CYTOTAXONOMIC STUDIES IN THE GENUS SYMPHYTUM. II.

CROSSING EXPERIMENTS BETWEEN SYMPHYTUM OFFICINALE L. AND SYMPHYTUM ASPERUM LEPECH.

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SUMMARY

Crossing experiments were carried out between the three cytotypes (2n=24, 2n=40 and 2n=48) of *Symphytum officinale* and that of *Symphytum asperum* (2n=32). The results indicate that morphologically closely related types are sometimes crossable, whereas in other cases no hybrids could be produced. On the other hand morphologically very distinct types could be crossed with great ease.
The significance of these studies for the elucidation of taxonomic problems is discussed.

1. INTRODUCTION

The species *Symphytum officinale* L. and *Symphytum asperum* Lepech. are able to intercross. The hybrid, *Symphytum* × *uplandicum* Nym. is widespread in Europe, a.o. in Great Britain (TUTIN 1956; WADE 1958). Cytological studies revealed that in the Netherlands *Symphytum officinale* is represented by three cytotypes: 2n = 24, 2n = 40, and 2n = 48 (GADELLA & KLIPHUIS 1967). The cytotype 2n = 48 is widespread in Europe, many populations in Burgenland (Austria), Czechoslovakia and Western Germany are tetraploid. The diploid plants seem to have a more restricted distribution, at least in the Netherlands. They are also known from Italy, Hungary and Eastern Germany. Hitherto, plants with the number 2n = 40 have been found in the Netherlands only.

The species *Symphytum asperum* was studied by STREY (1931) and BRITTON (1951), who found in material of garden origin the numbers 2n = 36 and 2n = 40 respectively. This could neither be confirmed by GRAU (1968), nor by the present authors, who found the number 2n = 32.

In view of the great ease with which both species are able to intercross, it seemed obvious to undertake combined cytological and morphological studies. Moreover, repeated back-crossing of the hybrid with both parents is said to occur (WADE).

In this paper some preliminary results of crossing experiments will be dealt with. Detailed morphological and biometrical studies of plants belonging to various populations and also of hybrids will be published later.

2. MATERIAL AND METHODS

The cytological methods are described in our previous paper (GADELLA & KLIPHUIS 1967).

For crossing experiments the flower buds were emasculated (before any pollen was shed, two days before anthesis). For these experiments, 7779 flower-buds were used. Emasculation of the flower is easy in view of the fact that the filaments are inserted on the corolla tube. With the aid of a pair of tweezers the corolla was removed.

At the time of anthesis the ripe pollen was placed on the stigma. This was repeated two days later. Contamination by pollen of other plants was prevented by enveloping bags. The seeds were harvested after 3–6 weeks.

3. RESULTS

For the crossing experiments the following cytotypes were used:

a. *Symphytum officinale*, 2n = 24; flowers white.
b. *Symphytum officinale*, 2n = 40; flowers purple.
c. *Symphytum officinale*, 2n = 48; flowers white, red and purple.
d. *Symphytum asperum*, 2n = 32; flowers blue.
e. *Symphytum "asperum"*, 2n = 36; flowers bluish. Plants of garden origin, probably not pure.

The following results were obtained (see *fig. 1*):

a. Self pollination experiments.
No seeds could be obtained in this way. This is true for all cytotypes.
b. *Symphytum officinale* 2n = 24 ♀ × *Symphytum officinale* 2n = 40 ♂.
Despite many attempts seeds could never be obtained in this way.
c. *Symphytum officinale* 2n = 40 ♀ × *Symphytum officinale* 2n = 24 ♂.
No seeds were formed.
d. *Symphytum officinale* 2n = 24 ♀ × *Symphytum officinale* 2n = 48 ♂.
It appeared that the colour of the flower of the plant used for pollination is of importance. If this plant is white-flowered, a hybrid can be obtained. This, however, is very difficult. Many attempts failed, but once a seed could be harvested which was able to germinate. The hybrid is white-flowering [which is to be expected, as the diploid plant (2n = 24) is always white-flowering, as far as we found up till now], and is characterized by the number 2n = 36. This vigorous hybrid did not produce viable seeds up till now. In view of the self incompatibility this is to be expected. Also seeds formed after so-called "open" pollination (i.e. the flowers were not enveloped by bags, so that insects could carry pollen from other plants of the experimental plot to the stigma of this triploid hybrid) did not germinate.

No seeds could be obtained if the plant used for pollination has red or purple flowers.

e. *Symphytum officinale* 2n = 48 ♀ × *Symphytum officinale* 2n = 24 ♂.
Once a hybrid could be obtained (2n = 36) after many unsuccessful attempts. Also in this case the tetraploid plant (2n = 48) was white-flowering. This hybrid has not flowered so far.

f. *Symphytum officinale* 2n = 40 ♀ × *Symphytum officinale* 2n = 48 ♂ and

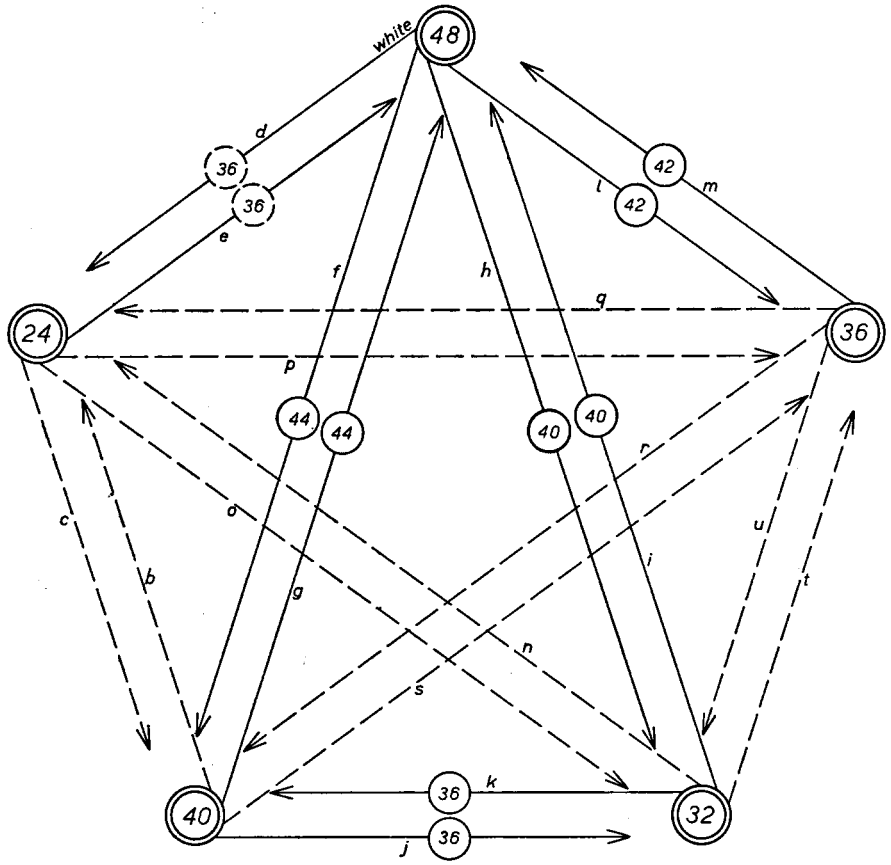

Fig. 1. Crossing-polygon, showing the relationships between *Symphytum asperum* Lepech. and *Symphytum officinale* L.
Double circles represent the five cytotypes (*S. officinale*: 2n=24, 40, 48; *S.asperum*: 2n=32, 2n=36 – – garden origin, Bucuresti – –).
Black lines connecting double circles indicate successful hybridisation, dotted lines unsuccessful hybridisation.
The direction of the arrows corresponds with the direction of pollination. Single circles, between the double circles, represent hybrids, with the chromosome number indicated.
The figures b/u refer to the descriptions given in the text.

g. *Symphytum officinale* 2n = 48 ♀ × *Sympyhtum officinale* 2n = 40 ♂.

In both cases f. and g. the cytotypes 2n = 40 and 2n = 48 can be crossed without difficulties. The hybrid is characterized by the number 2n = 44.

These hybrids can be reciprocally crossed with great ease. Their progeny, (also 2n = 44), is fully fertile.

Back-crossing of the hybrid 2n = 44, (obtained by the crossing of the

cytotypes 2n = 40 and 2n = 48) with the parental types (2n = 40 and 2n = 48 respectively) always resulted in hybrids with the chromosome numbers 2n = 42 and 2n = 46 respectively.

h. *Symphytum asperum* 2n = 32 ♀ × *Symphytum officinale* 2n = 48 ♂, (only the pollen of the flower with light purple corolla was used).

The hybrids, (flower bluish-purple), have the chromosome number 2n = 40. They were made easily.

i. *Symphytum officinale* 2n = 48 ♀ × *Symphytum asperum* 2n = 32 ♂.

The hybrid (2n = 40) has bluish-purple flowers and is produced easily.

j. *Symphytum asperum* 2n = 32 ♀ × *Symphytum officinale* 2n = 40 ♂.

Some hybrids 2n = 36 resulted from this cross. They have bluish-purple flowers.

k. *Symphytum officinale* 2n = 40 ♀ × *Symphytum asperum* 2n = 32 ♂.

The hybrids resulting from this experiment, have the same flower colour as the hybrids described under j. They are characterized by the chromosome number 2n = 36.

l. *Symphytum "asperum"* 2n = 36 of garden origin ♀ × *Symphytum officinale* 2n = 48 ♂. (Flowers white or light purple).

The hybrids, which have not flowered sofar, have the chromosome number 2n = 42.

m. *Symphytum officinale* 2n = 48 ♀ × *Symphytum"asperum"* 2n = 36 of garden origin ♂.

The hybrids have the chromosome number 2n = 42. Up till now (spring 1969) they did not come into flower.

n–u. all these crosses were unsuccessful:

n. *Symphytum officinale* 2n = 24 ♀ × *Symphytum asperum* 2n = 32 ♂.
o. *Symphytum asperum* 2n = 32 ♀ × *Symphytum officinale* 2n = 24 ♂.
p. *Symphytum "asperum"* 2n = 36 ♀ × *Symphytum officinale* 2n = 24 ♂.
q. *Symphytum officinale* 2n = 24 ♀ × *Symphytum "asperum"* 2n = 36 ♂.
r. *Symphytum officinale* 2n = 40 ♀ × *Symphytum "asperum"* 2n = 36 ♂.
s. *Symphytum "asperum"* 2n = 36 ♀ × *Symphytum officinale* 2n = 40 ♂.
t. *Symphytum "asperum"* 2n = 36 × *Symphytum asperum* 2n = 32 ♂.
u. *Symphytum asperum* 2n = 32 ♂ × *Symphytum "asperum"* 2n = 36 ♂.

Summarizing, the various cytotypes can be interpreted as follows:

1. 2n = 24: *S. officinale* L. subsp. *officinale* (diploid).
2. 2n = 32: *S. asperum* Lepech.
3. 2n = 36: a. *S. officinale* L. subsp. *officinale* (2n = 48) × *S. officinale* L. subsp. *officinale* (2n = 24).
   b. *S. officinale* L. subsp. *uliginosum* (Kern.) Nym. (2n = 40) × *S. asperum* Lepech. (2n = 32).
   c. *S. asperum* Lepech. (2n = 32) × [*S. officinale* L. subsp. *officinale* (2n = 48) × *S. asperum* Lepech. (2n = 32]*.

* This has to be tested experimentally.

4. 2n = 40: a. *S. officinale* L. subsp. *uliginosum* (Kern.) Nym.
   b. *S. officinale* L. subsp. *officinale* (2n = 48) × *S. asperum* Lepech. (2n = 32).
5. 2n = 42: a. *S. officinale* L. subsp. *uliginosum* (Kern.) Nym. (2n = 40) × [*S. officinale* L. subsp. *officinale* (2n = 48) × *S. officinale* L. subsp. *uliginosum* (Kern.) Nym. (2n = 40)].
   b. *S. officinale* L. subsp. *officinale* (2n = 48) × *S.* "*asperum*", garden origin (2n = 36).
6. 2n = 44: *S. officinale* L. subsp. *officinale* (2n = 48) × *S. officinale* L. subsp. *uliginosum* (Kern.) Nym. (2n = 40).
7. 2n = 46: *S. officinale* L. subsp. *officinale* (2n = 48) × *S. officinale* L. subsp. *officinale* (2n = 48) × *S. officinale* L. subsp. *uliginosum* (Kern.) Nym. (2n = 40)].
8. 2n = 48: *S. officinale* L. subsp. *officinale*, (tetraploid).

4. DISCUSSION

It is clear that *Symphytum officinale* is a very complex species consisting of at least three cytological races. The plants with 2n = 40 differ slightly from the diploids and tetraploids, (GADELLA & KLIPHUIS, 1967), and may be assigned to the subspecies *uliginosum* (Kern.) Nym.

The diploids and tetraploids are very similar morphologically. According to our results diploid and tetraploid plants seem to be crossable to an extremely limited extent. In the experimental garden a hybrid could be obtained only twice. In nature, in a mixed population, hybrids are never met with. Contrary, the plants with 2n = 40 and 2n = 48 chromosomes are fully interfertile. Their hybrids are also fertile and intercrossable. Crossing of these hybrids (2n = 44) with their parents (2n = 40 and 2n = 48) is possible.

Symphytum asperum is not variable in cytological respect, as far as is known up till now. Only the number 2n = 32 could be found. Other plants, with 2n = 36, which resemble *Symphytum asperum* to a certain extent, were obtained from the botanical garden of Bucuresti (Rumania). These plants could be crossed with *Symphytum officinale* 2n = 48 without difficulties, but it appeared impossible to cross them with *Symphytum asperum* 2n = 32. Both cytotypes 2n = 40 and 2n = 48 of *Symphytum officinale* are crossable with *Symphytum asperum* 2n = 32, resulting in hybrids with the number 2n = 36 and 2n = 40 respectively. These hybrids must be regarded as *Symphytum* × *uplandicum*, in spite of the fact that they differ slightly from each other and have a different origin. In view of the fact that tetraploid plants (2n = 48) of *Symphytum officinale* seem to be the more common in Europe, it is highly probable that the hybrid *Symphytum* × *uplandicum* with the chromosome number 2n = 40 is the commonest type of *Symphytum* × *uplandicum*. More research, however, is necessary, including detailed herbarium studies. This year, (1969), attempts will be made to intercross the hybrids *S.* × *uplandicum* 2n = 36 and 2n = 40. Also back-crossing of the hybrids *S.* × *uplandicum* 2n = 36 and 2n = 40 with the parents

Symphytum officinale 2n = 40 and 2n = 48, will be the objective of further investigation.

The plants from Bucuresti, (2n = 36), may possibly be regarded as a backcross from *Symphytum* × *uplandicum* 2n = 40 with *Symphytum asperum* 2n = 32. Backcrossing of the *Symphytum* × *uplandicum* 2n = 40 hybrids with *Symphytum asperum* 2n = 32 might prove this.

Also the hybrid *Symphytum* × *uplandicum* with the number 2n = 42 (obtained by crossing *Symphytum* "*asperum*" 2n = 36 of garden origin × *Symphytum officinale* 2n = 48) will be studied more in detail.

Taxonomically it is not easy to give an interpretation of these results. If the *Symphytum* × *uplandicum* hybrids are fertile, the species *Symphytum asperum* and *Symphytum officinale* are fully interfertile. The clearcut morphological differences indicate without any doubt that the taxa *S. officinale* and *S. asperum* represent distinct morphological species. The ease with which they hybridize (at least in the experimental garden) indicates that they belong to the same biospecies (sensu Mayr).

Symphytum officinale is very variable, which is also reflected in the hybrids uplandicum 2n = 36, 2n = 40.

In the Dutch province of Limburg some plants were found that could possibly be regarded as backcrosses of *Symphytum* × *uplandicum* with *Symphytum officinale* or with *S. asperum*. On the Dolsberg, near Wijlre (prov. of Limburg), some plants with the chromosome numbers 2n = 43, 44 were found. They are morphologically intermediate between *Symphytum officinale* 2n = 48 and *Symphytum* × *uplandicum* 2n = 40. Near Epen (prov. of Limburg) some plants were found with the chromosome number 2n = 36. The plants are more or less intermediate morphologically between *Symphytum asperum* 2n = 32 and *Symphytum* × *uplandicum* 2n = 40.

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