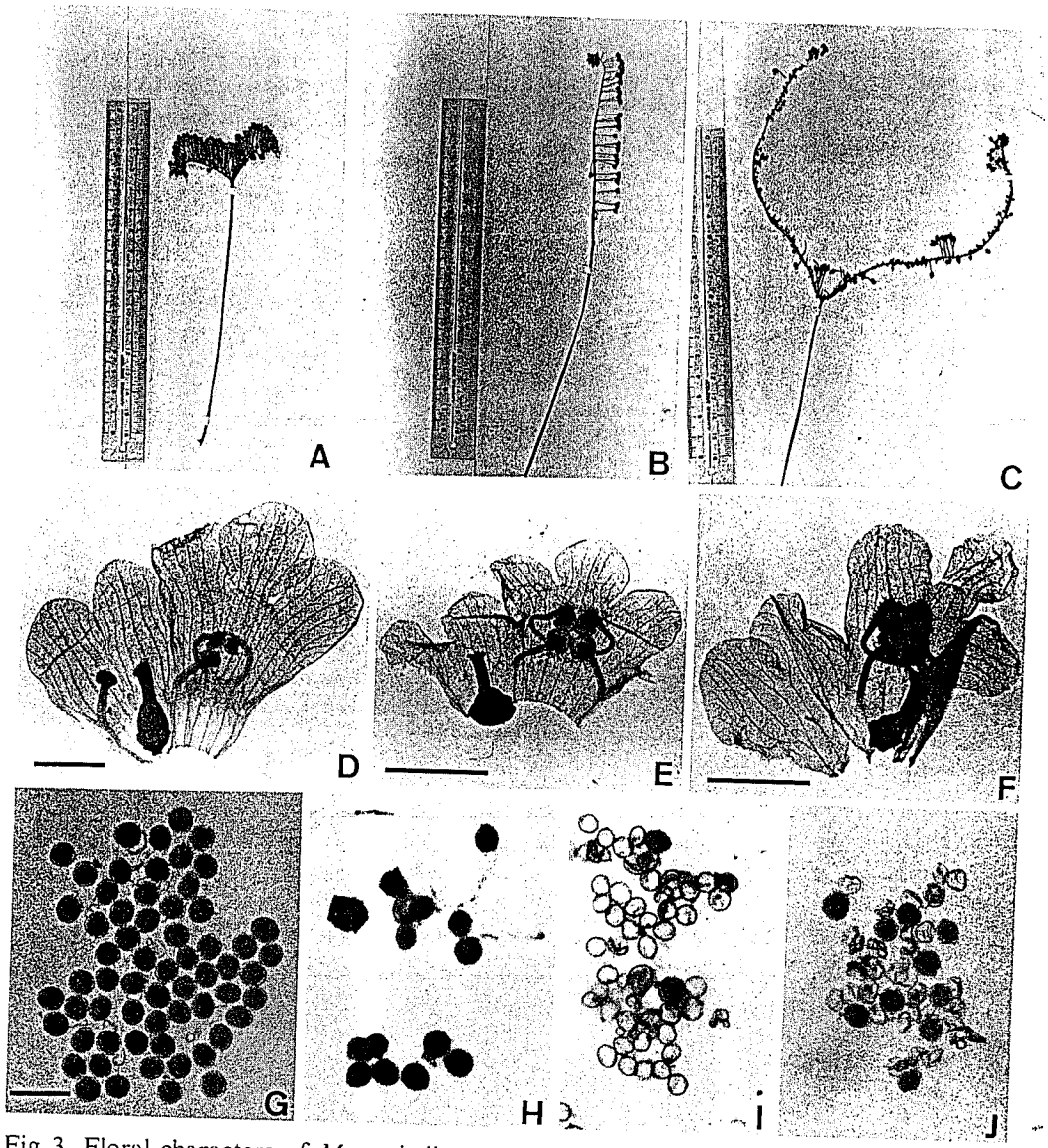

Fig. 3. Floral characters of *Monophyllaea* (cf. Table 1). *A*–*C* Inflorescences of parental species and hybrid. Note poor fruit set in the hybrid. *D*–*F* Dissected corolla with androecium and gynoecium. *G*–*J* Fertility of pollen grains. Note different stainability of *I* (2% fertility) and *J* (29%). *A, D, G M. hirtella. B, E, H M. horsfieldii. C, F, I, J* Hybrid. Bar: *D*–*F* 3 mm, *G*–*J* 50 μm

or three species. Many of the hybrid specimens showed intermediary characters between the parental species. This also applies to the chromosome number.

The pollen grains of some specimens of both *M. hirtella* and *M. horsfieldii* were observed to germinate within the anthers. The stigma seemed to be attached to the anthers at anthesis (Fig. 3 D and E). These facts suggest that these species fertilize by self-pollination (autogamy), as was pointed out by BURTT (1978). Actually, both species showed very high fruit set in the field in spite of very rare visit of insects.

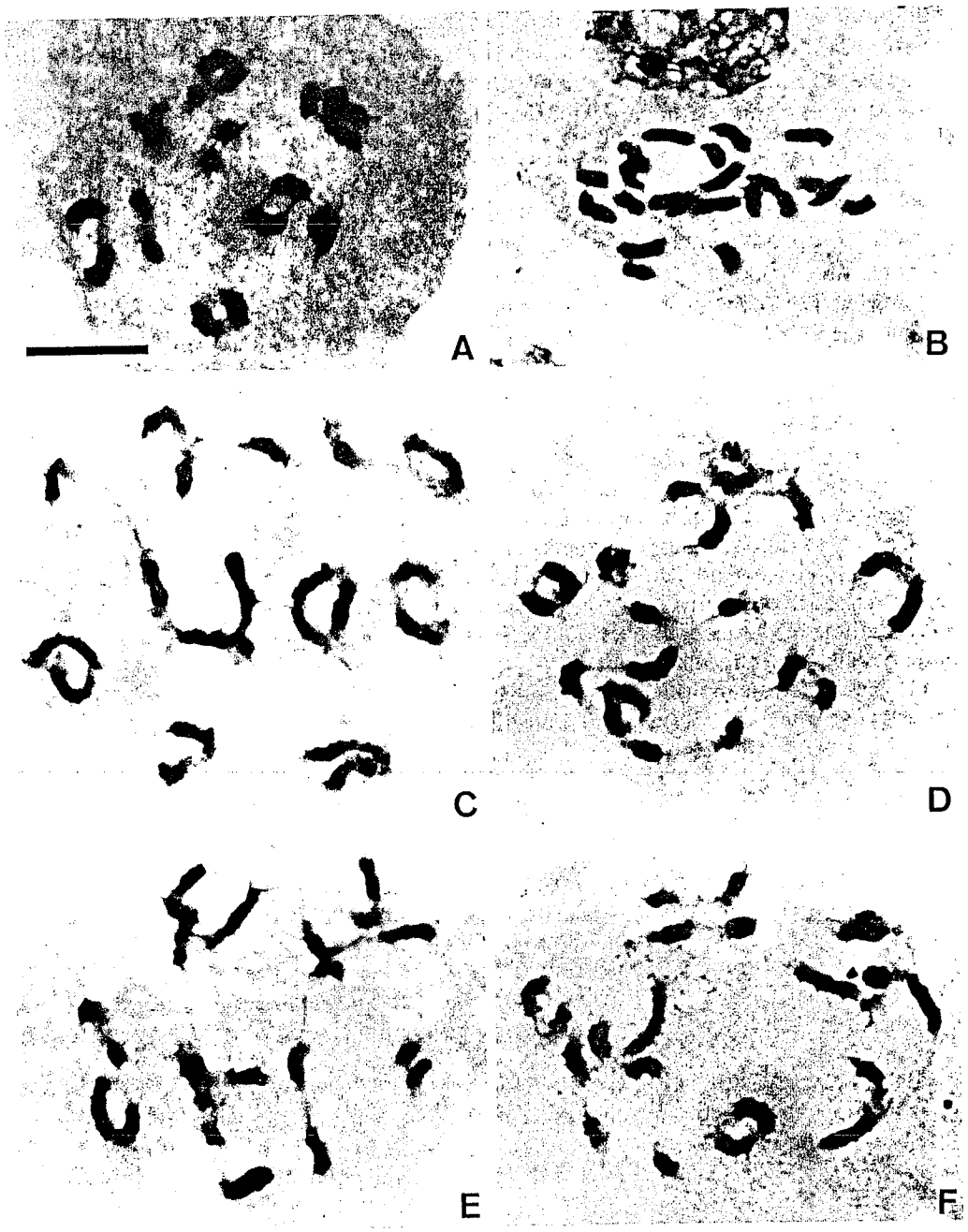

Fig. 4. Microphotographs of meiotic and somatic chromosomes. *A Monophyllaea hirtella*, diakinesis of PMC, $n=10_{II}$. *B M. horsfieldii*, late prophase, $2n=22$. *C–F* Hybrid, early diakinesis of PMCs. Many kinds of pairing configuration were observed, such as, $1_{III}+8_{II}+2_{I}$ (*C*), $9_{II}+3_{I}$ (*D*), $8_{II}+5_{I}$ (*E*), and $5_{II}+11_{I}$ (*F*). Bar: 10 μm

Fruits were filled with fertile seeds. Contrary to these features suggesting autogamy, the existence of hybrids indicates that outbreeding is also involved in the reproduction system. KATO & ICHINO (pers. comm.) observed that hymenopteran insects, e.g., *Halictidae, Vespidae* and *Anthophoridae*, rarely visited *M. hirtella* and hybrid

individuals. JANZEN (1971) discussed that euglossine bees probably promote outcrossing among neotropical plants by foraging long distances and by visiting the same plants repeatedly. The hymenopteran insects in this study site may act similarly. Further studies on reproductive biology are required to analyze the pollen flow.

Hybrids between parapatric species, *M. hirtella* and *M. horsefieldii*, seemed to have established their habitat at boundary regions between both parental species, and could reproduce mature offsprings by viable seeds. The hybrid specimen with 29% fertility of pollen grains is probably an offspring from an F_1 hybrid or from back cross between the hybrid and one of the parental species. The existence of such hybrids gives rise to the idea that genetical isolation between both parental species has broken down by the phenomenon of introgressive hybridization.

I would like to express my sincere thanks to Drs S. KAWAMURA and AMSIR BAKAR, the leaders of Sumatra Nature Study project (SNS), and other members of SNS for their kind support to our studies in Indonesia. Mr T. HAINALD, Head of Bureau of Science and Technology Cooperation, Indonesian Institute of Science (LIPI) accelerated the procedures of our entry to Indonesia. Special thanks are due to Drs M. HOTTA, Kagoshima University, T. KOHYAMA, Kagoshima University, M. KATO, Kyoto University, and T. ICHINO, Kagawa University, for cooperative work in the field. Dr HOTTA is primarily responsible for the identification of the plant species. Dr A. WEBER, University of Vienna, kindly discussed and critically read the manuscript.

This study was supported by a Grant-in-Aid for Overseas Scientific Survey from the Ministry of Education, Science and Culture, Japan (no. 59041037, 62041048).

References

BURTT, B. L., 1978: Studies in the *Gesneriaceae* of the old world 45: A preliminary revision of *Monophyllaea*. – Notes Roy. Bot. Gard. Edinburgh **37**: 1–60.

HOTTA, M., (Ed.) 1984: Forest ecology and flora of G. Gadut, West Sumatra. – Sumatra Nature Study (Botany), Kyoto Univ.

– (Ed.) 1986 a: Diversity and dynamics of plant life in Sumatra. Forest ecosystem and speciation in wet tropical environmens. – Sumatra Nature Study (Botany), Kytoto Univ.

– 1986 b: Check list of *Monophyllaea* in Sumatra. – In HOTTA, M., (Ed.): Diversity and dynamics of plant life in Sumatra. Forest ecosystem and speciation in wet tropical environments, pp. 127–128. – Sumatra Nature Study (Botany), Kyoto Univ.

JANZEN, D. H., 1971: Euglossine bees as long-distance pollinators of tropical plants. – Science **171**: 203–205.

KOHYAMA, T., HOTTA, M., 1986: Growth analysis of Sumatran *Monophyllaea*, possessing only one leaf throughout perennial life. – Pl. Sp. Biol. **1**: 117–125.

OEHLKERS, F., 1923: Entwicklungsgeschichte von *Monophyllaea horsfieldii*. – Beih. Bot. Centralbl. **39**: 128–151.

OKADA, H., 1984: Polyphyletic allopolyploid origin of *Ranunculus cantoniensis* (4x) from *R. silerifolius* (2x) × *R. chinensis* (2x). – Pl. Syst. Evol. **148**: 89–102.

RATTER, J. A., PRENTICE, H., 1967: Chromosome numbers in *Gesneriaceae* 3. – Notes Roy. Bot. Gard. Edinburgh **27**: 205–209.

WEBER, A., 1975: Beiträge zur Morphologie und Systematik der *Klugieae* und *Loxonieae* (*Gesneriaceae*) 1. Die Sproß- und Infloreszenzorganisation von *Monophyllaea* R. BR. – Bot. Jahrb. Syst. **95**: 174–207.

– 1976a: Beiträge zur Morphologie und Systematik der *Klugieae* und *Loxonieae* (*Gesneriaceae*) 2. Morphologie, Anatomie und Ontogenese der Blüte von *Monophyllaea* R. Br. – Bot. Jahrb. Syst. **95**: 435–454.
– 1976b: Beiträge zur Morphologie und Systematik der *Klugieae* und *Loxonieae* (*Gesneriaceae*) 3. *Whytockia* als morphologische und phylogenetische Ausgangsform von *Monophyllaea*. – Beitr. Biol. Pflanzen **52**: 183–205.

Address of the author: Hiroshi Okada, Department of Biology, College of General Education, Osaka University, Toyonaka, Osaka 560, Japan.

Plant Systematics and Evolution

Contents Vol. 169 No. 1–2 1990 ISSN 0378-2697-ESPFBP 169 (1–2) 1–121 (1990)

Abstracted/Indexed in:
Current Contents,
SCI, ASCA, and ISI/BIOMED,
Chemical Abstracts,
Current Awareness in Biological Sciences

Published March 13, 1990