**Cyto-genetical Studies in the Genus *Solanum*. I.
Autopolyploidy of *Solanum nigrum* Linn.***

By
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In the previous paper (11), the present author reported that there occurs two different karyological forms of *S. nigrum* in Taiwan (Formosa). One of these is the ordinary Black Nightshade which is represented by 36 chromosomes in the haploid phase, while the other is a very interesting one being characterized by 12 chromosomes. The latter is the commonest weeds of Taiwan widely distributing throughout the island. A large number of specimens of the latter type deposited in the herbaria in Taihoku are identified as *S. nigrum* L. without exception. The present paper deals with the results of a cytological study of both types with the comparison of morphological characters, and a survey of geographical distribution, bringing about the criticism upon the problem of polyploidy of this group of plants with reference to the origin of the species involved.

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Taxonomic aspects

Both types of wild plants display large variation in the habit of growth and in the size and the shape of vegetative organs, in response to the environmental condition under which they grow. But the seedlings of each type are all true to type, if they are raised under an identical condition such as in a glass house. The failure of the systematists in detecting the morphological difference in both types is probably due to the plasticity of plant. Close examination of both types of the plants, however, brought to light several characters, by which one can be distinguished from the other, and such distinction is easier, when flesh materials are used than herbarium specimens are consulted.

A taxonomic study of both types with an assistance of Mr. K. ODASHIMA brought the conclusion that the type with 36 chromosomes is the typical *S. nigrum*, whereas the other with 12 chromosomes must

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be a new species which was named *Solanum photeinocarpum* NAKAMURA et ODASHIMA (12).

In order to illustrate the difference of both types, the most contrasting characters are compared in Table 1.

Table 1. Contrasting characters of *S. nigrum* and *S. photeinocarpum*

	<i>S. nigrum</i> (n = 36)	<i>S. photeinocarpum</i> (n = 12)
Height	About 70 cm.	About the same.
Stem	Terete or subangular in cross section, covered with minute pubescence, spines indistinct.	Angular in cross section, rather glabrous, spines distinct.
Leaf	3 × 5–5 × 8 cm. large, dark green, midribs and veins being often tinged with purple color, thick, ovate or broad ovate and becoming obtuse or acute at the apex, covered with scattered hairs. Margin irregularly crenate or unevenly undulate.	3 × 6–9 × 13 cm. large, light green, thin broad lanceolate or elliptic, becoming usually acute or caudate-acuminate at the apex, rather glabrous. Margin irregularly dentate or nearly entire.
Inflorescence	Raceme, 6–9 flowers.	Umbel, 4–7 flowers.
Corolla	White, yellowish green at the base, 8–14 cm. in diam. Lobes 5, triangular.	White, light yellowish green at the base 6–11 cm. in diam., Lobes 5, oblong-ovate or narrow triangular.
Calyx	Cupulate. Lobes 5, subulate.	Cupulate. Lobes 5, oblong lanceolatis, becoming obtuse at the apex.
Stamens	Free, short, bright yellow.	Do.
Style	Same in length as stamens.	Do.
Fruit	Globose or oblate, 7–9 mm. in diam., purplish black, never shining.	Globose or oblate, 6–8 mm. in diam., shiny bluish black.
Seeds	About 40, 2–2.5 mm. in width.	About 70, 1.5–1.8 mm. in width.

Text-fig. 1 illustrates the leaf, flower, fruits and seeds of both types that were collected flesh from seedlings raised under an identical condition and the whole plants of both types are also shown. The measures given in this figure are graduated in millimetres.

In addition to the typical *S. photeinocarpum*, there is an other form which is generally classed into *S. nigrum*. This form belongs to the class n = 12. It is distinguished, however, by its wider leaves, larger flowers, racemose inflorescence and smaller fruits, often failing to set fruits. This plant is probably a variety of *S. photeinocarpum*.

Geographical distribution

To determine the natural range of occurrence of *S. nigrum* and *S. photeinocarpum*, a collection of seeds from various locales through-

out the empire was made by the assistance of the author's friends.¹⁾ The chromosome number and the morphological characters were studied with the plants which were raised from seeds in Taihoku. The specimens deposited in the Tanaka Herbarium, the Herbarium

Text-fig. 1. *Solanum nigrum* (A) and *Solanum photeinocarpum* (B). 1, leaves; 2, seeds and flowers; 3, fruits; 4, whole plants.

1) In this connection, the author wishes to express his indebtedness to Mr. Y. NAKAJIMA, Mr. M. ARIMA, Prof. E. FUKUSHIMA, Mr. Y. HAYASHI, Mr. M. SASAWO, Mr. S. SUZUKI, Mr. K. TAMAKI and Mr. Y. TAKIGUCHI.

of the Botanical Institute of the Taihoku Imperial University and the Herbarium of the Taihoku Botanic Garden were also examined

to make the survey more complete. The result is mapped in Text-fig. 2.

Text-fig. 2. Natural range of *S. nigrum* and *S. photeinocarpum* in Japan.

As the figure illustrates, the typical *S. nigrum* distributes in the northern part of Japan, from Karafuto to Kiushiu, while *S. photeinocarpum* is found in the south, from Kiushiu to Taiwan. One case of the occurrence of *S. nigrum* in Taiwan must be an exceptional case interpreted as follows: In this island, the occurrence of a large number of this plant is limited to the enclosure of the Shirin Horticultural Experiment Station ground. It was found to grow also in the nurcerries of our institute and RYÔ's garden at Seira-Gai of Tainan Province where a few individuals were found. The first discovery of this plant was done by the author and ODASHIMA last year. These

facts must indicate that the plant is an introduced origin recently reached to the island. As the Shirin Horticultural Experiment Station is the place where nursery plants of fruit-trees are always coming in from the Main Island and abroad, the seeds of *S. nigrum* must have been unconsciously introduced to this station and then scattered over with their plants to the other places.

The unexpected occurrence of *S. nigrum* in Taiwan is taken for granted as an exceptional case, the natural range of *S. nigrum* and *S. photeinocarpum* in Japan is geographically distinct inhabiting in the different climatic belts, i.e. the former, with high chromosome number, is distributed throughout the temperate region, while the latter with low chromosome number is found in the subtropical and tropical regions. From the examination of our herbarium materials,

similar relationship is found to be prevalent with other part of the world, since the specimens from Central Europe, Sweden, and North America represent the form of *S. nigrum*, while the specimens from the warm districts, such as India, the Philippines and Canary islands are linked closely, if not identical, with *S. photeinocarpum*. In Fukien province, China, *S. nigrum* and the other form which is assumed to be a variety of *S. photeinocarpum*, have been collected.

The plant materials raised from the seeds collected by Mr. TAMAKI in Shimonoseki have turned out to be *Solanum aggregatum* JACQ., first time recorded from Japan, probably introduced from abroad.

Cytological observation

A. Method

Root tips collected from the plants grown in pots were fixed in NAVASHIN's solution. The observation on meiosis was made from slides prepared by BELLING's smear method. The smeared materials used in the most of the cases were fresh young anthers, but in certain cases, the anthers which had been stored in 80% alcohol after they were fixed in CARNOY's solution has been used. The slides were heated for a short time after mounting. This treatment gave satisfactory result, the chromosomes being stained deeply and the cytoplasm being destained fairly well.

B. *S. photeinocarpum*

The counting of the chromosomes in the cells of root tips shows that this plant is represented by the lowest chromosome number of the genus *Solanum*, i.e. 24 (Text-fig. 3). The meiosis of the pollen mother cells are quite regular.

At diakinesis, 12 bivalent chromosomes are observed, which are characterized by one or two terminal chiasmata (Text-fig. 5). The chromosomes are arranged uniformly on the equatorial plate at the first metaphase (Text-fig. 7, Pl. 3, Fig. 1). They take

Text-figs. 3-4. Somatic chromosomes ($\times$ ca. 2,200). 3, *S. photeinocarpum*; 4, *S. nigrum*.

generally rod- or ring-shape following the mode of chiasma (Text-fig. 6). The division in anaphase takes place in usual way (Text-fig. 8, Pl. 3, Fig. 2). At the second metaphase, 12 chromosomes are counted again on each equatorial plate (Text-fig. 9, Pl. 3, Fig. 3). The anaphase division is regular with no laggard (Text-fig. 10).

Finally, normal tetrads and complete pollen grains (Plate 3, Fig. 10) are formed.

The chromosome behavior demonstrated above indicates clearly

that the present material is one of the diploid forms of the genus *Solanum*.

Text-figs. 5-10. Meiosis of the pollen mother cells of *S. photeinocarpum* ($\times$ ca. 1,700). 5, diakinesis; 6, side view of the chromosomes at the first metaphase, each chromosome being drawn apart from each other; 7, polar view of the chromosomes at the first metaphase; 8, the first anaphase; 9, the second metaphase; 10, the second anaphase.

C. *S. nigrum*

The counting of the somatic chromosomes of *S. nigrum* agrees with the results obtained by WINKLER (19) and JØRGENSEN (3). It showed 72 chromosomes in the cells of root tips (Text-fig. 4). Workers (3, 4, 14, 16, 17, 18, 19) who studied the chromosomes of the

Black Nightshade at the first metaphase reported that all appears as bivalents. But the chromosome behavior, especially the mode of conjugation of chromosomes, observed by the present author is different from the result obtained by the other workers. In diakinesis, the author found frequently quadrivalents and hexavalents besides bivalents. The number of hexavalents varied from 0 to 8 corresponding to the number of quadrivalents and bivalents. Examples of diakinesis with the three kinds of chromosomes are illustrated in Text-figs. 11-13, (Plate 3, Figs. 4-6). Among the chromosomes observed in Text-fig. 11 (Plate 3, Fig. 4), we can easily distinguish 8 hexavalents, 1 quadrivalent and 10 bivalents. In drawing this figure, the cytoplasm was slightly squeezed with the cover glass to arrange all the chromosomes on the same plane. In Text-fig. 12 (Plate 3, Fig. 5), there are 4 hexavalents, 4 quadrivalents and 16 bivalents. No hexavalent is found in Text-fig. 13 (Plate 3, Fig. 6), but it comprises 8 quadrivalents and 20 bivalents. The hexavalents and the quadrivalents appeared in diakinesis are found to fall into various types according to the number of conjugate chromosomes and the number and position of chiasmata, whereas the bivalent

chromosomes are characterized by one or two chiasmata, taking usually ring-shape, U-shape or dumb-bell shape.

Text-figs. 11-19. Meiosis of the pollen mother cells of *S. nigrum* ($\times$ ca. 1,700). 11-13, diakinesis chromosomes, showing the appearance of bivalents, quadrivalents (IV) and hexavalents (VI); 14, the first metaphase comprising 36 bivalents; 15, the first metaphase, showing that some of the chromosomes closely assemble in three; 16, the first metaphase chromosomes consisting of 17 bivalents, 5 quadrivalents (IV) and 3 hexavalents (VI); 17, the second metaphase having 36 chromosomes on each equatorial plate; 18, the second metaphase, showing the appearance of 2 tripartite and 2 dipartite chromosomes besides single ones; 19, the second anaphase.

At the first metaphase, some figures show 36 bivalents (Text-fig. 14 and Pl. 3, Fig. 7), while, in the other figures, some of the chromosomes assemble so closely that it is difficult to count accurately all the elements (Text-fig. 15, and Pl. 3, Fig. 8). The latter case is probably due to the existence of multivalent chromosomes. Text-fig. 16 illustrates the case that 3 hexavalents, 5 quadrivalents and 17 bivalents were found at a side view of the first metaphase. In anaphase the chromosomes separate regularly towards each pole.

The counting of chromosomes at the second metaphase is done easily as we can identify most of the chromosome units. In some cases, we count here 36 chromosomes on each equatorial plate (Text-fig. 17). However, the appearance of a few tripartite or dipartite chromosomes is not rare (Text-fig. 18, Pl. 3, Fig. 9). Such chromosomes is undoubtedly originated from the quadrivalents or hexavalents which were halved in the first meiosis. In the second anaphase, all the chromosomes separate regularly towards the poles (Text-fig. 19). It is followed by the formation of normal pollen tetrads and perfect pollen grains. (Pl. 3, Fig. 11).

Morphological observation

As we know that polyploidy brings about gigas forms in certain plants, it would be interesting to find out how the diversity of chromosome number in the present case gives influence upon the size of various parts of organs. The author measured one hundred each of flowers, fruits and seeds of *S. nigrum* and *S. photeinocarpum*, and obtained the result that those of *S. nigrum* is markedly greater than those of *S. photeinocarpum*. This is illustrated in Table 2 (See also

Text-fig. 1).

Text-fig. 20. Cross sections through a portion of leaves. A, *S. nigrum*; B, *S. photeinocarpum*. ($\times$ ca. 160).

Text-fig. 21. Stomata of the under side of leaves. A, *S. nigrum*; B, *S. photeinocarpum*. ($\times$ ca. 160).

Similar relationship was found also in the anatomical study of leaves. In this case, the epidermis and the palisade cells of *S. nigrum* were strikingly larger than those of *S. photeinocarpum*. (Text-fig. 20). The stomata of leaves were studied indirectly by the SUMP method. It showed that *S. nigrum* is larger in the size of stomata, but fewer in the number of them per unit area, in comparison with *S. photeinocarpum* (Text-fig. 21). The data are summarized in Table 3.

The pollen grains of both plants are practically complete-fertile, the occurrence of the empty ones being less than 4% in both cases.

Table 2. Size of flowers, fruits and seeds

	<i>S. nigrum</i>	<i>S. photeinocarpum</i>
Diameter of colloras	mm. 11.42	mm. 8.47
Fruits height	7.49	6.57
diameter	8.00	7.07
Seeds length	1.79	1.49
width	1.49	1.20

Table 3. Size and distribution of stomata

Species	Size		No. per unit area*
	Length	Dia-meter	
<i>S. nigrum</i>	39 ^μ	29 ^μ	7.9
<i>S. photeinocarpum</i>	23	20	16.0

* The unit area in this case means the area of field of the microscope which was obtained by the combination of Zeiss AP. 40 X K 15 at the tube length of 160 mm.

The size of pollen grains, however, differs corresponding to the chromosome numbers of the plants. (Pl. 3, Figs. 10 and 11). The measurement of the diameter of pollen grains showed that *S. nigrum* is about 35 micra, while *S. photeinocarpum* is about 25 micra.

Discussion

In *S. nigrum* the question of chromosome conjugation is very important. Yet, with the exception of the work of JØRGENSEN (3), little effort has been made to gain a comprehensive view of cytology. WINKLER (18, 19), VILMORIN and SIMONET (16, 17), and TOKUNAGA (14) reported that 36 bivalent chromosomes appear at the first meiosis. JØRGENSEN and CRANE (4) counted the same chromosome number in the meiosis of the pollen mother cells in the *nigrum* tissue of certain graft hybrids. In his later study, JØRGENSEN (3) compared the meiosis of haploid, diploid, triploid and tetraploid forms of *nigrum*, and he considers that the presence of trisome or tetrasome is possible in the diploid form, though he could not find any evidence. In *S. nigrum*, the appearance of 36 bivalents is a possible case, but, as proved, in the present study there is the other case that hexavalents and quadrivalents occur besides bivalents. In an extreme case, the present author counted 8 hexavalents at diakinesis, and he found also tripartite or dipartite chromosomes at the second metaphase. It is a known fact that the association of non-homologous chromosomes is brought about in certain plants by the influence of abnormal environmental factors, such as low temperature, or by the occurrence of segmental interchange. But, it is not the case in *S. nigrum*, because, the association of chromosomes does not take place at random, resulting alway bivalents, quadrivalents or hexavalents, and the meiosis is carried out quite regularly, followed by the formation of complete pollen grains.

Judging from the occurrence of a large number of hexavalents, the author considers that *S. nigrum* is an autohexaploid which was brought about by the multiplication of 12 basic chromosomes. An

evidence which supports this view is obtained in the experiment of JØRGENSEN (3). According to him, a few trisomes were observed as well as bivalents and univalents in the meiosis of his haploid *nigrum*. Such chromosome behavior is quite strange if the plant is a true haploid, but it is natural if we assume that the plant is autotriploid which was raised parthenogenetically by the development of the haploid egg cell of the autohexaploid parent.

The variation in the pairing of chromosomes in meiosis, also seems to be parallel to that observed in autotetraploid tomatoes. In these plants, some workers (2, 7, 19) observed that most of the chromosomes were bivalents, while the others (5, 6, 13) reported that there occurs a large number of quadrivalents. According to HUMPHREY (2), there are usually 12 tetrasomic groups at middle diplotene, more or less associated though the number of units increases following the advancement of meiotic stages, giving finally a large number of bivalents and a few quadrivalents. At the metaphase of the first division, the chromosomes are contracted so closely as it is difficult to distinguish the individual chromosomes, but there seems to be a tendency to clump in four. UPCOTT (15) assumes that increasing or decreasing in the number of units in these cases are due to either influence of environmental condition or special characters of genotypes.

In *Solanum nigrum*, the multiplication of the chromosome number is associated with the increase of the size of various parts of the organs. This fact was illustrated clearly by WINKLER (19) with his tetraploid *nigrum*. In this particular plant, flowers, pollen grains, palisade cells and stomata are markedly larger in comparison with those of the diploid parent. According to JØRGENSEN (3), petals and leaves of *Solanum nigrum*, show different characters when the chromosome number is doubled or halved. They become narrower and thinner in the haploid, and broader and thicker in the triploid and the tetraploid. Analogous cases are found with respect to *S. photeinocarpum* and *S. nigrum*. In the former, with lower chromosome number, the parts of organs such as flowers, pollen grains, fruits, seeds, palisade cells and stomata are strikingly smaller in comparison with those of the latter, and the petals and leaves are also decidedly narrower and thinner.

In studying the distribution of the diploid and the tetraploid forms of *Vaccinium uliginosum*, HAGERUP (1) states that polyploid forms may be ecologically changed so as to grow in other climates and formations where the diploid form will not thrive. Analogous facts were reported by MANTON (8, 9) with *Biscutella laevigata* and *Nasturtium officinale* and by MATSUURA (10) with *Fritillaria cam-*

schatensis. The natural occurrence of *S. photeinocarpum* and *S. nigrum* with reference to the chromosome numbers resembles the case of *Nasturtium officinale* in which the diploid form is distributed throughout the south of Europe, whereas the tetraploid form is found in the north.

All facts enumerated above seem to show that there is an intimate relationship between *S. photeinocarpum* and *S. nigrum* in connection with the evolution of species. Close resemblance in general appearance of both forms and the difficulty of identification, easily overlooked by the systematists, suggest also the possibility of this supposition, although it is hard to say that the latter was derived directly from the former. To make this point clear, it is necessary to study the affinity of chromosomes in F_1 progenies from a cross between both plants. The study on this line is now in progress, as the author succeeded to obtain several seeds by cross-pollinations, which will be brought to the experiment of the coming season.

Summary

1. Among the plants generally termed as *S. nigrum* by the systematists the author found two types, karyologically distinct. One of them is $n = 12$, and the other $n = 36$.

2. Close examination proved that both types are not exactly alike, differing in many points of their morphological characters. The type with 36 chromosomes is the typical *S. nigrum*, but, the other with 12 chromosomes deserves to receive a new specific name, *S. photeinocarpum*.

3. *S. nigrum* is generally distributed throughout the northern part of Japan, whereas *S. photeinocarpum* occurs in the south. *S. nigrum* rarely found in Taiwan is of recent introduction, and its distribution is limited only to a few locales.

4. The chromosome behavior observed in meiosis proved that *S. photeinocarpum* is a diploid form of the genus *Solanum*, while, *S. nigrum* is an autohexaploid.

5. Size of the vegetative and reproductive organs of *S. nigrum* is markedly larger than that of *S. photeinocarpum*.

6. The comparison of morphological characters and the survey of geographical distribution suggest that, there is an intimate relationship between both types with respect to the evolution of species.

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Explanation of Plate 3

All the photomicrographs were taken by the aid of LEITZ's "Macca" attachable camera from the smeared preparations which were stained with aceto-carmin. Figs. 1-9 were taken at the combination of ZEISS AP. 120 objective and K. 15 ocular. They were enlarged to give a magnification of ca 1,400 on reproduction. Figs. 10-11 were taken at the combination of ZEISS AP. 40 objective and K 15 ocular, giving a magnification of ca 300.

Figs. 1-3. Meiosis of the pollen mother cells of *S. photeinocarpum*. 1. The first metaphase, illustrated in Text-fig. 7; 2, the first anaphase, showing normal division of chromosomes; 3, the second metaphase, illustrated in text-fig. 9.

Figs. 4-9. Meiosis of the pollen mother cells of *S. nigrum*. 4-6, diakinesis, showing the occurrence of quadrivalents, hexavalents and bivalents, illustrated in Text-figs. 11-13; 7, the first metaphase with 36 bivalents, illustrated in Text-fig. 14; 8, the first metaphase, showing that some of the chromosomes closely assemble in three, illustrated in Text-fig. 15; 9, the second metaphase with tripartite and dipartite chromosomes, illustrated in Text-fig. 18.

Fig. 10. Pollen grains of *S. photeinocarpum*.

Fig. 11. Pollen grains of *S. nigrum*.

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