植物研究雑誌
J. Jpn. Bot.
67: 10–14 (1992)

Chromosome Number of *Rubus* ×*pseudosieboldii* (Rosaceae)

Yoshikane IWATSUBO[a], Hisashi MASAKI[b], Osamu KUME[c],
Yoshimasa ARAKI[a] and Naohiro NARUHASHI[a]

[a]Department of Biology, Faculty of Science, Toyama University, Gofuku, Toyama, 930 JAPAN;
[b]Shinnanyo Senior High School, Doi, Shinnanyo, Yamaguchi, 746 JAPAN;
[c]Kagawa Prefectural Eastern Forestry Office, Teramine, Higashiueta-cho, Takamatsu,
Kagawa, 761-05 JAPAN.

オオフユイチゴ（バラ科）の染色体数

岩坪美兼[a]，真崎　久[b]，久米　修[c]，荒木克昌[a]，鳴橋直弘[a]

[a]富山大学理学部生物学教室　930 富山市五福 3190；
[b]山口県立新南陽高等学校　746 新南陽市土井 1－8－1；
[c]香川県東部林業事務所　761－05 高松市東植田町寺峰 1210－3

(Received on June 10, 1991)

Chromosome counts of *Rubus* ×*pseudosieboldii* Makino collected in Honshu and Shikoku Islands showed two different numbers, 2n = 42 and 2n = 56. The two types of plants were widely distributed over the whole region investigated. This taxon has been regarded as a spontaneous hybrid between *R. sieboldii* Blume (2n = 28) and *R. buergeri* Miquel (2n = 56). The plants with 2n = 56 therefore seemed to be produced by a cross between an unreduced gamete of *R. sieboldii* and a reduced gamete of *R. buergeri*. Plants with 2n = 42 were ordinary hybrids with chromosome numbers intermediate between the parental plants.

Rubus ×*pseudosieboldii* Makino has been regarded as a spontaneous hybrid between *R. sieboldii* Blume and *R. buergeri* Miquel (Makino 1926, Ohwi 1965, Kitamura and Murata 1981), and sometimes grows with either of the two species. In Japan the three taxa are distributed in the central and western parts of Honshu, Shikoku and Kyushu (Naruhashi and Satomi 1972).

The chromosome number of *R. sieboldii* is known to be 2n = 28, and that of *R. buergeri* to be 2n = 56 (Jinno 1951a, 1958). These numbers were ascertained by the authors using both plants cultivated in the garden of Toyama University and plants collected at several sites in Honshu and Shikoku (unpublished). The expected chromosome number of the hybrid between the two species is 2n = 42, whereas, the number of *R.* ×*pseudosieboldii* reported by Jinno (1951b, 1958) was 2n = 56. This number seems to be unreasonable in hybrids between *R. buergeri* and *R. sieboldii* and we therefore decided to examine *R.* ×*pseudosieboldii* cytologically to determine the cause of the

discrepancy.

Materials and methods

Chromosome numbers were examined in 86 plants of *Rubus* ×*pseudosieboldii* collected in Honshu and Shikoku, Japan (Tables 1 & 2). The root tips of each plant were pretreated with a 0.002M 8-hydroxyquinoline solution for one hour at room temperature, subsequently kept at 5°C for 15 hours in a refrigerator. After fixation in a 1:3 acetic acid and ethyl alcohol mixture for 40 minutes, these were immersed in 1N HCl for a few hours, and then hydrolyzed in 60°C 1N HCl for 6 minutes. The root tips were stained with 1.5% lacto-propionic orcein, and the squash method was employed for observation.

Voucher specimens designated in Tables 1 and 2 are preserved in the herbarium of the Department of Biology, Faculty of Science, Toyama University.

Results and discussion

Chromosome counts of 86 plants of *Rubus* ×*pseudosieboldii,* whose collection sites and chromosome numbers are listed in Tables 1 and 2, showed two different numbers, $2n=42$ and $2n=56$. Features of the chromosomes were as follows:

1) *Rubus* ×*pseudosieboldii* with $2n=42$ (Fig. 1A)

Twenty three plants (27% of the examined plants) had a chromosome number of $2n=42$. The variation in chromosome length in each complement appeared gradual; the longest chromosome was about 1.2 μm, and the shortest about 0.7 μm. The chromosomes of the complement had centromeres situated in median, submedian and subterminal positions. This type of plant was widely

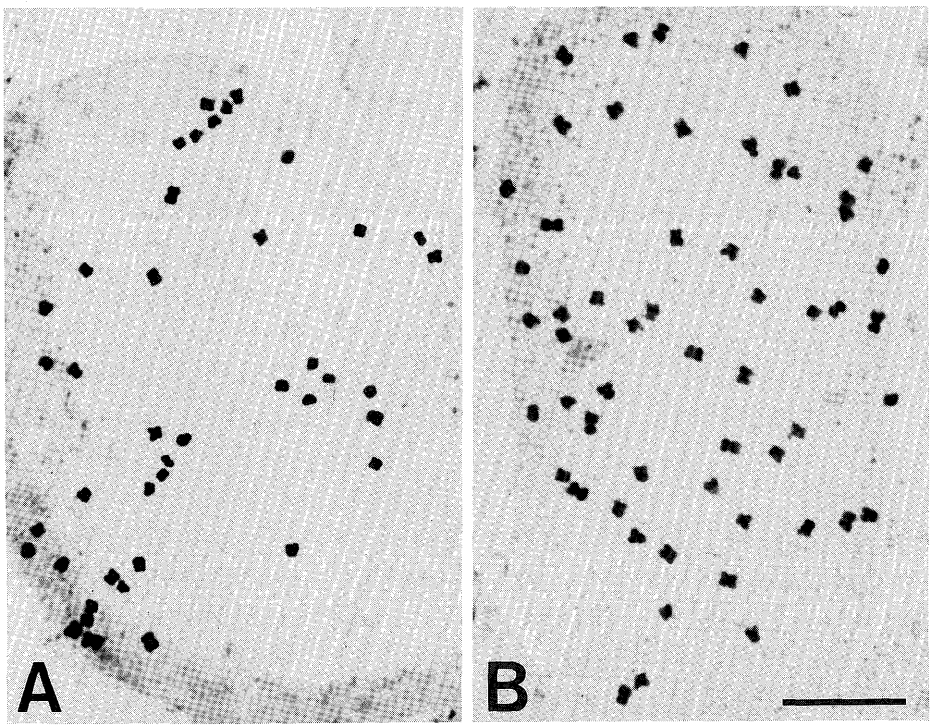

Fig. 1. Somatic metaphase chromosomes of *Rubus* ×*pseudosieboldii* Makino. A: $2n=42$, B: $2n=56$. Bar represents 10 μm.

Table 1. Distribution of *Rubus* ×*pseudosieboldii* with 2n=42.

Mie Pref., Watarai-gun, Futami-machi, Futamigaura, 30m*; Y. Araki**; AA44***
Mie Pref., Watarai-gun, Nansei-machi, Takahama, 3m; Y. Araki; 910328F
Mie Pref., Watarai-gun, Nanto-machi, Tanahashikamado, 3m; Y. Araki; 910328J
Mie Pref., Kitamuro-gun, Kiinagashima-machi, Tonose, 5m; Y. Araki; AA41
Mie Pref., Shima-gun, Isobe-machi, Natsukusa, 40m; Y. Araki; 910328E
Wakayama Pref., Shingu-shi, Ikeda-machi, 40m; Y. Araki; AA39
Wakayama Pref., Shingu-shi, Sehara, 30m; N. Naruhashi; 90102722
Wakayama Pref., Arita-gun, Hirokawa-machi, Shikagase, 100m; Y. Araki; AA38
Wakayama Pref., Arita-gun, Hirokawa-machi, Shikagase, 75m; N. Naruhashi
Hyogo Pref., Sumoto-shi, Takehara; T. Kobayashi
Yamaguchi Pref., Nagato-shi, Oumijima, Oumi, 10m; H. Masaki; 90111217
Yamaguchi Pref., Nagato-shi, Oumijima, Oohibi, 0m; H. Masaki; 90111219
Kagawa Pref., Mitoyo-gun, Oonohara-machi, Iseki, Uchinono, 250m; O. Kume; K1250
Kagawa Pref., Mitoyo-gun, Toyohama-machi, Wada, Innai, 30m; O. Kume; K1285
Kagawa Pref., Ookawa-gun, Shirotori-machi, Iza, Yojiyama, 30m; O. Kume; K1341
Kagawa Pref., Ookawa-gun, Shirotori-machi, Iza, Yojiyama, 30m; O. Kume; K1343
Kagawa Pref., Ookawa-gun, Shirotori-machi, Iza, Komatubara, 10m; O. Kume; K1344
Kagawa Pref., Ookawa-gun, Shido-machi, Oda, Kamaidani, 50m; O. Kume; K1345
Ehime Pref., Minamiuwa-gun, Johen-machi, Shimizu, 7m; Y. Araki; 901019B
Tokushima Pref., Anan-shi, Gakuhara-machi, 20m; Y. Araki; 901021L
Tokushima Pref., Kaihu-gun, Yuki-machi, Saichi, 20m; Y. Araki; 901021K
Kochi Pref., Sukumo-shi, Sakanoshita, 5m; Y. Araki; 901021F
Kochi Pref., Tosashimizu-shi, Misaki, 25m; Y. Araki; 901021B

*above sea level, **collector, ***voucher specimen

distributed over the entire region investigated (Table 1).

2) *Rubus* ×*pseudosieboldii* with 2n=56 (Fig. 1B)

A chromosome number of 2n=56 was found in sixty three plants (73% of the examined plants). The length of the chromosomes in the complement gradually varied from about 1.3 μm to about 0.7 μm and the centromeres were located in median, submedian and subterminal positions. This type of plant was prevalent over the entire region (Table 2).

As a rule, hybrids will have a chromosome number intermediate between the parental species. Some hybrids with higher chromosome number than the expected ones have been reported, however. In the experiment on hybridization between *Ranunculus cantoniensis* DC. (2n=32) and *R. silerifolius* Lév. (2n=16), Fujishima (1984) found a plant with 2n=32. The F_1 plants had a chromosome complement composed of a half set of *R. cantoniensis* and a full set of *R. silerifolius*. The same phenomenon was found by Agarwal (1988) in the F_1 hybrids of *Musa acuminata* Colla. (2n=22) × *M. rubra* Wall. (2n=22), where chromosome numbers of 2n=33 or more, contrasted with an expected 2n=22 in the hybrids. Examination of the F_1 hybrids showed that the chromosome complement resulted from the fusion of an unreduced female gamete in *Musa acuminata* and a reduced gamete of *M. rubra*. The author suggested that the phenomenon of such restitution nuclei formation as found in his experiment played an important role in the occurrence of present day triploid bananas the genomic constitution of which are AAB and ABB. In natural hybrids a similar phenomenon was reported in *Duchesnea* (Naruhashi et al. 1986, Naruhashi and Takano 1987). In Japan, this genus has two species (Hara and Kurosawa 1959): *Duchesnea chrysantha* Miquel (2n=14); *D. indica* Focke (2n=84), and the hybrid between them. The expectated chromosome number of the hybrid is 2n=49, which is the

Table 2. Distribution of *Rubus* ×*pseudosieboldii* with 2n = 56.

Mie Pref., Owase-shi, Motoyukino, 200m*; N. Naruhashi**; 90102732***
Mie Pref., Toba-shi, Toba, 10m; Y. Araki; AA36
Mie Pref., Watarai-gun, Kisei-machi, Nisiki, 100m; N. Naruhashi; 90102836
Mie Pref., Watarai-gun, Nanto-machi, Kawachi, 50m; Y. Araki; 910328I
Mie Pref., Kitamuro-gun, Kiinagashima-machi, Tonosu, 3m; Y. Araki; AA40
Mie Pref., Kitamuro-gun, Kiinagashima-machi, Tonosu, 5m; Y. Araki; 9103280
Mie Pref., Kitamuro-gun, Kiinagashima-machi, Kori, 50m; N. Naruhashi; 90102835
Mie Pref., Kitamuro-gun, Miyama-cho, Kizu, 20m; N. Naruhashi; 90102834
Mie Pref., Kitamuro-gun, Miyama-cho, Watari, 10m; N. Naruhashi; 90102833
Wakayama Pref., Kainan-shi, Uetani, 260m; N. Naruhashi; 90102616
Wakayama Pref., Shingu-shi, Kakitani, 20m; N. Naruhashi; 90102725
Wakayama Pref., Tanabe-shi, Akizu-cho, Aoki, 40m; N. Naruhashi
Wakayama Pref., Tanabe-shi, Kamiakizu, Kizetsukyo, 60m; N. Naruhashi
Wakayama Pref., Nishimuro-gun, Nakaheji-machi, Toragamine-touge, 480m; N. Naruhashi
Hiroshima Pref., Saeki-gun, Miyajima-machi, 10m; Y. Araki; 901022B
Hiroshima Pref., Saeki-gun, Miyajima-machi, 10m; Y. Araki; 901022C
Hiroshima Pref., Saeki-gun, Miyajima-machi, 10m; Y. Araki; 901022E
Hiroshima Pref., Saeki-gun, Miyajima-machi, 15m; Y. Araki; 901022F
Hiroshima Pref., Saeki-gun, Miyajima-machi, 15m; Y. Araki; 901022G
Yamaguchi Pref., Houfu-shi, Mukoushima, Oda, 130m; H. Masaki; 90110909
Yamaguchi Pref., Kudamatsu-shi, Kasadoshima, Ogou, 50m; H. Masaki; 90110502
Yamaguchi Pref., Kudamatsu-shi, Kasadoshima, Honura, 40m; H. Masaki; 90111520
Yamaguchi Pref., Tokuyama-shi, Ooshima, 40m; H. Masaki; 90111111
Yamaguchi Pref., Abu-gun, Abu-cho, Udago, 100m; H. Masaki; 90111215
Yamaguchi Pref., Kuga-gun, Nishiki-cho, Usago, 300m; H. Masaki; 90111939
Yamaguchi Pref., Kuga-gun, Yuu-cho, Zenitsuboyama, 220m; H. Masaki
Yamaguchi Pref., Kumage-gun, Hirao-cho, Hannyaji, 120m; H. Masaki; 90111213
Yamaguchi Pref., Toyoura-gun, Houhoku-cho, Horikoshi, 90m; H. Masaki; 90111735
Kagawa Pref., Kanonji-shi, Awai-machi, Takenari, Awai-jinja, 70m; O. Kume; K1252
Kagawa Pref., Kanonji-shi, Awai-machi, Takenari, Awai-jinja, 70m; O. Kume; K1253
Kagawa Pref., Takamatsu-shi, Nishiueta-machi, Fujio-jinja, 120m; O. Kume; K1330
Kagawa Pref., Kita-gun, Miki-machi, Kaniwa, Sanban, 150m; O. Kume; K1392
Kagawa Pref., Nakatado-gun, Kotohira-machi, Kawanishi, Ushiyaguchi, Kotohirayama, 170m; O. Kume; K1254
Kagawa Pref., Nakatado-gun, Manno-machi, Sumishiyonishi, Shiota, 150m; O. Kume; K1294
Kagawa Pref., Nakatado-gun, Manno-machi, Yoshino, Gomo, 220m; O. Kume; K1295
Kagawa Pref., Mitoyo-gun, Oonohara-machi, Iseki, Uchinono, 290m; O. Kume; K1251
Kagawa Pref., Ookawa-gun, Hiketa-machi, Iseki, 55m; Y. Araki; 901021M
Kagawa Pref., Ookawa-gun, Hiketa-machi, Minamino, Sakamoto, 70m; O. Kume; K1287
Kagawa Pref., Ookawa-gun, Hiketa-machi, Kureha, Jooko, Tachibanadani, 60m; O. Kume; K1288
Kagawa Pref., Ookawa-gun, Hiketa-machi, Omi, Chikamori, 50m; O. Kume; K1333
Kagawa Pref., Ookawa-gun, Hiketa-machi, Kawamata, Kooji, Kawamata-damu, 190m; O. Kume; K1335
Kagawa Pref., Ookawa-gun, Hiketa-machi, Kawamata, Kooji, 80m; O. Kume; K1338
Kagawa Pref., Ookawa-gun, Hiketa-machi, Kawamata, Kooji, 70m; O. Kume; K1339
Kagawa Pref., Ookawa-gun, Hiketa-machi, Kawamata, Kawamatakami, Ishigami-jinja, 60m; O. Kume; K1340
Kagawa Pref., Ookawa-gun, Ookawa-machi, Minamigawa, Nakajou, 90m; O. Kume; K1327
Kagawa Pref., Ookawa-gun, Ookawa-machi, Tazura, Wareishi, 90m; O. Kume; K1328
Ehime Pref., Yawatahama-shi, Yahatahama, Shiroura, 30m; Y. Araki; 901018G
Ehime Pref., Yawatahama-shi, Kurinoura, 5m; Y. Araki; 901018H
Ehime Pref., Iyo-gun, Futami-machi, Takanokawa; N. Saitoh
Ehime Pref., Iyo-gun, Nakayama-machi, Ichinari, 200m; Y. Araki; 901018D
Ehime Pref., Ochi-gun, Namikata-machi, Katagamihigashi, 10m; Y. Araki; 901018B
Ehime Pref., Ochi-gun, Kikuma-machi, Aketanagasaka, 30m; Y. Araki; 901018C
Tokushima Pref., Kaihu-gun, Kaihu-machi, Chonai, 20m; Y. Araki; 901021I
Tokushima Pref., Kaihu-gun, Kaihu-machi, Chonai, 10m; Y. Araki; 901021J
Kochi Pref., Sukumo-shi, Sukumo, 20m; Y. Araki; 901021E
Kochi Pref., Susaki-shi, Hara-machi, 5m; Y. Araki; 901020G
Kochi Pref., Tosashimizu-shi, Dogamori, 80m; Y. Araki; 901020B

Kochi Pref., Tosashimizu-shi, Kotobuki, 20m; Y. Araki; 901020E
Kochi Pref., Tosashimizu-shi, Misaki, 7m; Y. Araki; 901021A
Kochi Pref., Tosashimizu-shi, Misaki, 25m; Y. Araki; 901021C
Kochi Pref., Tosashimizu-shi, Misaki, 20m; Y. Araki; 901021D
Kochi Pref., Hata-gun, Oogata-machi, Tosashirahama, 25m; Y. Araki; 901018M
Kochi Pref., Hata-gun, Oogata-machi, Tosashirahama, 40m; Y. Araki; 901018N

*above sea level, **collector, ***voucher specimen

number intermediate between the two species. Natural hybrids, however, have two different chromosome numbers, 2n = 49 and 2n = 56. Moreover, hybrids with 2n = 56 are found about three or more times as frequently as plants with 2n = 49. While plants with 2n = 49 are regarded as being ordinary hybrids, those with 2n = 56 are considered to be the result of fusion of a haploid gamete of *D. indica* and an unreduced gamete of *D. chrysantha*. Considering the occurrence of the two types of *Rubus* ×*pseudosieboldii*, it seems that plants with 2n = 56 are also produced by the fusion of an unreduced gamete of *R. sieboldii* and a reduced gamete of *R. buergeri*. Plants with 2n = 42 are produced by the fusion of normal reduced gametes. As in natural hybrids of *Duchesnea*, the majority of plants of *R.* ×*pseudosieboldii* also have a chromosome complement composed of a half set of one parent plant and a full set of the other parent plant. The reason for this fascinating phenomenon awaits clarification.

We wish to express our gratitude to Messrs. Tomoki Kobayashi and Noriyasu Saitoh for collecting plants and to Dr. David E. Boufford for checking the English text.

References

Agarwal P. K. 1988. Cytogenetical investigations in Musaceae. IV. Cytomorphology of an interspecific triploid hybrid of *Musa acuminata* Colla ×*M. rubura* Wall. Cytologia **53:** 717–721.

Fujishima H. 1984. Cytogenetical studies on F_1 hybrids between *Ranunculus cantoniensis* DC. and *R. silerifolius* Lév. Jpn. J. Genet. **59:** 205–214.

Hara H. and Kurosawa M. 1959. On the *Duchesnea indica* group. J. Jpn. Bot. **34:** 161–166.

Jinno T. 1951a. The chromosome numbers in *Rubus* I. La Kromosomo **9–10:** 360–361.

——— 1951b. The chromosome numbers in *Rubus* II. Jpn. Journ. Genet. **26:** 133–135.

——— 1958. Cytogenetic and cytoecological studies on some Japanese species of *Rubus* I. Chromosomes. Bot. Mag. Tokyo **71:** 15–22.

Kitamura S. and Murata G. 1981. Coloured illustrations of woody plants of Japan, Vol. II, p. 81. Hoikusha, Osaka (in Japanese).

Makino T. 1926. A contribution to the knowledge of the Flora of Japan. J. Jpn. Bot. **3:** 45–48.

Naruhashi N., Itahashi T. and Iwatsubo Y. 1986. Chromosome numbers of *Duchesnea* in Toyama Prefecture, Japan. La Kromosomo **II-42:** 1330–1335.

Naruhashi N. and Satomi N. 1972. The distribution of *Rubus* in Japan – 1. Distribution maps. Ann. Rep. Bot. Gard. Facult. Sci. Univ. Kanazawa **5:** 1–21.

Naruhashi N. and Takano H. 1987. Chromosome numbers and distribution of *Duchesnea* (Rosaceae) in Gifu Prefecture. Acta Phytotax. Geobot. **38:** 155–160.

Ohwi J. 1965. Flora of Japan, rev. ed., p. 746. Shibundo, Tokyo (in Japanese).

要 旨

オオフユイチゴ（*Rubus* ×*pseudosieboldii*）はホウロクイチゴ（*R. sieboldii*）とフユイチゴ（*R. buergeri*）の自然雑種と考えられている．しかし，ホウロクイチゴ2n=28，フユイチゴ2n=56，オオフユイチゴ2n=56という神野による染色体の報告は，オオフユイチゴの雑種性に疑問を抱かせたので，ここで染色体の再調査をおこなった．その結果はオオフユイチゴ86産地のうち，42本の染色体をもつもの23ケ所（Table 1），56本の染色体をもつもの63ケ所（Table 2）であった．42本の染色体を持つオオフユイチゴは通常の雑種と考えられるのに対して，56本の染色体を持つものはホウロクイチゴに生じた非還元配偶子とフユイチゴの減数した配偶子との交配による雑種であると推定された．