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Chromosome Determinations of Some Ryukyuan Orchid Species*

By

H. Y. NAKASONE and S. MOROMIZATO**

The Ryukyu Islands consist of approximately 72 islands which extend southwest for 374 miles between Japan and Taiwan. Although these islands are located within the North Temperate Zone, the climate is semi-tropical with high humidity and mild winters. Winter temperatures range from a low of 5°C to as high as 18°C. The mean daily temperature of February, the coolest month, is 10°C. The mean daily maximum for July, the warmest month, is around 31°C.

Precipitation ranges from 1,325 mm (53 inches) to 4,875 mm (115 inches) with heaviest rainfall in May and June. Being located in the typhoon belt, a large part of the precipitation comes with the typhoons whenever they occur.

Amidst these background of geographic and climatic factors (Fact Book, 1962) is found a divergent array of orchid plants, indigenous and endemic. Hatusima and Amano (1958) list approximately 108 orchid species. They make no distinction among introduced, indigenous and endemic flora, but undoubtedly, some are of recent introductions while others are indigenous to this region. Further examination of this list indicates that approximately 15 have specific names associated with the Ryukyus. Some of these are undoubtedly endemic species.

While a few botanically inclined people have made attempts to collect and grow these native species, the culture of orchids as a hobby and as a commercial crop is relatively new in the Ryukyus and preference has been for the introduced varieties. Little attempt has been made to evaluate the value of these species as a horticultural crop. Even less has been done from the academic standpoint. Academically, the value of these species can only be measured in terms of the types and depth of scientific investigation. Studies in the ecology and distribution of the various native genera and species, together with studies in the cytology and cytogenetics of these plants could contribute to the overall knowledge of species relationships and evolutionary trends. Practically, chromosome counts have been found to aid in the selection of desirable plants for hybridization. Meiotic studies have explained the nature of sterility and fertility among hybrids.

To stimulate interest in such studies, the senior author attempted a preliminary study of the chromosome numbers together with a resident faculty during his tour of assignment (1962-63) as a member of the Michigan State University Group (Office of International Programs, MSU) located at the University of the Ryukyus. The little time available for this investigation and the condition of the plants in the collections limited the extent of this study both in depth and scope. It is hoped that other investigators with similar interest would confirm this work and continue this study to include all the known and available native orchid species, not only

^{*} Published with the approval of the Director, Hawaii Agricultural Experiment Station, University of Hawaii, as Technical Paper. No. 718. This study was done at the University of the Ryukyus when the senior author served as a Horticultural Specialist with the Michigan State University Group (1962-63).

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in establishing their chromosome numbers but also in the area of karyotype studies. Botanically, the preservation of these native species should be a major concern. With limited land area and advancing agricultural technology, virgin areas are being rapidly cleared of natural vegetation for crop production. Already a number of species are approaching extinction.

Materials and Methods

Cytological techniques used in this study follow the methods outlined by Kamemoto, *et al.* (1961). These authors describe in detail the methods of preparing the various solutions such as aceto-orcein stain, 8-oxyquinoline solution, fixing fluids, tissue softener and how to use them.

They list three choices of fixing fluids and two of tissue softeners. In this study the modified Carnoy's fixative and the 1:1 mixture of concentrated hydrochloric acid and ethyl alcohol tissue softener were used.

In all cases root-tip smear method was employed.

Observations and Discussion

Table 1 tabulates the list of orchids by their binomials and the vernacular used in the Ryukyus. This is followed by the figure references to the photographs and the general distribution range as reported in the literature. Each species is discussed separately below.

1. Staurochilus luchuensis (Iriomote ran)—This genus is listed with a single species by Hatusima and Amano (1958) and as the vernacular indicates, it is found growing wild on Iriomote Island, one of a group of southern islands very sparsely populated with much of the land in a natural state of vegetation (Table 1). It is commonly grown by the people as an ornamental garden flower.

The plant and the flowering spike (Fig. 1) show resemblance to the Vanda. The flowers are medium reddish-brown with buff cross striations. According to Amano (personal contact), it is cross-compatible with the Vanda. Figure 11 shows its chromosomes and the number is reported in Table 2 as 2n=38. This genus has not been reported in the Chromosome Atlas (Darlington and Wylie, 1956).

Binomial	Vernacular	Figure Reference	e Distribution
Staurochilus luchuensis Fukuyama	Iriomoteran	. 1	Iriomote Island, Ryukyus Range unknown
Cymbidium aloifolium Sw.	Ken ran	2	Japan to Southeast Asia
Aerides japonicum Reichb. f.	Nago ran	3	Southern Japan, Amami Oshima to Okinawa
Neofinetia falcata Hu	Fū ran	4	Generally Far East, no specific reports on range
Luisia liukiuensis Schltr.	Ryukyu Bō ran	—	Ryukyu Islands, Range unknown
Calanthe liukiuensis Schltr.	Ryukyu ebine	—	Ryukyu Islands, Range unknown
Calanthe furcata Bateman	Tsuru ran	_	Japan to Southeast Asia Abundant in Philippines
Bletilla striata Reichb.	Shī ran	_	Japan, Ryukyu Islands, Taiwan and China
Dendrobium moniliforme Sw.	Sekikoku (Take ra	n) —	Southern Japan, Ryukyus, Taiwan and China

 Table 1. Botanical and vernacular names, figure references and distribution of some Ryukyuan orchids

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2. Cymbidium aloifolium (Ken ran)—Withner (1959) states that there are around 50 species in the genus Cymbidium. Distribution range is shown in Table 1. Early collections of C. aloifolium were made in Ceylon and Java (Lingley, 1830).

In the Ryukyus this species is said to have been the most popular among the native orchids. Figure 2a shows the plant and an enlargement of the flower spike is shown in Fig. 2b. Flowers are attractive with yellow petals suffused with reddish brown color emanating from the center of the flower.

The chromosome number for *C. aloifolium* has already been reported by Mehlquist (1952) as 2n=40. Darlington and Wylie (1956) list the *x* number for the genus as 20. Chromosome number given in Table 2 confirms Mehlquist's count of 40. The chromosomes shown in Fig. 10 are relatively long and somewhat difficult to spread. They stain well but clumping of chromosomes as shown at the upper and lower left side of the cell seems almost characteristic. The lower left clump appeared in many cells examined.

3. Aerides japonicum (Nago ran)—There are listed around 40 species in the genus Aerides distributed from India and Molluccas to Japan (Withner, 1959). According to Veitch (Vol. II, 1894), A. japonicum is the smallest of the Aerides with stem length never exceeding a few inches. As shown in Table 1, this species is found in Southern Japan (Kagoshima area), abundantly in Amami Oshima and a few in Okinawa. Figure 3 shows this plant in bloom. Flower spike is short with miniature white flowers. Chromosome determination was not made because of poor sample roots. However, Miduno and others as reported by Withner (1959), have established the number to be 38. The x numbers for Aerides given in the Chromosome Atlas are 19 and 20. Other species with 40 chromosomes have been reported.

4. Neofinetia falcata (Fu ran)—This is a small epiphytic orchid indigenous to the Far Eastern areas. Literature concerning this plant is meager. Basic number for this genus does not seem to be recorded. Figure 4 shows the plant with a spike of small white flowers. Figure 5 shows the chromosomes and the number is given in Table 2 as 38.

5. Luisia liukiuensis (Ryukyu Bo ran)—According to Hawkes (1961), Luisia is a genus of approximately 35 species distributed in the Asiatic and Indonesian tropics. This genus is closely allied to the Vanda. L. liukiuensis has so far been reported only in the Ryukyus (Table 1).

Chromosome number for L. liukiuensis is given in Table 2 as 38 and the chromosomes are shown in Fig. 6. The Chromosome Atlas gives 20 as the x number for this genus, based on Miduno's count for L. boninensis (2n=40). Chromosome determinations for Luisianda uniwai (intergeneric hybrid between Luisia teretifolia and Vanda Miss Agnes Joaquim) made by the senior author consistently showed 38 chromosomes. Chromosome number for Vanda Miss Agnes Joaquim has been established as 38. The intergeneric hybrid is free-seeding and shows

Species	Figure References	Chromosome Number (2n)	Reported Numbers*
Neofinetia falcata	5	38	Not reported
Luisia liukiuensis	6	38	x = 20
Calanthe liukiuensis	7	40	x = 20
Calanthe furcata	8	40	x=20, C. $furcata=40$
Bletilla striata	9	ca38	x=16, 18; B. striata=32; B. formosana=36
Cymbidium aloifolium	10	40	x=20; C. aloifolium=40
Staurochilus luchuens	is 11	38	Not reported
Dendrobium monilifor	·me —	ca 38	x=10, 19

 Table 2.
 Chromosome numbers, figure references and reported numbers of eight Ryukyuan orchids

* x numbers and somatic chromosome numbers as reported in the Chromosome Atlas (Darlington and Wylie (1956). Specific references are cited in the text. no apparent aberrations in fertility, indicating equal contributions of 19 chromosomes from each parent. This latter study and present study reported here for L. *liukiuensis* indicate that *Luisia* may have x numbers of 19 and 20.

6. Calanthe liukiuensis (Ryuku ebine) and C. furcata (Tsuru ran)—These two species are treated together as discussion deals with the same genus. This genus is composed of around 150 species which are native to areas extending from South Africa and Madagascar through Asia to New Guinea and the Fiji Islands (Hawkes, 1961). This range covers both tropical and sub-tropical climates. Izumi (1956) states that there are around 11 species distributed from Kagoshima to Hokkaido but C. furcata is considered to be one of the best of the Japanese Calanthes. While this species is rather widely distributed and especially abundant in the Philippines, C. liukiuensis appears to be restricted to the Ryukyus (Table 1). Further studies are necessary to establish its distribution range.

These two species were examined and the chromosome numbers are given in Table 2 as 40 for both. Figures 7 and 8 represent chromosomes for *C. liukiuensis* and *C. furcata*, respectively. Miduno's determinations as given in the Chromosome Atlas for *C. discolor*, *C. furcata* and several other species indicate 20 as the x number.

7. Bletilla striata (Shi ran)—Bletilla is a genus of seven known species, largely terrestrial and native to Japan, Taiwan and China (Hawkes, 1961). B. striata is indigenous to this area. The plant somewhat resembles the Spathoglottis in general appearance.

The Chromosome Atlas gives x numbers of 16 and 18 for the genus, based upon Miduno's determinations for *B. striata* as 2n=32 and *B. formosana* as 2n=36. The chromosome number of 38 given in this paper is not conclusive. The chromosomes are relatively long and among these long chromosomes were few very small ones which appeared to be fragments of chromosomes (Fig. 9). Supernumerary chromosomes have been reported by Kosaki (1958) for the Dendrobiums. A larger sampling with more detailed study is needed to clarify these differences in numbers before a typical number can be established.

8. Dendrobium moniliforme (Sekikoku or Take ran)—Dendrobium is a large genus distributed over a wide area from the Western and Southern Pacific through Australia to Asia. Distribution of *D. moniliforme* appears to be restricted as shown in Table 1. In the Ryukyus this species is largely confined to the northern part of Okinawa, according to Amano (personal contact).

Cytological studies of Dendrobiums have been done by Kosaki (1958), Vajrabhayn and Randolph (1960), Kosaki and Kamemoto (1961) in the United States; Miduno (1940) and Ito and Matsuura (1957) in Japan. While the Japanese workers have reported a few species with chromosome number of 40, the typical number appears to be 38. Chromosome number for D. moniliforme reported in this paper is around 38. Additional studies are required to confirm this number.

Summary

Chromosome numbers of eight orchid species native to the Ryukyus are reported in this paper. x numbers of the genera represented here are also discussed. In addition to the chromosome numbers, binomials and vernacular names are listed. Distribution of the genera and species investigated is briefly discussed.

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Explanation of Plates

Plate I

- Fig. 1. Plant and flowering spikes of Staurochilus luchuensis (Iriomote ran).
- Fig. 2. Plant and enlarged flower spike of Cymbidium aloifolium (Ken ran).
- Fig. 3. Plant and flowering spike of Aerides japonicum (Nago ran).
- Fig. 4. Plant and flowering spike of Neofinetia falcata (Fū ran).

Plate II

- Fig. 5. Chromosome figure of Neofinetia falcata (Fu ran).
- Fig. 6. Chromosomes of Luisia liukiuensis (Ryukyu Bō ran).

- Fig. 7. Chromosomes of Calanthe liukiuensis (Ryukyu ebine).
- Fig. 8. Chromosomes of Calanthe furcata (Tsuru ran).
- Fig. 9. Chromosomes of Bletilla striata (Shì ran).
- Fig. 10. Chromosomes of Cymbidium aloifolium (Ken ran).
- Fig. 11. Chromosomes of Staurochilus luchuensis (Iriomote ran).

琉球産蘭数種の染色体数決定(摘要)

ヘンリー, Y. 仲宗根・諸見里秀宰

琉球原産の9種の蘭分布について、簡単に論じた。これら9種のうちイリオモテラン (Staurochilus luchuensis) とリュウキュウボウラン (Luisia liukiuensis) の2種は琉球固有のものであると考えられる。

6 属, 8 種の染色体数を決定し、その結果を報告した。これらの種のn数は 19 または 20 の何れかである。またこれらの属のx数についても簡単に論じた。

PLATE I





